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Milk and Milk Products in Human Nutrition

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Preface

Milk is the sole source of nutrition for mammals for a period from several days to a few years, depending on species. The complex biology of mammalian milks impacts early growth and development, and may provide a foundation for health throughout the entire lifespan.

Human milk is aimed at being the sole source of nutrition in early infancy, but if breastfeeding is not possible milk substitutes, in general based on cow’s milk protein, need to have a composition fulfilling the same goal of serving as the sole source of nutrition during the first months of life and confer as close as possible the overall health benefits that human milk provides to the infant.

In many populations, milk continues to play a major role in a healthy, balanced diet throughout life. During childhood, pregnancy and adulthood, intake of cow’s milk has important beneficial effects on linear growth, bone development and oral health. Cow’s milk has been especially effective in prevention and treatment of undernutrition in low-income countries. Potentially adverse effects of cow’s milk intake, like increased risk for type 1 diabetes and certain cancers, or negative aspects of dairy fats continue to be under debate in the absence of convincing evidence.

The workshop covered three sessions with excellent presentations of invited lecturers and vivid discussions typical for the Nestlé Nutrition Institute workshops. The first session covered Milk during Pregnancy and Infancy, the second session Milk during Childhood in Low- and High-Income Countries, and the last session General Aspects of Milk: Milk in Adult Nutrition. Together, the three sessions covered most aspects of milk during the life cycle in a global perspective.

This publication includes all the presentations together with the discussions following each of them. The concluding remarks provide a short summary and conclusions drawn from the deliberations of the workshop.

Roger A. Clemens
Olle Hernell
Kim Fleischer Michaelsen
Foreword

Following the workshop on the ‘Biology of Human Milk’ held in 1988, the present 67th workshop was the first one focusing on the health aspects of milk during and beyond the breast milk feeding period, reflecting the major role that milk plays in a healthy, balanced diet across the lifespan. Breast milk is unique, and in the ideal situation, is the sole source of nutrition in early infancy. Breast milk substitutes therefore have to be chosen carefully depending on their suitability for the infant. However, since 1988 the scientific world has reached the consensus that the performance of the breastfed infant rather than the composition of human milk should be the reference for the innovation of breast milk substitutes. The benefits of milk in the diet during the weaning and toddler periods were debated in this workshop, as well as the benefits for school age children and throughout adolescence and adult life. The benefits may be different at different ages.

Amongst the most important beneficial effects summarized during the workshop were:

- Milk remains an important source of dietary calcium, protein, energy, vitamins, minerals, growth factors and other bioactive components in both, low- and high-income countries. Milk particularly contributes to dietary vitamin D intake, especially when fortified.
- Milk is a crucial part of the diet for child growth and development. There is a clear association with linear growth, although the mechanisms are yet to be fully elucidated.
- Dairy fats contain a range of lipids that may have health-promoting properties including omega-3 LC-PUFA, gangliosides, sphingolipids, etc.

Impact of ingestion of trans-fatty acids or selected saturated fatty acids on health and the association of risk for type 1 diabetes and milk intake were discussed in a balanced manner.

This workshop, held in Marrakech, Morocco, in March 2010, brought together an outstanding group of scientific experts in the field and participants from 30 countries who contributed largely to the lively and intense discussions.
We want to thank the three chairpersons, Prof. Roger Clemens from the USA, Prof. Olle Hernell from Sweden and Prof. Kim Fleischer Michaelsen from Denmark, all highly respected experts in the field of pediatric nutrition, for putting together this outstanding scientific program.

Our special thanks go to Mr. Badr Nassili and Ms. Sophia Jala and their Nestlé Maghreb team in Morocco for their efficient logistic support and for hosting this workshop in the beautiful environment of Marrakech.

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Milk Intake, Calcium and Vitamin D in Pregnancy and Lactation: Effects on Maternal, Fetal and Infant Bone in Low- and High-Income Countries

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Abstract
Calcium and vitamin D are essential for bone growth and maintenance. Among the bone-forming minerals, dietary calcium supply is close to biological requirements and may be limiting in some parts of the world where there are few rich dietary sources of calcium, particularly for children and women during pregnancy and lactation. Animal milk is a rich source of calcium and, in countries where milk is fortified with vitamin D, a contributor to dietary vitamin D intake. Current evidence indicates that, in the human, there are physiological mechanisms that support the necessary calcium fluxes across the placenta and mammary gland and that are unresponsive to increases in calcium intake. This applies across the range of dietary calcium intakes recorded in healthy individuals. In contrast, although there is unlikely to be an additional requirement for vitamin D during pregnancy and lactation, many women have poor vitamin D status. This places them at risk of osteomalacia and their infants at risk of rickets, osteomalacia, compromised skeletal growth and other outcomes. There needs to be increased awareness among policy makers, health professionals and the public about the importance of safe UVB sunshine exposure and consumption of dietary vitamin D by women of reproductive age at risk of vitamin D deficiency.

Introduction
Calcium and vitamin D are essential for bone growth and maintenance. Among the bone-forming minerals (Ca, P, Mg, Zn), dietary calcium supply is close to biological requirements and may be limiting in some parts of the world where there are few rich dietary sources of calcium, particularly for
children, and women during pregnancy and lactation [1]. Similarly, the vitamin D status of many women and young children is compromised by low UVB skin exposure and by factors that increase vitamin D usage, even in tropical countries with abundant sunshine [2]. Milk from domesticated ruminants, primarily cow, sheep, goat, camel and buffalo, are major sources of dietary calcium and, in countries where commercially available milk is fortified, of dietary vitamin D. This short review summarizes the data on calcium and vitamin D supply for mothers and young children in low- and high-income countries and considers the evidence on the implications for maternal, fetal and infant bone health.

**Calcium as a Bone-Forming Mineral**

Bone consists of mineral embedded in a collagen matrix. The mineral phase closely resembles hydroxyapatite, and is composed of crystals of predominantly calcium, phosphorus and water together with a number of other components that are present in lower quantities, such as Mg, Zn, carbonate and citrate. All but 1–2% of the body's calcium is contained within the skeleton, unlike the other bone-forming minerals which are widely distributed in many tissues [3]. Calcium forms part of essential cell-signaling systems throughout the body, and these are exquisitely sensitive to changes in intra- and extracellular calcium concentrations. The skeleton acts as a buffer to avoid life-threatening swings in calcium concentration elsewhere in the body. The release and accretion of skeletal calcium is one arm of a physiological response to maintain ionized calcium in the circulation within narrowly defined limits and to smooth out the peaks and troughs of calcium flux that occur naturally during 24 h, for example following a calcium-rich meal or an overnight fast. Consequently, any factor that compromises the body's supply of calcium for a prolonged period is likely to affect the mineral content of the skeleton, either by reducing the potential for bone mineral accretion and repair, or by inducing bone mineral mobilization to meet the body's extraskel-etal requirements for calcium [4].

**Calcium Requirements for Infancy, Pregnancy and Lactation**

Calcium is an essential nutrient, provided purely from dietary sources. There are two routes of calcium absorption from the intestine: active absorption under the control of the active metabolite of vitamin D (see below) and passive absorption. There are obligatory endogenous losses of calcium from the intestinal secretions, urine and sweat. Excess calcium is excreted into urine, and renal calcium reabsorption also becomes more efficient when physiological needs for calcium retention increase [5].
The skeleton of a neonate contains about 25 g of calcium and that of an adult woman contains about 800–1,000 g of calcium [1, 3]. The accretion of calcium by the fetus occurs predominantly in the second half of pregnancy with the highest rates of 200–300 mg/day occurring in the last trimester. Similar daily accretion rates occur in the first weeks of life and gradually decrease during infancy. These are mirrored by the calcium supply from breast milk which averages around 200–300 mg Ca/day during exclusive breastfeeding [1]. A woman who delivers a singleton infant and breastfeeds exclusively for 6 months therefore transfers about 200–300 mg Ca/day to her infant either in utero or via breast milk for around 9 months of the reproductive cycle, and continues to transfer significant quantities for as long as breastfeeding is continued. This represents more than 100 g for a typical Western woman who has 2 children and exclusively breastfeeds each for at least 6 months and around 1,000 g for a mother who breastfeeds each child on demand for 2 years with complementary feeding from about 6 months and who has 7 or more children, as is typical in many low-income countries.

**Vitamin D Requirements for Infancy, Pregnancy and Lactation**

Vitamin D is a prohormone which is converted in the body to an active metabolite, 1,25-dihydroxyvitamin D [1,25(OH)2D] that is essential for maintaining calcium homeostasis by the orchestration of intestinal calcium absorption, bone mineral turnover and renal calcium reabsorption [2, 6, 7]. There are two forms, vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol), that differ in some aspects of structure and metabolism, but both have prohormone activities. Vitamin D3 is produced in the skin under the action of UVB light of wavelength 290–315 nm. It enters the circulation bound to D-binding protein and is transported to the liver where it is converted to the long-lived metabolite 25-hydroxyvitamin D (25OHD). This also circulates bound to D-binding protein and is converted to 1,25(OH)2D in the kidney and is then secreted and has endocrine action in those tissues involved in the regulation of calcium and phosphate homeostasis (intestine, skeleton, parathyroid glands and kidney). 1,25(OH)2D is also involved in cellular processes in many other tissues, and 25OHD is taken up by these cells for intracellular production and utilization of the active metabolite.

For those individuals that have regular exposure to adventitious UVB sunlight exposure on face, hands and lower arms, endogenous synthesis of vitamin D3 is the primary source of vitamin D [4, 7, 8]. However, in temperate countries, UVB is not present in sunshine during the winter months, and there are other factors, such as pollution, indoor lifestyle and conservative dress, that also reduce an individual’s UVB skin exposure, even in tropical countries where UVB is present in sunshine year-round [4]. In addition, melanin reduces the penetration of UVB into the skin, and people with darkly pigmented skin
require longer periods of UVB exposure to achieve an equivalent amount of cutaneous vitamin D synthesis to fair-skinned people. Dietary sources of vitamin D$_3$ and vitamin D$_2$ are important providers of the prohormone when skin exposure to UVB sunlight is limited [7]. After absorption, oral vitamin D is transported to the liver where it is converted to 25OHD.

During gestation and breastfeeding, vitamin D and its metabolites are transferred by the mother to her offspring via the placenta and breast milk. These are relatively small quantities compared to maternal concentrations, and there is no evidence that the requirement of the mother for vitamin D is increased in absolute terms by pregnancy and lactation [9, 10]. However, hypovitaminosis D [a plasma concentration of <25 nM (10 ng/ml) 25OHD] is common in many parts of the world, including among pregnant and lactating mothers and young children [2]. This increases the risk of rickets and osteomalacia, and high prevalence rates of clinical vitamin D deficiency are widely reported, particularly among people originating from Africa, Asia and Middle East living in temperate countries and those living in tropical countries who have limited skin exposure to UVB sunshine [2].

**Dietary Sources and Intakes of Calcium**

Ruminant milks are among richest sources of dietary calcium when expressed as a percentage of weight consumed; cow's milk contains approximately 120 mg Ca/100 g. Other calcium-dense sources include small fish consumed with bones and materials used as condiments and culinary ingredients, such as lime used for the production of tortilla in Central America, dried baobab leaf used to prepare steamed millet and dried baobab fruit used to flavor porridges in West Africa [11]. Some cereals, such as tef, green leaves, seeds, nuts and fruits also contain measurable amounts of calcium, all of which make small but useful contributions to the overall calcium intake in populations where milk and milk-products are scarce or not part of the local cuisine.

Relative absorption efficiency from most diets is generally around 30% or less, but is greater when calcium intakes are low and during times of high physiological need [5]. Calcium absorption from animal milks is considered to be high relative to most other sources, averaging around 30–35%; the absorption of calcium from breast milk exceeds that from animal milks [5]. Calcium absorption from plant sources is considered to be low compared to animal sources because of the chelation properties of phytates and oxalates present in a plant-based diet. Calcium-rich water is also a significant source of dietary calcium, with an absorption similar to milk in healthy individuals with normal gastric acid production, but this fact is not widely appreciated and calcium from drinking water is generally not recorded in dietary surveys. Some countries require or permit calcium fortification of the food supply, for example in the UK there is mandatory fortification of white flour with calcium, and many
Milk, Calcium and Vitamin D in Pregnancy and Lactation

Food products contain calcium as a fortificant. Dietary calcium supplements in tablet or powder form are also sold in many countries. Dietary calcium intakes vary widely between individuals in the same population and between different populations, generally reflecting the consumption of animal milks (see below): a typical adult range is 300–1,500 mg Ca/day and is proportionately lower for children. Intakes at the low end of the range are well below international recommendations and are close to the biological calcium requirement for pregnant and lactating mothers and their offspring [1, 11].

Breast milk is the sole source of calcium for the exclusively breastfed infant and remains the predominant source after the introduction of complementary foods in populations where animal milk and milk products are not commonly available or avoided [12]. As described earlier, intakes of breast milk calcium average around 200–300 mg/day. However, there is considerable variation between women and between different parts of the world [13], such that the intake of calcium by healthy exclusively breastfed children can range by as much as 5-fold [13]. The accumulating evidence from observational and intervention studies has shown that the concentration of calcium in breast milk does not reflect the mother’s dietary calcium intake during pregnancy or lactation [1] even among women with a very low calcium intake [14, 15] but is related to the concentration of other constituents of human milk, notably casein, phosphate and citrate [16].

Dietary Sources and Intakes of Vitamin D

There are few rich dietary sources of vitamin D that are eaten regularly [2]. Among the most common are oily fish, egg yolks and to a lesser extent, meat. Fish liver oils and synthetic preparations of vitamin D are used as a dietary supplement; these make a significant contribution to vitamin D intakes in those individuals who use them regularly [17]. Many countries have mandatory or voluntary fortification of the food supply with vitamin D, although the fortification vehicle varies: for example, in the US and some other countries ‘door-step’ milk is fortified; in the UK, there is mandatory fortification of margarine [7, 17]. Animal milks contain small, but measurable, quantities of vitamin D and its metabolites. However, unless fortified with vitamin D, animal milks are not a rich source of vitamin D and make only a small contribution to overall dietary intakes. Other commercially available products, such as breakfast cereals and fruit juices, and aid foods, such as wheat-soy blend and multiple micronutrient mixes, are also often fortified with vitamin D.

The intakes of vitamin D vary between individuals and populations depending on the consumption of these rich sources of vitamin D: an average of 4 µg/day in the UK from a variety of sources including fortified margarine and cereals compares with 7 µg/day in the USA and Japan, the former largely due to the significant number of consumers of vitamin D supplements and vita-
Prentice

D-fortified milk in the USA, the latter because of the high consumption of oily fish in Japan [2, 17]. Dietary intakes of vitamin D from the plant-based diets of subsistence-farming populations in low-income countries are likely to be very low.

Breast milk contains measurable quantities of vitamin D and its metabolites, but the predominant sources of vitamin D for the exclusively breast-fed infant in the first months of life are from body stores built up in utero and later from skin exposure to UVB sunshine. The concentrations of vitamin D and its metabolites appear to be unrelated to the vitamin D status of the breastfeeding mother, except when she consumes high doses of supplemental vitamin D [7, 9, 10].

**Animal Milk Consumption in Low- and High-Income Countries**

Animal milks and milk products are major contributors to calcium intakes; populations with a ready supply of animal milks have considerably higher average calcium intakes than those where the supply is limited. The most reliable information about the likely contribution of animal milks to the diets of people in low- and high-income countries comes from the regular reports from the Food and Agricultural Organization on supply per capita in different countries of the world. The most recently available was conducted in 2005 (http://faostat.fao.org/site/368/). These demonstrate wide disparities in the availability of animal milks around the world, with the supply per capita in many African and Asian countries being very low. Calculating the potential amount of calcium that could be provided by animal milks in each country demonstrates that, when averaged over all members of the respective populations, there is sufficient animal milk available in countries of North America, Europe and Australasia to provide most, if not all, of the calcium needed to meet international dietary recommendations but that this is not the situation in most countries in Africa and Asia [18]. In such countries, the average total calcium intake from all sources generally falls well below international recommendations. As described earlier, the consumption of animal milks makes only a small contribution to dietary vitamin D intake, unless the country has a program of fortifying purchased animal milks and milk products with vitamin D.

**Implications of a Low Calcium Intake for Maternal and Infant Bone Health**

In theory, the additional calcium required by a pregnant or lactating mother to support the skeletal growth of her fetus during gestation and her infant during breastfeeding could be obtained by an increase in one or more of the...
following: dietary calcium intake, intestinal calcium absorption, renal calcium reabsorption and maternal bone mobilization. Also plausibly, maternal physiological adjustments to provide the essential supply of calcium to the offspring could be compromised in women with a low customary calcium intake with potential health consequences for herself and her baby. In the last 10–15 years, there have been a number of detailed studies that have investigated calcium and bone metabolism in human pregnancy and lactation and the extent to which mothers on a low calcium intake would benefit from greater calcium intakes during their reproductive lives. As has been summarized in detail elsewhere [1, 19–21], these studies have demonstrated that both pregnancy and lactation are associated with increased maternal bone turnover and a net efflux of bone mineral into the extracellular compartment, combined with enhanced intestinal calcium absorption in pregnancy and greater renal reabsorption in some women during lactation. Measurable decreases in whole-body and regional bone mineral content are observed [13, 22, 23]. Such decreases are of sufficient magnitude to make a significant contribution to the calcium required for fetal bone accretion or breast milk production [1, 21, 23]. These changes appear to be reversed, and bone mineral replenished, once breastfeeding becomes less intensive or is stopped altogether. By the time a woman has ceased breastfeeding for several months, there is little evidence of a lasting reduction in skeletal mineral content that can be related directly to pregnancy or lactation rather than to changes in bodyweight or advancing age [23, 24].

These studies have found no evidence of a relationship between the magnitude of these changes and the mother’s calcium intake [22, 23, 25]. Decreases in parathyroid hormone, bone resorption markers and small increases in maternal bone mineral density have been reported in pregnant and lactating women receiving calcium supplements, but these are in line with the expected biological response to the ingestion of additional calcium and are of a similar magnitude to the changes observed in control women given calcium supplements [21, 25]. Most intervention studies among pregnant and lactating women with very low calcium intakes have not identified any benefit of the extra calcium for fetal bone mineral accretion, birth size or infant skeletal growth and development [14, 15]. Some studies have suggested a temporary increase in bone mineral density immediately after birth in the infants of mothers with a low calcium diet supplemented with calcium during pregnancy, but these increments have not been observed in infants studied some weeks after delivery [14, 26]. A very low calcium intake in early life may also be implicated in the development of rickets reported among young African and Asian children where primary vitamin D deficiency has been discounted and calcium deficiency is suspected [18, 27]. Other health detriments unrelated to the skeleton have been hypothesized for mothers with a very low calcium intake and their offspring, most notably an increased risk of pre-eclampsia and eclampsia during pregnancy and a greater prevalence of cardiovascular
risk factors later in childhood: the limited data are summarized elsewhere [28, 29], but definitive comment awaits further evidence from ongoing studies.

**Implications of a Low Vitamin D Supply for Maternal and Infant Bone Health**

Vitamin D deficiency causes rickets in children and osteomalacia in children and adults. Both are disorders of poor mineralization of collagen, of the growth plates at the end of long bones and bone osteoid, respectively. Other sequelae include hypocalcemia, cardiac complications, myopathy, poor dental development and, in the newborn, craniotabes [10]. Vitamin D deficiency in the mother during pregnancy is associated with vitamin D deficiency in the infant [7, 10]. There is also evidence to suggest that poor vitamin D status of the mother at levels above those associated with clinical deficiency are associated with small birth size of the offspring, reduced linear growth in infancy and lower bone mineral accretion by 9 years of age [1, 7, 30]. Other health detriments have been suggested in terms of immune function and insulin sensitivity/glucose handling, and are currently being explored in a number of detailed intervention studies which will report in the next few years.

**Animal Milk Intake in Pregnancy and Lactation**

It is well recognized that generalized maternal undernutrition affects the size and development of the fetus, and hence the bone mineral accretion of the offspring. Infants born in less affluent societies tend to be smaller and have lower bone mineral content than their more affluent peers [14, 21]. There are data that suggest an association between the consumption of calcium-rich foods, including dairy products, in pregnancy and fetal growth [31, 32] and the bone mineral density of the offspring aged 6 years [33]. It is difficult to distinguish between the effects of these foods on the nutrient quality of the whole diet and those of calcium per se, given that animal milks are rich sources of growth factors and many essential nutrients such as Mg, Zn, essential fatty acids and water-soluble vitamins. Interpretation is even more complicated in studies from the USA, Canada and other countries where commercially available animal milks are fortified with vitamin D and where the regular consumption of such products may partly determine maternal vitamin D status.

**Conclusions**

Calcium intake and vitamin D status are low in many parts of the world. Animal milks make valuable contributions to dietary intakes of many nutri-
Milk, Calcium and Vitamin D in Pregnancy and Lactation

ents, including calcium. Skin exposure to UVB sunlight is the predominant source of vitamin D for most people, but dietary sources of vitamin D are important when UVB exposure is limited due to climate, latitude or lifestyle; milk and milk products that are fortified with vitamin D contribute to vitamin D intakes. Calcium is a bone-forming mineral, and vitamin D is required for regulation of calcium homeostasis and bone mineralization; both calcium and vitamin D are essential for the growth and maintenance of a healthy skeleton. Current evidence indicates that, in the human, there are physiological mechanisms that support the necessary calcium fluxes across the placenta and mammary gland and that are unresponsive to increases in calcium intake during pregnancy and lactation. This applies across the range of calcium intakes recorded in healthy individuals. In contrast, although there is unlikely to be an additional requirement for vitamin D during pregnancy and lactation, many women around the world have poor vitamin D status, which places them at risk of osteomalacia and their infants at risk of rickets, osteomalacia, compromised skeletal growth and other outcomes of vitamin D deficiency. There needs to be increased awareness among policy makers, health professionals and the public in both low- and high-income countries about the need for safe UVB sunshine exposure and provision of dietary vitamin D to women of reproductive age at risk of vitamin D deficiency.

Acknowledgments

I am grateful to all members of my research group in Cambridge, UK, and Keneba, The Gambia, past and present, who have been involved in many of the studies that have contributed to the evidence summarized in this paper. I would also like to thank Dr Gail Goldberg and Ms Annika Rasijeff for their help in preparing the manuscript.

References

Prentice

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Discussion

Dr. Mølgaard: Concerning calcium, as far as I can see there is nearly no relation between calcium intake and the outcome in these studies. What is the mechanism behind this utilization of calcium, or are there any factors that have an influence on it like vitamin D? Could there be other nutrition factors or other things that have an influence on this regulation, because it’s a huge difference in calcium intake and you have the same outcome.

Dr. Prentice: There are two parts to the answer to that question, I think. One is about what the mechanism is, and that is still being examined at the moment. It is clear that a whole range of different hormones that are specific to pregnancy and lactation, prolactin for example, are part of this story, as is the hypoestrogenemia of lactation. Parathyroid hormone-related peptide, which is produced by the mammary gland in late pregnancy and lactation, appears to be at least one component of the mechanism that drives the skeletal changes, but the likely mechanism is really very complex. The other part of the answer is the relatively small biological stress on the mother of pregnancy and lactation per unit time in relation to her own size. The results from human and animal studies have shown large species differences in the amount of calcium that needs to be transferred each day during pregnancy and lactation relative to maternal size. For small mammals, the amount is huge in relation to the calcium in the mother's skeleton, and these animals are very dependent on the dietary calcium intake for reproductive success. These animals also have physiological changes including bone mobilization, but these are very dependent on calcium supply, and there are mechanisms to bring in more calcium, such as increases in parathyroid hormone production, if it is needed. That does not appear to be the case in the human for calcium, in common with several other aspects of human nutrition in pregnancy and lactation. One can rationalize this in terms of the fact that the amount of calcium needed on a daily basis is actually very small compared to the mother's own size, but the mechanism has still yet to be fully understood [1–3].

Dr. Thorsdottir: I have a question about parathyroid hormone levels as an indicator for vitamin D deficiency. What could you tell us about this possibility?

Dr. Prentice: Obviously secondary hyperparathyroidism, high PTH, in white elderly women particularly, is a well-recognized risk factor for osteoporosis. As I illustrated during my talk, parathyroid hormone will increase when any aspect of the calcium-phosphorus-vitamin D physiological system is perturbed such that calcium is needed to maintain homeostasis, and this can lead to bone mineral loss. So in an elderly person, high PTH is a risk factor for osteoporosis, and for people living in European countries that is often associated with poor vitamin D status as opposed to low calcium intake, and so it has been used as a marker as you say. However, the reason for a high PTH is very variable from one person to another. For example, it is highly dependent on calcium intake. Women in The Gambia have an elevated parathyroid hormone concentration all through life, which is not related to vitamin D status or to fracture risk. So, across populations PTH is not useful as a marker of vitamin D sufficiency. In pregnancy and lactation it is not useful. Parathyroid hormone is suppressed during pregnancy and suppressed during lactation, because parathyroid hormone-related peptide
has taken over. Therefore, using PTH as a marker of nutritional status in that situation is hopeless. In adolescence, PTH will rise because calcium requirements go up and calcium absorption needs to go up. Physiologically you would expect to see high levels of PTH, so it is not a marker of status in adolescence either [4].

Dr. Anderson: Concerning the 25-hydroxy levels, is there a downside to getting much above the 70 nM level? The other thing that puzzles me is fortification. You mentioned that the UK, US, and Japan have very different levels in plasma. Is there any population health evidence that that makes a difference? The third question then is why is not vitamin D fortification of milk much more widely used, especially in the UK?

Dr. Prentice: The first question was on toxicity or at least on detrimental/adverse effects of high levels of 25-hydroxy vitamin D. I don’t actually have any evidence to go into that particular discussion. The Gambian people have abundant tropical sunshine and relatively little restrictions on skin UVB exposure because of local customs of dress. This is changing at the moment, but certainly in the years when I have been studying there the people are out in the sunshine most of the day with their face, arms and parts of their upper bodies uncovered, so they have 25-hydroxy vitamin D levels which I believe reflect endogenous skin synthesis in somebody with unrestricted year round UVB exposure. We rarely see a 25-hydroxy vitamin D level in those individuals who have a concentration of above 150 nM, so I have expressed concern that moving the lower level for defining sufficiency to 80 or 90 nM, which is an average figure for The Gambia would mean that the average level required for a healthy population would exceed the upper range that I see in endogenous synthesis. As to whether that is toxic or not, I know there are many people who are looking very hard at that question at the moment. Your third question was about fortification with milk and why we do not do it in the UK. What was the middle question? Health consequences of the differences in dietary intake? These are very difficult to tease out because the data I presented were from national surveys in the three countries, and they don’t tell you anything about 25-hydroxy D status. However, in the US, we certainly know that 25-hydroxy vitamin D concentrations of the general population are much better than in the UK, but then it is a sunnier country, at a lower latitude. I do think this helps to put into context the debates that there are between the Americans and Europeans about defining vitamin D adequacy, because we still have such a major problem of rickets and osteomalacia which is hardly seen in the US, although in Canada you are seeing it again. In the US, the debate has shifted from how we can prevent rickets/osteomalacia to how we can optimize health, whereas in Europe we have got a frank clinical problem that needs to be addressed and I think that changes the rhetoric. Why don’t we fortify milk? You almost have to go back 50 years and ask the recommendation committees at the time but it was to do with quality control or the difficulty of controlling the quality, with respect to the amount of vitamin D that was put into milk. There were some cases of hypercalcemia in infants that occurred at about the time that milk was fortified in the UK, which at the time were put down to the fortification. This assumption has been challenged since, but nevertheless vitamin D was taken out of milk because they felt it was not possible to maintain consistent levels in milk, but that by adding it to margarine you could even out any peaks and troughs. I have to say having seen some of the data from the US, quality control of vitamin D fortification of milk is a problem there too. However, milk is still a vehicle that could be used for population fortification in the UK, but it would not reach our ethnic minorities, because they don’t traditionally drink milk. So, if we are going to fortify the food chain for them, we need to think of something else [5–7].

Dr. Mohan: There has been a concept that low maternal calcium intake affects the growth of the baby, but the study which you presented fails to show a response of
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calcium supplementation. Is it then necessary to supplement mothers with calcium?

Dr. Prentice: In my talk I described the results from my studies, but also showed you a summary table of studies that have been conducted in lactation elsewhere. Most of the women in these studies would have had good vitamin D status. In the UK, our pregnant women would have had reasonable vitamin D status, but it would have been seasonal, it would have been low in the winter and high during the summer. In The Gambia, vitamin D status would have been high as I was just explaining to Dr. Anderson. The aspect of whether our conclusions about low calcium intake in pregnancy and lactation hold in the face of low vitamin D status is a very important question that needs to be answered. But the studies that I think you may be referring to are from India. There is a very famous study from Pune that has looked at providing calcium-rich foods during pregnancy on the growth of the child. However, calcium-rich foods provide the mother with many other aspects of good nutrition, including calories and protein and I think we would all expect an undernourished fetus to grow better under those circumstances. The studies that I have been showing have been trying to tease out whether it is calcium itself that is limiting. Now, if you increase the nutrition of everything else and don’t increase calcium you are likely to run into problems as well and so thank you for raising the question. 30 min isn’t very long to talk about all these things, but adequate nutrition throughout pregnancy and lactation is essential, calcium is part of that. It was whether a woman’s needs for calcium go up even higher that I was addressing [1, 8].

Dr. Mouane: Is there an optimal body skin exposure to sunshine? We have more and more women with Islamic clothes, and these women often ask about that.

Dr. Prentice: Of course ‘optimal’ is always a very difficult word because it implies that you know where the peak comes before it starts to become a problem again but if you ask me about ‘minimum’ I could possibly answer that question a little better. One of the misconceptions I think is that one needs to have a lot of UVB exposure on 100% of the body, I mean that you have to be immodest and out in the sunshine, increasing the risk of burning and melanoma, especially for fair skin people. Neither of those is true because regular, adventitious (in other words walking about doing your every day business) UVB exposure on the face, top of the head, hands and lower arms, should be sufficient. It is very difficult, because the evidence is really not there, to put it down to minutes but ‘approximately 15 min a day in summer sunshine, don’t go pink; if you are beginning to go pink, put your sunscreen on’, that is the message [7].

Dr. Sankaranarayanan: Could you explain dietary interference in calcium absorption?

Dr. Prentice: In terms of the absorption pathway, calcium shares it with iron to some extent, so iron and calcium are thought to be antagonistic to each other. There are many components particularly from plant sources, that chelate calcium in the gut and prevent it from being absorbed. We have Lindsey Allen in the audience, and she has much more knowledge of this than I have, so maybe you could ask her outside of the session? Nevertheless, for the people that I work with in Sub-Saharan Africa, most of the calcium comes from plant foods which are likely to bind calcium and there clearly must be mechanisms where their bodies release this calcium and absorb it. So I think to some extent the absorption of calcium must be regulated by the gut and by physiological needs as well as by dietary factors such as the composition of the food that the calcium is delivered with, but I absolutely take your point that absorption may be a problem for some individuals [9].

Dr. Johansson: Among the varieties of dietary calcium sources, which ones course over the other?
Dr Prentice: For an individual who has a normal gastric acid secretion, the evidence would suggest there is very little difference between them. There may be very small differences but they are only very small differences. For elderly people who have problems secreting enough acid, that is not the case, but in pregnant and lactating women that shouldn’t be an issue [9].

Dr Johansson: The variation of calcium in saliva is also very narrow. It is very close to 1 mM independent of the status of the person. So there seems to be a similar mechanism for the secretion of all these fluids.

Dr Prentice: As I was saying, there is now a mechanism to explain why the concentration of calcium drawn into the mammary gland ready for breast milk production is independent of extracellular calcium concentration and therefore of dietary intake. This is because the calcium is incorporated into casein and citrate complexes, and these are the drivers apparently [10].

Dr Makrides: Is there a difference in the metabolism or utilization of the vitamin D that is produced in the skin and vitamin D taken orally?

Dr Prentice: Thank you very much, fascinating question and one that there is not an answer to. We know that, in terms of vitamin D3 and vitamin D2 that are taken orally, they are both absorbed in a very similar way but that the metabolism is different, involving different pathways. However they are both antirachitic, i.e. they prevent rickets. The question whether oral vitamin D3 is different from synthesized D3, I am sure that it is unlikely that by the time it has reached the liver and has formed 25-hydroxy vitamin D3 the body could tell the difference. But the absorption of oral vitamin D3 is into the chylomicron transport system, whereas endogenously synthesized D3 diffuses out of the skin into the circulation where it is transported bound to D-binding protein. So, although the metabolism and utilization of 25-hydroxy vitamin D3 is probably the same from both sources, the mode of delivery to the liver is different. Whether this affects the efficacy of oral D3 vs. skin D3 has not, as far as I know, been looked at, and it would be good for somebody to answer that question [11, 12].

Dr Adrianasolo: Is calcium deficiency in the lactating mother affecting her breast milk calcium?

Dr Prentice: As far as we can tell from our evidence the answer is no, the breast milk calcium concentration of the mother is being determined by other factors in her milk, the protein and citrate content particularly, and not by the mother’s dietary calcium intake [10].

Dr Haschke: I was interested in your data on calcium content in breast milk. As you indicated, there is a continuous decline in the concentration until 72 weeks of age. Are we doing something wrong when we increase the calcium concentration in follow-up formulas because of the (CODEX-based) regulatory environment in most countries?

Dr Prentice: It’s a very interesting question. We did a study in Nigeria amongst families where there was a child with probable calcium deficiency rickets as opposed to vitamin D deficiency rickets, and looked at the breast milk of the mother when she was suckling a younger sibling, compared to age-matched women in the community. We did show a small but significant difference in breast milk calcium concentration between those mothers. So there may be something in the variation from one mother to another that might predispose a child to calcium deficiency rickets, and we are looking at that at the moment. However, your question about the physiological change in breast milk composition, it is not just calcium that goes down over time, of course, it is protein and many of the other nutrients. For a child who is exclusively breastfeed- ing, the volume will be going up at the same time and so the total amount of calcium that a child is consuming at 6 months from breast milk will be similar or greater than at 1 month and will tail off as breastfeeding begins to tail off. So, the extent to which
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formula milks mimic those changes would need to factor in volume as well as concentration, but I have no experience in the setting of Codex recommendations [13].

Dr. Boukari: My question is about calcium and vitamin D status and the development of chronic disease. I know that the topic is huge, but can you just give us some evidence about, for example, the role of calcium and vitamin D in the development of immune or allergic disease?

Dr. Prentice: This is in relation to calcium and vitamin D intakes in pregnancy and the programming of the child for later disease. The evidence on vitamin D is associative largely, there have been relatively few supplementation studies, there are some ongoing at the moment in the UK and the US that are looking at this in a prospective fashion. The answers have not come out. There really isn’t a short answer for me to give you, and maybe we can talk about this later, but I think the best answer I can give is that there are plausible mechanisms that are being actively looked at, and this is an area of huge intense research at the moment and we just have to wait for the results.

References

Human Milk vs. Cow’s Milk and the Evolution of Infant Formulas

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Abstract

Until the early 20th century, a wet nurse was the only safe alternative to breastfeeding, one reason being that each species has a unique composition of its milk. When techniques for chemical analyses of milks and assessment of the energy requirements of infants became available during the 19th century, reasonably safe breast milk substitutes started to be developed. Successively, these were developed into modern infant formulas during the 20th century using human milk composition as reference and cow’s milk as protein source. Even with a composition similar to human milk there are differences in performance between formula-fed and breastfed infants. Novel ingredients and new techniques within the dairy industry will contribute to minimize these differences and so might techniques in molecular biology allowing large scale production of recombinant human milk proteins. This technique may be used for production of bioactive substances present in low concentrations in human milk but absent from bovine milk with proven effect on nutrient utilization or other health benefits. For formulas containing novel ingredients with potent biological activities produced with new techniques it will be extremely important that their safety and efficacy are rigorously evaluated because ‘functional effects’ are not necessarily the same as health benefits.

Introduction

‘A child is to get a sufficient quantity of good nourishment, if it is to thrive well. The best food for it is, no doubt, the mother’s milk. We therefore find that children thrive well suckled by their mother’s milk, tho’ that should not stand all the proofs which are required towards approving that of a nurse’. These words of wisdom are from what is considered the first comprehensive textbook of pediatrics, The Diseases of Children and their Remedies, first
published in 1764 by the Swedish physician Nils Rosén von Rosenstein, often named the father of pediatrics [1]. At that time, there was no other safe alternative than a wet nurse if the mother could not breastfeed her infant. Rosén von Rosenstein and his colleague David Schulz von Schulzenheim, professor of obstetrics, were well aware of the fact that merely substituting breast milk for milk from other species, including milk from cows, could have fatal consequences. This was clearly expressed by von Schulzenheim in his inaugural speech to The Swedish Royal Academy of Sciences 1760 [2], although the reasons were not fully understood. Rosén von Rosenstein in his textbook suggested that should the wet nurse happen to get her period, it would not be good for the baby to suck her milk: ‘therefore it would be much safer, if some other female should suck the nurse’s milk on those days, and the child in the meantime, to be fed with clear whey, which is to be prepared of coagulated milk and eggs: such whey I mean, as we get in preparing egg cheese’. 250 years ago, that was the best recipe of a breast milk substitute available, which certainly did not meet the standard of today’s infant formulas, which are defined as foodstuffs intended for particular nutritional use by infants during the first months of life and satisfying by themselves the nutritional requirements of such infants until the introduction of appropriate complementary feeding [3].

Principal Differences between Human Milk and Cow’s Milk

The composition of milk is unique to each species. Human milk contains 9 g protein/l to be compared with 34 g/l in cow’s milk and 120 g/l in rat’s milk. The fat content is similar or about 38 g/l in human and cow’s milk but as high as 150 g/l in rat’s milk. With respect to lactose, the content differs less, 70 g/l in human, 48 g/l in cow’s and 30 g/l in rat’s milk [4, 5]. In principle, there is an inverse relationship between these differences in protein and energy content and the time it takes for the offspring to double its birthweight. Bovine milk protein is dominated by the casein fraction, which constitutes 80% of total protein, while the whey protein fraction constitutes 20%. The corresponding figures for human milk are 40 and 60%. Also, within the casein fraction the relative proportion of the various subclasses differ between bovine and human milk. α-S1 caseins constitutes the largest fraction in bovine milk, while β-caseins by far dominate in human milk. With respect to the whey protein fraction, the differences are as striking. The concentration of α-lactalbumin is twice as high in human milk as in bovine milk and the iron-binding protein lactoferrin, which second to α-lactalbumin is the dominating whey protein in human milk, constitutes only a minor fraction in bovine milk. In contrast, β-lactoglobulin, the predominant protein in bovine whey, is completely absent from human whey. IgA is by far the major immunoglobulin fraction in human milk, but in bovine milk IgG is present in 10-fold higher concentration than
Human Milk and Evolution of Infant Formulas

IgA, and the total immunoglobulin fraction is much lower than in human milk [4]. The specificity of the secretory IgA antibodies reflects environmental exposure of the mother and confers significant antimicrobial protection on the infant [6]. Goldman [7] suggested that overall the variation of antimicrobial and immune-modulating agents in milk, e.g. immunoglobulins, iron-binding proteins, lysozyme, oligosaccharides, leukocytes, cytokines, etc. (see below), seem to serve to compensate for development delays in early postnatal production of antimicrobial factors among various species, and vary depending on type of placenta, maturity of the offspring, lactation pattern and environment of the species.

Likewise, it is clear that lactation has evolved to minimize the energy cost to the mother while maximizing the utilization of energy and nutrients by her offspring, thus promoting the chance of survival of both. Recent genome studies comparing the bovine genome with 6 other species including the human lends support to this concept. Milk and mammary genes are more conserved and have evolved more slowly than other genes in the bovine genome despite selective breeding to optimize milk production. The most divergent proteins in the lactome are those with nutritional or immunological attributes, suggesting continuing selection of these genes to meet the nutritional and microbial challenges incurred by diverse environments and reproductive strategies. The most conserved genes were those coding for proteins of the milk fat globule membrane, supporting a key role in milk fat secretion [8]. It is quite clear that these and other differences between human and bovine milk have fundamental consequences reflected not only by protective effects against infections and immune development but also by an amino acid profile in human milk, which is better adapted to the needs of the human infant [9], and a lower potential renal solute load, which is essential because of the not fully developed renal function at birth [10].

Diversity in milk composition does not seem to be explained mainly by diversity of the encoded milk proteins; and although gene duplication may contribute to species variation, this is not a major determinant [11]. Thus, other regulatory mechanisms must be involved because, as mentioned, there are clear differences not only between species but also within a species as well as between milks collected from the same dam – or mother (table 1), which makes it difficult to define what is a human milk or a bovine milk, in turn impacting on using human milk as reference and bovine milk as raw material for formula production.

The History of Infant Formulas

The availability of satisfactory infant formulas is a comparatively recent development. Until the 20th century, there was virtually no safe and reliable alternative to breastfeeding, and few infants not suckled by mothers or wet
nurses survived the first year. It was not until the mid-19th century when chemical techniques were developed allowing analysis of the gross chemical composition of milk from various species that it became clear that every species has a unique composition of its milk. Towards the end of the century, pasteurization was adopted by the dairy industry and several physicians attempted to develop adequate substitutes for breast milk. The first marketed preparation, ‘Soup for infants’ was patented in 1867 by the German chemist Justus von Liebig who modestly termed it ‘the perfect infant food’. It consisted of a mixture of wheat flour, cow’s milk and malt flour, cooked with a little potassium carbonate to reduce acidity [15]. The commercial success of this formula raised the interest of competitors, but most of these early efforts were unsuccessful, and little attention was paid to the nutrient requirements of infants. An important step in the development of infant feeding occurred at the end of the 19th century when Heubner and Rubner published their calorimetric method of feeding, which made it possible to feed infants according to their energy requirements and was the beginning of modern studies of infant metabolism [16].

Eventually, substitutes for human milk were developed from milk of other mammals through numerous modifications into the complex formulas that are available today. Successive improvements in the understanding of the chemical and nutrient composition of milks, particularly of human and cow’s milk were the basis for these developments. The composition of human milk became the ultimate reference. The modern era of single formulas of known composition as complete foods for infants began in 1915 when Gerstenberger and colleagues developed an artificial milk in which the fat content was adapted to simulate human milk. The fluid mixture, which became SMA (synthetic milk adapted), contained about 4.6% fat, 6.5% carbohydrate and 0.9% protein [17]. In 1961, the first whey-dominant formula was launched and a decade later, in 1972, came the first Codex Alimentarius standard for infant formulas.

<table>
<thead>
<tr>
<th>Human milk</th>
<th>Bovine milk</th>
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<tr>
<td>• Between mothers</td>
<td>• Genetic variation (both casein and whey protein polymorphisms), which may affect digestibility, nutrient absorption, allergenicity, bioactivity [12]</td>
</tr>
<tr>
<td>• During a feed</td>
<td>• Seasonal variation [13]</td>
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<tr>
<td>• During the day</td>
<td>• Over time [13]</td>
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<tr>
<td>• With lactational stage</td>
<td>• Lactation stage [14]</td>
</tr>
<tr>
<td>• With gestational length</td>
<td>• With type of feed</td>
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<tr>
<td>• With the mother’s diet</td>
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It is fair to say that the major goals have been met; formulas do have a nutrient composition similar to human milk, and in high-income countries they are generally safe, effective and affordable for mothers who are unable to continue breastfeeding. In spite of that, there are still differences between breastfed and formula-fed infants also in high-income countries, both with respect to short-term and long-term outcomes [18–20] (table 2). The gold standard has therefore changed. It is now generally agreed that the performance of the breastfed infant, e.g. physiological (growth pattern and body composition), biochemical (plasma and other tissue markers, including metabolomics) and functional (immune responses, neurodevelopment and morbidity) is a more relevant reference than the composition of human milk [10, 19]. Questions remaining to be answered are what breastfed infants? Exclusively or partially breastfed infants? And for how long and at what age should the comparisons be made [20–22]?

**Recent Modifications of Infant Formulas**

The difference in performance between breastfed and formula-fed infants with respect to susceptibility for infections, immune responses, blood pressure, the risk to develop obesity and certain diseases, including health effects far beyond infancy [18, 22] (table 2) has put into focus the many ‘biologically active’ compounds in milk with proven or potential ‘functional’ effects. To add such components to formulas to achieve a performance more similar to breastfed infants has led to recent modifications; e.g. addition of bovine α-lactalbumin improving protein quality and thus allowing reduced protein concentration and a growth pattern more similar to breastfed infants [9, 23], certain bovine milk proteins with antimicrobial and immune-modulating properties such as lactoferrin [24, 25], nucleotides to improve immune function [10, 25, 26], long-chain polyunsaturated fatty acids affecting

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**Table 2. Differences in performance between breastfed and formula-fed infants [18]**

<table>
<thead>
<tr>
<th>Compared to formula-fed infants breastfed infants have</th>
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<tbody>
<tr>
<td>• Different growth pattern</td>
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<tr>
<td>• Fewer infections (gastrointestinal infections, acute otitis media)</td>
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<td>• Reduced risk for celiac disease</td>
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<td>• Reduced risk for obesity</td>
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<td>• Reduced risk for type 1 diabetes</td>
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<tr>
<td>• Lower blood pressure?</td>
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<td>• Lower total and LDL cholesterol?</td>
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<td>• Better cognitive achievements?</td>
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21
neurodevelopment and immune function [20, 27] and oligosaccharides as prebiotics [10, 28, 29]. Formulas based on hydrolyzed protein are not targeting the composition of human milk but an allergy-preventing effect similar to breastfed infants, or are used for treatment of cow’s milk protein allergy [30, 31]. Whether addition of probiotic bacteria targets the composition of human milk or merely a gut microbiota, stool composition and immune functions similar to breastfed infants is under debate [29, 32]. There is no consensus on the true health benefits of most of these modifications, which is illustrated by the fact that there is as yet no recommendation on their inclusion in infant formulas, although should the manufacturer choose to do so, the amounts allowed are regulated for long-chain polyunsaturated fatty acids, nucleotides and oligosaccharides [3].

Even with these most recent modifications, differences in performance between breastfed and formula-fed infants still remain [18]. Hence, with new knowledge novel ingredients will be identified and new technology within the dairy industry developed to produce them, and both scientific and market interests will drive further modification of formulas. It seems likely that the next step in modification will focus further on the so-called ‘functional’ compounds in human milk, i.e. compounds that are not considered nutrients themselves but with the potential to affect nutrient utilization or confer additional ‘functional effects’ on breastfed infants, making formula-fed infants perform more similar to breastfed infants. Such compounds may be lipids, e.g. sphingomyelin, which is a constituent of the milk fat globule membrane and the major phospholipid in human milk with potential effects on gut maturation and signal transduction [30], and gangliosides affecting gut microbiota and neurodevelopment, the latter possibly via their carbohydrate moiety, sialic acid, important for neurodevelopment reflected in effects on learning and memory [34]. There will most likely be further development of oligosaccharides added to formulas since those available today, even if they affect gut microbiota composition and stool consistency, are of much less complexity and variety than those in human milk. Interestingly, the oligosaccharides may exert their function as decoy receptors for pathogens both as free and protein bound. For instance, breastfeeding-associated protection against calicivirus diarrhea is associated with high levels of 2-linked fucosylated oligosaccharides in the milk, and human calicivirus strains, including Norwalk virus, use gut 2-linked fucosylated glycans as receptors. Recently, it was shown that milk of mothers who are non-secretors, and thereby lack 2-fucosylated oligosaccharides in their milk, had little inhibitory activity against binding to mucosal biopsies. Interestingly, the same was true for free oligosaccharides from milk of secretor mothers, having 2-fucosylated oligosaccharides in their milk, had little inhibitory activity against binding to mucosal biopsies. Interestingly, the same was true for free oligosaccharides from milk of secretor mothers, having 2-linked fucosylated glycans, while the milk proteins bile salt-stimulated lipase (BSSL) and mucins MUC1 and MUC4 accounted for virtually all the inhibitory activity. These proteins have in common that they have O-glycosylated tandem repeat sequences offering multiple binding sites [35], which these viruses obviously need. Thus, also the
backbone to which the decoy receptors are attached is important. In analogy, we recently showed that BSSL is the main, or the only glycoprotein in human milk that potently binds dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN) and blocks DC-SIGN mediated trans-infection of CD4+ lymphocytes with human immunodeficiency virus type 1, probably by offering multiple Lex binding sites [36]. Other new bovine milk fractions of potential use in formulas are discussed by Lönnerdal in his chapter. In a more remote future, other bovine milk fractions may be enriched in bioactive substances such as enzymes, enzyme inhibitors, growth factors, cytokines, chemokines, binding proteins and immunoglobulins [37]. However, there will always be limitations to what can be achieved by modification of milks from other species. Some functions are species specific as are some compounds. As mentioned, the composition of the oligosaccharide fraction is very different in bovine milk compared to human milk, and some functional compounds and effects in human milk are absent from bovine milk.

With modern techniques in molecular biology, it is now possible to produce, also on a large scale, recombinant human milk proteins, which can be added to formulas with the potential to further reduce the gap in performance between breastfed and formula-fed infants. Examples of this are lactoferrin and lysozyme; in a recent study, a combination of both was found to shorten the duration of infectious diarrhea [38]. Another example is the fat-digesting enzyme BSSL. BSSL is secreted by the pancreas of all species studied and from the lactating mammary gland into the milk in some species, notably the human but not the bovine. BSSL is a key enzyme in neonatal fat digestion and thought to be the main reason for the more efficient use of fat from human milk than from infant formulas [39]. In a recent phase 2 placebo-controlled double-blind clinical trial in preterm infants, recombinant human BSSL was found to improve weight gain when added to an infant formula at a concentration typical for human milk. In another phase 2 study with similar design, the same principal effect was observed when recombinant BSSL was added to pasteurized human milk (Swedish Orphan Biovitrum, press release 2010-04-21, 2010-05-06). Since pasteurization inactivates BSSL, the addition restored the level of active BSSL in the milk. This illustrates its potential value for a further formula modification, particularly of formulas intended for very low birthweight infants.

New ingredients, some with potent biological activities and produced with new techniques are like to be expensive and they may also confer a safety risk. Moreover, a proven biological activity may not necessarily confer a health benefit on the recipient infants. Therefore it is – and will be even more in the future – extremely important to rigorously ascertain safety and efficacy before formulas with such ingredients are launched on the market, and the cost-benefit must be considered for formulas intended for infants at large, and particularly for formulas intended for infants in low-income countries.
References

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Human Milk and Evolution of Infant Formulas


Discussion

Dr. Garg: Whenever we compare human milk with formula, we just supplement formula with one thing and see the effect on the outcome. Human milk has so many components that affect the same outcome, so maybe adding multiple components would give a beneficial effect that has not been shown with milk formula supplemented with a single factor.

Dr. Hernell: You are right. Human milk, as all milks, is very complex in its composition. We already know that there are differences in outcome between breastfed and formula-fed infants. To find out why that is so, we need to identify and characterize components in human milk that we think might have functional effects, and verify a potential effect if possible first in vitro and in animal models before we finally move on to randomized clinical trials. As I mentioned, many components have been added to infant formulas for a good purpose, and have also been proven to have an effect. However, for some of them the effect is not necessarily the same as a health benefit. To ascertain health benefits that make the addition of a new ingredient justified, we need to do proper clinical studies to prove the health benefit effect. The problem is that for that we need well-designed, sufficiently powered randomized studies, and these are very expensive. However, such studies are needed to prove efficacy as well as safety of new ingredients, even if they are present in human milk.
Dr. Garg: Regarding DHA in breast milk. To what extent is it beneficial to term infants?

Dr. Hernell: I think DHA is one of the components in human milk, and its concentration is reflected by the mother’s diet. I think most of us have been convinced that DHA in formulas like in human milk has a health benefit effect in terms of improved development of visual function and neurodevelopment, at least in preterm infants, and there are studies suggesting that exogenous DHA is also beneficial to term infants, even beyond infancy. Judged by recent meta-analyses and systematic reviews, the results are not as convincing as we used to think. Perhaps the effect is restricted to subgroups of infants that have not yet been clearly identified? It may also be that the effect is there for larger groups of infants but many studies have been underpowered, and there are so many other factors that interfere with neurodevelopment. Moreover, current methodology may not be sensitive enough to reveal small but significant effects.

Dr. Sankaranarayanan: What would be the ideal omega-6 to omega-3 ratio in formula milk?

Dr. Hernell: I do not think we know the optimal ratio, if there is one. When the comments were written by ESPGHAN, the n-6:n-3 ratio was set to 5–15:1 because this range was considered representative of human milk. Perhaps the ratio does not need to be the same in formulas with and without added DHA.

Dr. Anderson: You talked about the modification of the infant formula again by adding many bioactives, and mentioned that there are great differences in protein composition and quantity in milk from different species. Formulas are different from human milk in not only protein quantity but also many aspects of composition. How important is the fact that infant formulas have a higher quantity of protein?

Dr. Hernell: The concern about the protein content in infant formulas has been that high protein content in early life may result in increased risk of obesity, and that’s a major reason why it is of interest to reduce the protein content in infant formulas to come as close as possible to the concentration in human milk. However, even if the concentration has been successively reduced, there is still a difference. The way to achieve a reduced protein concentration has been to improve the quality of the protein, allowing a reduction in the total concentration and still meet the needs of the infants. This method has been used by Nestlé and other formula manufacturers. They increased the proportion of α-lactalbumin, the major whey protein in human milk which has an excellent amino acid profile. Both quality and quantity are important. I have only touched on the potential biological effects of the various milk proteins besides being a source of amino acids. One can assume that these functional effects vary considerably with the quality of the proteins and also with peptides formed from some of them during their digestion.

Dr. Haschke: The lactalbumin-enriched formula was developed by Niels Raiha in 2000 [1]. Four clinical trials, which have recently been analyzed in a meta-analysis [2], indicate that growth of infants fed that formula was similar to that of breastfed infants and to the WHO growth standard for breastfed infants. Fortunately, the GINI study [3] now indicates that at 6 years of age, children who were fed a modern partially hydrolyzed formula (NAN-HA) during infancy, had weight, height, and BMI similar to exclusively breastfed infants. It seems that feeding with modern infant formulas with low protein concentration results in growth similar to that of the breastfed infant, which is important from the obesity perspective. Breastfeeding is now considered to protect from childhood obesity.

Dr. El Barbary: Is breast milk adequate for feeding preterm infants?

Dr. Hernell: That’s a very good question. The answer depends on how much preterm the baby is. If you have a very preterm infant, the mother’s milk will not meet...
Human Milk and Evolution of Infant Formulas

the needs of all nutrients of the infant, for instance of protein and calcium. Therefore, there is a need to fortify the milk or to use a formula intended for preterm infants. However, there are studies showing that with respect to long-term outcomes, preterm infants fed breast milk are doing better than those fed formula. How to optimize the nutrition of preterm infants is, in my opinion, an area where we need to learn much more. The more preterm the infant is, the more limited is our current knowledge. To meet the nutritional needs and provide the other benefits of breast milk will be the challenge.

*Dr. Haralappa:* To treat allergic diseases of infants, would you add hydrolyzed protein and probiotics to the formula?

*Dr. Hernell:* For the treatment of cow’s milk protein allergy, I think most of us would recommend a formula based on extensively hydrolyzed protein. It has been more of a discussion what to recommend for prevention of allergy. Most scientific bodies, for instance ESPGHAN and AAP do not recommend any preventive strategy unless the infant is at high risk of developing allergy. For high-risk infants, breastfeeding is still the first recommendation. To recommend a formula is more difficult. It is debated how effective such prevention is and if formulas based on partially hydrolyzed protein have comparable effects to those of formulas based on extensively hydrolyzed protein, and if some formulas based on partial hydrolysates are more effective than others. I think we need more studies to give firm recommendations when it comes to prevention. With respect to probiotics, the data are still too inconclusive to give recommendations.

*Dr. Sankaranarayanan:* I agree with you that breast milk is one of the best prebiotics. But the amount of oligosaccharides in breast milk, is it optimal? Are there any studies? And what would be the ideal amount of oligosaccharides in infant formula?

*Dr. Hernell:* In human milk, the oligosaccharides are the third largest fraction, so they constitute a large fraction. They are also very complex in structure, and the composition varies between mothers, depending on, for instance, what blood group antigens they are expressing. The optimal concentration as well as composition is unknown, and it also depends on what effect you are looking for. I gave the example with calcivirus and the milk lipase BSSL; some mothers confer a protective effect via their milk and others do not depending on the glycosylation pattern. So far, it has not been possible to copy the oligosaccharides in milk, and to do this would be very expensive, I guess. What formula manufacturers do instead is to try to find other sources of oligosaccharides which are much less complex and much less diverse. How much to add to a formula is also a matter of how much are you allowed to add because that is specified in the infant formula directive, at least in Europe.

*Dr. Klassen:* A comment to the question related to partial hydrolysates. I wanted to bring to your attention that very recently two meta-analyses have been published on the preventive effect of hydrolysates. I want to underline the point Dr. Hernell made that the effect has to be demonstrated in clinical trials for each single hydrolysate. Some hydrolysates were shown to actually reduce the risk of development of atopic dermatitis to the level of breastfed children, so I think this is a somewhat important effect.

*Dr. Mouane:* I have a question about protein hydrolysates. You told us the degree of prevention depends on the hydrolysates, so what is the right one? Could you be more precise?

*Dr. Hernell:* I think the best answer you can get right now is conveyed in Dr. Klassen’s statement.

*Dr. Klassen:* It’s basically looking at the scientific evidence, as Dr. Hernell said in his talk. We should consider evidence from randomized placebo-controlled clinical trials. To my knowledge, clinical trials are selected for meta-analyses according to
quality criteria. Thus, it is only the clinical data that really count, in my view. It is not sufficient to have just animal data. This might be a good indicator, but can be limiting in establishing the evidence.

Dr. Haschke: The meta-analysis you are asking for comes out in the May 2010 edition of *Journal of Pediatric GI and Nutrition*, so you can make up your mind based on this.

Dr. Jongpiputvanich: I have read in the literature that breast milk also contains probiotics. Would you like to comment on that? And what is the mechanism behind breast milk probiotics?

Dr. Hernell: Before I started, I deleted one slide. That was actually a slide showing the front page of a paper in *BMJ* that we wrote in 1980; it was called ‘Human milk banking. To heat or not to heat.’ It says in the abstract that our study shows that human milk contains pathogens, but they seem to make no harm. From there, we have in 30 years switched from thinking that they do not cause harm to that they may even be beneficial and are meant to be there. But how they get there, I think is still an open question. If you read the paper, we found that breast milk samples at that time could contain 105 CFU/ml bacteria, but such milk didn’t cause any harm to the baby. When we instructed mothers to carefully clean the nipple and the areola before sampling, the bacterial counts were significantly reduced but not to zero. How for instance bifidobacteria present in human milk get into the milk, I think is still an open question. My guess would be from the outside.

Dr. Gibson: You gave an excellent summary table of the various effects of various additives that are being put into infant formulas. Testing the efficacy of some of these things has proven very difficult, particularly in the case of term infants where they have blood reserves on the one hand and on the other hand they have endogenous capacity, and I am thinking particularly of say DHA, the omega-3 fats and nucleotides. What are your thoughts about the efficacy of some of these things? Are they cosmetic or do they play a real role?

Dr. Hernell: I think if you look at nucleotides and read the literature, it’s very difficult to say yes they are beneficial, they do have a health consequence. On the other hand, if you look at subpopulations of infants, it may be that they do benefit from exogenous nucleotides, but I don’t think it is something that you need to add to an infant formula intended for healthy term infants, that’s how I read the literature. Concerning DHA, you know that better than I do, I think it’s pretty much the same; it’s very difficult to prove that you really have a health consequence in healthy term infants, which does not necessarily exclude such an effect.

References


Whole Cow’s Milk in Early Life

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Abstract

Cow’s milk is a major food for young children. Whole cow’s milk is known to be detrimental to infants, mainly due to its low iron content. The negative association with iron status led to recommending the introduction of formula feeding in infancy during the weaning period or when breastfeeding ceased. More recently, the literature suggests that consuming whole cow’s milk in infancy has unfortunate effects on growth, especially weight acceleration and development of overweight in childhood. These issues are discussed in the following chapter. Other suggested reasons for the avoidance of whole cow’s milk in infancy are touched upon, such as milk protein allergy and high renal solute load. The hypothesis about early cow’s milk introduction in the pathology of certain diseases, mainly through the peptide β-casomorphin-7, is briefly reviewed, showing that there is no clear evidence for the suggested associations. The chapter gives a recent example of introducing formula at 6 months of age instead of whole cow’s milk in infants’ diet in Iceland. Several aspects of consuming whole cow’s milk in infancy can be found in recent reviews.

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Cow’s Milk in Infancy and Iron Deficiency

One of the most negative aspects of cow’s milk consumption in infancy is its association with diminished iron stores and increased probability of iron deficiency (ID). Iron has many important roles in the human body, and its deficiency can have a great impact. Among other things, iron plays a part in oxygen distribution, the body’s immune system and brain functions. ID in its most severe form presents as ID anemia (IDA). Chronic IDA in infancy can lead to long-term effects lasting into adolescence and adulthood [1]. Intervention early in life is therefore vital. This is especially important after the first months of life, to prevent worsening of iron status after the depletion...
of iron stores from birth. Infants are born with sufficient iron stores to last for at least 4 months [2].

There has been an increased focus on the long-term effects of ID in later years. Most studies report that differences in behavior and developmental skills persist in the majority of iron-deficient anemic infants even after iron treatment [1, 3], even though it has been questioned whether social and other environmental factors may act as confounders.

Various factors control iron absorption; it can even vary considerably between individuals as it is regulated by iron status. A study of iron absorption in breastfed infants showed that children with serum ferritin <12 µg/l had significantly higher iron absorption than children with higher serum ferritin levels [4]. This is consistent with iron absorption regulation in adults [5]. Iron in food is divided into two groups, heme and non-heme iron. Heme iron is found in meat and animal-derived food. Heme iron is easily absorbed relatively independent of diet composition. Non-heme iron, on the other hand, is not so easily absorbed. Non-heme iron is influenced by several dietary factors, such as ascorbic acid (vitamin C) that enhances absorption and calcium that hinders its absorption. Several other factors like tannins, phytates, phosphates, soy protein products and various dietary fibers have been reported to inhibit non-heme iron absorption.

Protein-rich food can either increase or decrease iron absorption. The different kinds of amino acid combinations in protein are the reason for the various effects protein can have on iron absorption. Hurrel et al. [6] argued that cysteine is the only amino acid that has been demonstrated to increase iron absorption. Meat and fish are rich in cysteine, but cow's milk is not. This suggests that cow's milk consumption can hinder iron absorption in infants because of the amino acids in its protein construction. Additionally, whey proteins, which predominate in human milk, are more easily digested than casein protein from cow's milk [6]. Cow's milk proteins are also believed to induce blood loss through feces. However, this blood loss is not thought to be of clinical significance. Nevertheless, fecal blood loss is an aggravating factor of ID among infants fed cow's milk, but not among breastfed infants [7].

**Infant Formula instead of Whole Cow's Milk**

In the literature, unmodified cow's milk is deemed to have great negative impact on infants' iron status. The two relevant factors are the amount of cow's milk consumed and the age when consumption begins. Cow's milk and human milk are both low in iron (0.2–0.5 mg/l), but the bioavailability of iron from human milk is more favorable for an infant's intestine than iron from cow's milk. Human milk is a much better choice than cow's milk for infants. Moreover, breastfeeding for 25 weeks or more has been shown to positively impact hemoglobin level [8]. However, prolonged exclusive breastfeeding (>6
Whole Cow’s Milk in Early Life

months) has been associated with ID [9]. Others have concluded that ID is not of concern when cow’s milk is given to infants after 6 months of age if the complementary foods are rich in iron [10]. However, this matter is still under discussion, and the many factors associating cow’s milk with worse iron status in infancy have to be considered. Cow’s milk contains approximately four times more calcium than human milk [11], which in addition to the protein type and content of cow’s milk negatively influences iron absorption from food.

In the last century in Iceland, the custom was to give cow’s milk when breastfeeding decreased or stopped after 6 months of age. This was in accord with the official advice on infant feeding practices [12]. The study on nutrition in Icelandic infants that was carried out between 1995 and 1997 showed a high prevalence of ID, i.e. more than 40% of the children had serum ferritin values below reference (<12 µg/l), confirming an independent negative association with cow’s milk. The infants consuming more than 460 g/day of cow’s milk at 9 and 12 months were found to have lower iron status indices, indicative of deficiency [12]. An association between ID and cow’s milk consumption was still evident at 2 years of age, but when the velocity of weight growth from birth was included in the statistical model, it was the only significant predictor for ferritin [13]. Furthermore, low iron status at 1 and 2 years of age contributed to worse iron status at 6 years of age [14]. In the same children, who were longitudinally followed, worse fine motor development scores at 6 years of age were independently associated with ID at younger ages (fig. 1) [15, 16]. The observed association between low iron store values at 1 and 2 years of age and the children’s development scores at the age of starting school shows that the relation may be found even in populations of developed

![Fig. 1. Iron status vs. development at 6 years. Children, ID and with depleted iron stores (DIS) at 1 year had lower scores on fine motor development test. The Icelandic developmental inventory evaluates children’s motor and verbal development by collecting information from their mothers on 208 standardized questions [16].](image-url)
countries with high rates of breastfeeding and, besides early whole cow’s milk consumption, relatively good quality complementary feeding. Persistent effects on sleep and neurofunctions in children formerly with IDA have been suggested to contribute to reduced potential for optimal development [17].

Following this study, the recommendations for infant nutrition were modified, and from 2003 Icelandic iron-fortified follow-on milk was recommended from 6 months to 2 years of age instead of unmodified cow’s milk (table 1). Since then, the iron status of Icelandic infants has improved enormously: 5.8 vs. 41% iron-depleted, 1.4 vs. 20% iron-deficient and 0 vs. 2.7% iron-deficient anemic [18]. This was observed in a recent study evaluating the effects of the new recommendations. The follow-on milk composition was according to the current Icelandic directive with higher iron and vitamin C content and lower calcium and protein concentrations than whole cow’s milk (table 1), which led to increased intake of iron at 12 months and vitamin C at 9 months. This is therefore the most likely reason for the improvement in iron status of 12-month-old children.

Whole Cow’s Milk and the Consequences of the High Protein Concentration

More recently, another concern about early cow’s milk consumption emerged as the high protein content of cow’s milk may stimulate rapid growth in bodyweight and development of overweight. High protein intake early in life has been associated with higher body mass index (BMI) later in childhood. The study on nutrition in Icelandic infants carried out between 1995 and 1997 and the longitudinal follow-up at 6 years of age showed that

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Icelandic follow-on milk</th>
<th>Whole milk</th>
<th>Min. for follow-on milk</th>
<th>Max. for follow-on milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ (kcal)</td>
<td>280 (67)</td>
<td>280 (67)</td>
<td>251 (60)</td>
<td>335 (80)</td>
</tr>
<tr>
<td>Protein, g</td>
<td>1.8</td>
<td>3.4</td>
<td>1.35</td>
<td>3.6</td>
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<tr>
<td>Carbohydrates, g</td>
<td>7.2</td>
<td>4.5</td>
<td>4.2</td>
<td>11.2</td>
</tr>
<tr>
<td>Fat, g</td>
<td>3.5</td>
<td>3.9</td>
<td>1.98</td>
<td>5.2</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>0.75</td>
<td>–</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>90</td>
<td>114</td>
<td>47.25</td>
<td>126</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>9</td>
<td>–</td>
<td>4.8</td>
<td>–</td>
</tr>
<tr>
<td>Vitamin D, µg</td>
<td>1.2</td>
<td>–</td>
<td>0.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>
boys in the highest quartile of protein intake as a percentage of energy intake at the age of 9–12 months had higher BMI (17.8 ± 2.4) at 6 years than the lowest (15.6 ± 1.0) and second lowest (15.3 ± 0.8, p = 0.01) quartiles [19]. The energy intake did not differ between the groups. In the recent Icelandic study on infant nutrition, the protein intake at 9 and 12 months of age had decreased compared to the one 10 years earlier. However, the protein intake (g/kg) at 9 months was positively related to weight growth velocity from 8 to 12 months (r = 0.204, p = 0.019) but not to length gain (r = 0.134, p = 0.124). Protein intake was still higher for non-breastfed infants, compared with a breastfed reference group (table 2). It has been suggested that the association between high protein intake early in life and adiposity later in childhood is mediated through increased IGF-I concentration [20]. Results from another study also suggest that high protein intake increases IGF-I concentrations in healthy infants who have no signs of malnutrition, and whose protein intakes far exceed requirements [21]. The increased IGF-I concentration can then increase muscle mass and adipose tissue and therefore lead to higher BMI. A large European study, European Childhood Obesity Project, indicated that feeding infants with formula with reduced protein content could normalize growth relative to a breastfed reference group, and that modification of infant feeding practice has important potential for long-term health promotion [22].

Cow's milk protein has been shown to have various negative effects on infants’ health. Besides the mentioned effects, i.e. that protein fractions in cow’s milk may have a negative effect on iron absorption [11] and may lead to overweight by a protein intake above optimal, it is a burden on several organs. Cow’s milk protein has been shown to be a significant etiological factor for constipation when consumed in high doses but also in lower doses in allergic infants and young children [23]. Furthermore, it has been shown that the high protein concentration in unmodified cow’s milk combined with an immature renal function can cause fluid losses and dehydration [24]. During illness, such as fever and diarrhea, water loss is greater through evaporation and fecal losses; therefore, water balance maintenance is of great importance to prevent dehydration. As whole cow’s milk has more protein and minerals than infants need, the excess is excreted in the urine. The high renal solute load increases the risk of negative water balance in febrile illness [25].

<table>
<thead>
<tr>
<th>Age</th>
<th>Breastfed E% n</th>
<th>Non-breastfed E% n</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 months</td>
<td>2.33 ± 0.78 12.6 75</td>
<td>2.83 ± 0.80 14.5 75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12 months</td>
<td>2.91 ± 1.21 14.0 29</td>
<td>3.30 ± 1.14 15.5 119</td>
<td>NS</td>
</tr>
</tbody>
</table>
Icelanders generally consume high levels of protein, and this also applies to infants.

The previous tradition of cow’s milk consumption among Icelandic infants during the second half of infancy, along with the high initiation rate and long duration of breastfeeding among Icelandic mothers, made Iceland different from its neighboring countries. As mentioned, in 2003 the current recommendation on infant nutrition was launched, recommending Icelandic follow-on milk from 6 months to 2 years of age. The protein content of Icelandic follow-on milk is more similar to breast milk than unmodified cow’s milk as it contains 1.8 g of protein per 100 g, as compared to on average 1.3 g of protein per 100 g in breast milk and 3.4 g per 100 g in Icelandic cow’s milk (table 1).

**Allergy and Cow’s Milk Protein**

Cow’s milk protein is the most frequently encountered dietary allergen in infancy when the immune system is relatively immature and susceptible to sensitization from environmental antigens. The prevalence in early childhood is 2–5%, but decreases to 0.1–0.5% in adulthood. The majority of affected infants acquire natural tolerance to cow’s milk protein before the age of 3 years. However, around 20% of patients will remain allergic for a longer period. The best prevention for cow’s milk allergy is breastfeeding; however, exclusive breastfeeding does not completely eliminate the risk since traces of cow’s milk protein can be transferred into the milk from the mother’s diet. Allergies to milk can be classified into immunoglobulin E (IgE)-mediated allergy and non-IgE-mediated allergy. Non-IgE-mediated immune reactions are not as well defined as the former and more difficult to recognize. Correct diagnosis of cow’s milk allergy is crucial since restricted diet can reduce the quality of life and lead to serious detrimental effects, especially in infants and young children [26]. β-Lactoglobulin is the major whey protein in cow’s milk, but it is not expressed by the human mammary gland, and is one of the proteins involved in allergic reactions to cow’s milk [27].

**Cow’s Milk and Less Evident Hypothesis on Diabetes Type 1 and Autism**

Some studies have found a statistical association between type 1 diabetes and early introduction of cow’s milk. However, such an association is not observed in all studies. The potential association was the issue of a scientific report by EFSA on the potential health impact of β-casomorphins and related peptides [28]. Several studies have investigated distinct cow’s milk proteins to identify the possible diabetogenic factors in milk. The suggested
milk antigens are, for example, bovine serum albumin (BSA), bovine insulin, β-lactoglobulin and β-casein and fractions thereof. The role of BSA in type 1 diabetes was questioned since several authors could not confirm specifically enhanced humoral or cellular responses to BSA in diabetic patients compared with controls. When genetic risk determinants were included, antibodies to BSA, β-lactoglobulin, whole cow’s milk and islet cell antibodies were not independently associated with the risk of type 1 diabetes in a multivariate, logistic regression analysis. Elevated levels of antibodies to β-casein were demonstrated in some patients with type 1 diabetes, but not in others. The role of β-casein as a causative factor in diabetes development remains unclear. A diabetogenicity of β-casein A1, A2 and B has been suggested. Ecological studies have linked β-casomorphins, derived from β-casein A1 and B, with type 1 diabetes. Ecological studies have the shortcoming of being unable to establish cause-effect relationships, and they cannot adjust for possible confounding factors. They may indicate a hypothesis but do not demonstrate cause-effect relationships. The content of β-casein A1 + B in milk produced in the observed countries with high or low prevalence of type 1 diabetes does not explain differences in occurrence of type 1 diabetes. Based on the EFSA review of available scientific literature, a cause-effect relationship between the oral intake of β-casomorphins or related peptides and etiology of diabetes cannot of course be established.

Other dietary risk factors for type 1 diabetes include diet at a young age, i.e. use of soy milk formulas, consumption of wheat or gluten toxic chemicals and high growth rate have also been proposed as risk factors. Preschool day care has been suggested as protective. That theory has been linked to the age-dependent modifying influence of infections on the developing immune system. Dietary risk factors seem to be important in type 1 diabetes, but clear evidence is lacking.

A link between β-casomorphins and disorders of the central nervous system has also been suggested in the literature – autism, ventilation disorders and sudden infant death syndrome. The assumption is that β-casomorphins might be absorbed from the infant’s gastrointestinal tract, and pass easily through the blood-brain barrier because of the infant’s immature central nervous system. It has not been possible to prove these assumptions. The hypothesis is that in infants with abnormal respiratory control and vagal nerve development, opioid peptides derived from milk might induce depression of the brainstem respiratory centers, leading to apnea and sudden infant death. However, infants fed either formulas or human milk have a similar risk of developing sudden infant death syndrome, which does not support the hypothesis. Therefore, no evidence for such a relationship could be found during the review. Based on a hypothesis about genetically based peptidase deficiency and increased intestinal permeability, it has been suggested that casein-derived peptides are associated with autism. However, recent data do not provide any support for such a relationship.
Conclusions

The evidence for avoidance of whole cow’s milk in infancy mainly involves the risk of iron insufficiency and its consequences among infants receiving high amounts of cow’s milk. Additionally, there is discussion of whether children given whole cow’s milk early in life have accelerated growth and are more likely to develop overweight and obesity in childhood than their peers. These theories seem to gain support from recent studies. High protein intake following milk consumption is thought to be the main reason for this association. The high protein and mineral concentration are also reasons for possible adverse effects on water balance among sick infants with fever. The evidence, if any linking cow’s milk to diabetes and serious illness originating in the central nervous system are weak and should not be regarded as a public health concern. Well-established evidence-based guidance should be promoted and all possible research encouraged, increasing the value of infant nutrition recommendations. A recent review described the determinants for early introduction of cow’s milk, i.e. mother’s low education and socioeconomic status and not following the main guidelines for infant nutrition [29], indicating that research is needed to increase the likelihood of compliance with the recommendations.

References

Whole Cow's Milk in Early Life

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Discussion

Dr. Manafe: I would like to know your view on giving diluted cow’s milk to infants before the age of 6 months.

Dr. Thorsdottir: Before the age of 6 months, if the mother is not able to breastfeed her child, it’s usually recommended to use infant formula, which has a lower protein content than cow’s milk. Diluted cow’s milk sweetened with sugar was used many years ago.

Dr. Sarwar Ferdous: On the Asian subcontinent, most of the children develop lactose deficiency by the age of 3 years. So if children are fed milk right from the beginning and then again milk from the age of 6 months, these children will develop lactose deficiency and suffer from diarrheal disease or constipation if they continue to be fed with cow’s milk. Moreover, they never develop proper food habits even after the age of 5 or 6 years and only consume milk. The other thing is that if the child consumes more than 400 or 500 ml a day, he/she will develop intestinal bleeding. Ultimately the
result is iron deficiency and a total change in the food habit. Could you make a comment on that?

Dr. Thorsdottir: You are correct of course that cow's milk is associated with iron deficiency. There are many reasons for that. It's both the low content and bioavailability of iron in cow's milk; also the high calcium content in cow's milk is related to bad absorption, so there are several reasons for the development of iron deficiency and iron deficiency anemia. Regarding allergy, if there is a risk for allergy, and there is a relative with allergy to cow's milk, it is not recommended to use whole-protein formula but hydrolyzed formula. The issue of allergy is pretty difficult because according to the immunology literature it can be equally bad to avoid totally an allergen or a protein causing allergy and to update very early, so there seem to be windows of opportunity to slowly increase the variety of diet; maybe that would be the best way to prevent allergy.

Dr. Garg: This is regarding the effect of iron supplementation on growth in iron-replete children. In some community-based trials in developing countries [1, 2], infant nutrition was supplemented with iron beyond 6 months. These areas were mainly malaria-infested areas, and it was shown that in the children who had been given iron, malaria incidence was higher than in those children who were iron deficient. I understand that malaria may not be a concern in the area that you deal with, but is it possible that iron supplementation in iron-replete children causes more infections (viral, bacterial or other protozoal), and that may be one of the reasons why their growth at 6–12 months of age deteriorates?

Dr. Thorsdottir: You mean if they are iron sufficient?

Dr. Garg: Yes.

Dr. Thorsdottir: Your hypothesis was if they get more infections. That is not what we have seen.

Dr. Begum: The literature shows that the iron status of the infant is proportional to maternal iron status. If the mother is iron deficient, then the baby will be iron deficient. In your study, you showed that those infants who are fed cow's milk are iron insufficient, but did you measure maternal iron status in your study?

Dr. Thorsdottir: No, unfortunately we did not. At the maternity care, they measure iron status regularly, but we did not.

Dr. Begum: So, we can't say that cow's milk is responsible for iron insufficiency in the newborn or infant if we can't study maternal iron status.

Dr. Netrebenko: I would like to make a comment to the last two questions. First of all, you have seen the improvement in serum ferritin in infants who consume less cow's milk. Additionally, these infants have a low protein intake and they have a decrease in insulin growth factor. Thus, a decrease in cow's milk consumption improves iron status and slightly decreases growth of these infants. Here, we see a relationship between growth and improvement in serum ferritin. Would you agree?

Dr. Thorsdottir: We saw in this cohort an independent association with growth during the first year and a lower ferritin value, so there is a negative association. But the influence of whole cow's milk was stronger, also an independent association.

Dr. El Barbary: Is there any study comparing growth parameters in those infants who are iron supplemented and those who are not? And the second question, do you recommend iron supplementation in infants who are exclusively breastfeed for 6 months?

Dr. Thorsdottir: There are very few infants that are exclusively breastfed for 6 months, so we don't recommend iron supplementation, and I think it's not very common in Europe. There are a few examples such as Denmark, but it's not common. Regarding your second question, as we do not supplement iron, we do not have any comparison here, but if we compare or if we associate iron status indexes and growth we see that the iron-depleted children grow slower.
Dr. Lönnerdal: I would like to comment on both the previous question and a couple of previous ones on iron and growth. It has been shown in several studies now that iron supplementation can have an adverse effect on growth. Those results are from Sweden, Honduras, Indonesia and India. In the Swedish cohort, there was very little illness, but in the other studies there was an impairment in growth which was not related to the illness, it was a specific effect of iron causing reduced growth. We don’t know why that is, and we are looking at various possibilities that iron given to those that already have adequate iron status has an adverse effect. This is the first study I have seen with iron fortification having an adverse effect on growth. We have to be aware that we need to subdivide these populations; virtually all studies on iron fortification or iron supplementation have been done in populations where iron status is relatively low, and therefore you can expect a positive outcome when giving iron. What you have to do is to subdivide your cohort into those who are iron replete from the beginning and those who are iron deficient, and then you can see if there is an adverse effect by giving iron to the iron-replete population. That is why we found it in Sweden as most infants were iron replete; however, in most cases, the proportion of iron replete infants is small, and if you put them together the overall outcome is likely to be positive.

Dr. Haque: My question concerns the protein content of milk, especially β-casein. You said that A1/β-casein is implicated in diabetes mellitus, so what is your suggestion for the consumption of unmodified cow’s milk for the adult? The other question is, is A1/β-casein present in human milk, and if it is what is the concentration of A1/β-casein in human milk?

Dr. Thorsdottir: I think this is just an observation and we do not exactly know if there is a real association. Regarding your second question, I would guess that this has not been measured, that would be my honest answer. Casein in general is very low in human milk compared to cow’s milk.

Dr. Haque: You mentioned that A1/β-casein is in some way related to the development of diabetes mellitus.

Dr. Thorsdottir: Yes, I mentioned it; I just showed it as an ecological observation. There is no evidence for a cause-effect relationship.

Dr. Saldanha: In my country, we usually give infant formula until 1 year and then cow’s milk from there on. Do you think there is enough economic and scientific evidence to continue feeding children with follow-on formulas until 3 years of age?

Dr. Thorsdottir: It’s a difficult question because in this particular population I was telling you about, we had a bad iron status also at the age of 2 years. Maybe it’s remnant from the bad iron status around 1 year of age, and follow-on milk is prepared in a way that it could be recommended until the age of 2 years. The protein intake is not very low, it’s 1.8 g per 100 g, and according to the general diet of the very young children at 1 year of age, you eat meat, fish, eggs and different kinds of dairy foods, so I think there is no risk in recommending the follow-on formula until the age of 2 years.

Dr. Hernell: I was quite surprised to see the real good effect of the new recommendations on iron status in your country. You have actually abolished iron deficiency at 12 months of age, and I think your figures are even better than we have in Sweden; we have at least a few percent using conventional criteria, which could be questioned during infancy. So my question is, do you think that the iron levels in the new follow-on formulas are too high?

Dr. Thorsdottir: According to the Codex, they are still low, but anyway it’s different from the former status, and I think it was discussed a lot before this was decided. We had to be within the Codex regulation above the minimum, but of course I cannot really answer this question.
Dr. Okai Brako: If we talk about whole cow’s milk, are we talking about pasteurized cow’s milk or milk straight from the cow?

Dr. Thorsdottir: Pasteurized cow’s milk.

Dr. Bhattacharya: The first question from the gentleman from Bangladesh has actually raised a very important issue. I have some experience in working in Bangladesh, and there is a huge number of children who at present in that part of the world suffer from nutrition-related problems for various complex reasons. The question he has asked contains certain answers, but I am afraid those are very robust answers, so for this forum at some point if I am given the opportunity I might try to make some comments on those issues which I believe are very important for that part of the world.

Dr. Thorsdottir: Concerning malnutrition in children all around the world, I think the best would be if we had some local evidence from most of the places. It’s really hard to translate the research from Iceland to your country, and we really have to admit that we want true evidence-based scientific research for each and everyone. In my opinion, and we see it from the literature, when we come to public health guidelines for infant, child and family nutrition, it’s very important to have some local information.

Dr. Kapur: You haven’t talked about complementary feeding from 6 months onward. What is your view on the benefits of promoting complementary feeding vs. follow on milk?

Dr. Thorsdottir: I think especially in the developing countries it might be wise to breastfeed exclusively as WHO has recommended until the age of 6 months. We have also followed that recommendation in the Nordic countries, but I think it’s especially important in developing countries, and weaning food has to be added gradually and slowly. I think this applies to areas all over the world. I also think the circumstances, the hygienic procedures and so on have to be as good as possible, and it might be wise to try to continue breastfeeding beyond 1 year of age, up to 2 years, and even longer if that suits the family in question. The cultural differences are so large that we cannot give out any very hard laws or regulations about complementary feeding. In general, as recommended by the WHO, it should be formula after breastfeeding and then gradually increasing porridges, fruits, vegetables and other kinds of food. But I understand, and we have seen it in the surveys, that there are cultural differences which we have to take into consideration.

References


Biological Effects of Novel Bovine Milk Fractions

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Abstract

Novel dairy fractions have been isolated and are now commercially available. Several of them have been shown to have biological activities in various test systems. \(\alpha\)-Lactalbumin was first isolated to provide a good source of tryptophan, often the first limiting amino acid in infant formulas, but has then been shown to be digested into smaller peptides with antimicrobial and prebiotic activities, immunostimulatory effect and acting as enhancers of mineral absorption. Lactoferrin bioactivities include antibacterial and antiviral effects, regulation of immune function, stimulation of intestinal proliferation and differentiation and facilitating iron absorption, but these activities may have been limited due to earlier contamination with LPS. Lactoferrin free of lipopolysaccharide may prove to be more effective with regard to exerting these activities. Osteopontin is a heavily phosphorylated and glycosylated protein that modulates immune function and stimulates Th1/Th2 switching, and, possibly, also affects bone mineralization and growth. Biological activities of lactoferrin may be facilitated by osteopontin. Milk fat globule membranes are a fraction that has previously been excluded from infant formulas, but components of this fraction have been shown to exhibit antimicrobial activities and to prevent infection. Further clinical studies are needed on infants fed formulas with these components incorporated.

Introduction

Human milk is known to contain a wide range of proteins providing various biological activities in the newborn infant [1]. These bioactivities range from enhancing immune function, antibacterial and antiviral activities, facilitating nutrient absorption, promoting bone growth, and supporting infant development. Many of these protein components have been believed to be unique to human milk, and in a sense they are, but it is possible that some bovine
counterparts may exert some of the biological activities provided by human milk proteins. In recent years, dairy technology has improved considerably, and bovine milk protein fractions are now available on a commercial scale. While some of these protein fractions have only been tested in laboratory scale experiments and in animal models, several of them have been evaluated in clinical trials on infants. In this article, some of these novel bovine milk protein fractions, namely α-lactalbumin, lactoferrin, milk fat globule membrane (MFGM) proteins and osteopontin, will be reviewed with regard to their functionality and possible use as ingredients in infant formula.

**α-Lactalbumin**

One of the major whey proteins in human milk is α-lactalbumin, which constitutes 10–20% of the total protein content of mature breast milk [1], and even more in colostrum and preterm milk. The proportion of α-lactalbumin in bovine whey is considerably less than that in human whey, and since cow’s milk protein consist of ~82% casein, α-lactalbumin is only 2–5% of the total protein content. α-Lactalbumin has a molecular weight of about 14,100 Da, and the sequence homology between human and bovine α-lactalbumin is 74% [2]. It has an amino acid composition that corresponds very well to the amino acid requirements of infants, and it is relatively high in tryptophan (5%), lysine (11%) and cysteine (6%) and can therefore complement other proteins that have lower contents of these essential amino acids. The protein content of infant formula is often considered to be higher than needed, as reflected by higher blood urea nitrogen, plasma amino acids (particularly the insulinogenic branched-chain amino acids) and insulin in formula-fed as compared to breastfed infants. However, when the protein content of infant formula is lowered, tryptophan becomes a limiting amino acid. Therefore, several studies have evaluated the effects of adding bovine α-lactalbumin or α-lactalbumin-enriched fractions when lowering the protein content of the formula [2]. We found that plasma tryptophan levels were similar to breastfed infants and significantly higher than in infants fed control formula [3].

α-Lactalbumin binds one calcium ion tightly, which aids to render the molecule a tight, compact globular structure; removal of this calcium ion results in unfolding and the molecule becomes considerably larger and more flexible. This may affect the digestibility of α-lactalbumin (see below). α-Lactalbumin also has a second calcium-binding site, which may not be occupied in vivo [2]. This site is capable of binding other divalent cations, e.g. zinc, iron and manganese, but native α-lactalbumin isolated from human milk does not contain any significant quantities of these ions. It is possible, however, that during digestion, smaller peptides with capacity of forming complexes with cations are formed. We have found increased zinc and iron absorption from formulas enriched with bovine α-lactalbumin in infant rhesus monkeys [4], suggest-
ing that this may occur. In our clinical study on infants fed α-lactalbumin-enriched formula (see above), we also found that indicators of iron status were higher than in infants fed control formula [3].

During the digestion of α-lactalbumin in the gastrointestinal tract, smaller peptides are formed which may have physiological activity in the small intestine, or in other tissues of the body, if they are absorbed intact (fig. 1). Several of these peptides (amino acids 50–52, 99–103, and 104–108) have been shown to inhibit angiotensin I-converting enzyme in vitro [2]. It is not known, though, whether these peptides are physiologically active in vivo. An immune-stimulating peptide, GLF, consisting of Gly-Leu-Phe (amino acids 51–53, symbols GLF), has been shown to be formed, and at concentrations that may be of physiological relevance [5]. GLF increases phagocytosis by human macrophages and stimulates polymorphonuclear neutrophil oxidative metabolism, which are important for killing pathogens [6]. Specific binding sites for GLF have been found on human phagocytic cells [7]. In vitro digestion of bovine α-lactalbumin by human neonatal gastric fluid resulted in formation of GLF, and it has also been found in gastric aspirates from young infants, showing that it can also be formed in vivo [6]. It is possible that GLF stimulates macrophages in epithelial villi of the small intestine, or that it acts upon macrophages in breast milk. Phagocytes in colostrum (~80% macrophages) have been shown to kill enteropathogenic *Escherichia coli* (EPEC), possibly explaining our observation that infant formula enriched with bovine α-lactalbumin protected against induced EPEC infection in infant rhesus monkeys [8].

Antibacterial peptides have also been shown to be formed during digestion of α-lactalbumin. In vitro digestion of α-lactalbumin with trypsin and chymotrypsin, or chymotrypsin alone, resulted in three different peptides which were active against Gram-positive bacteria [9]. It is not yet known whether these peptides are formed in vivo. Brück et al. [10] used batch culture and a
two-stage continuous culture system to evaluate the effect of α-lactalbumin-enriched formula on mixed populations of human gut bacteria. α-Lactalbumin supplementation resulted in a significant reduction in potentially pathogenic bacteria (Bacteroides, Clostridia, E. coli) and making the microflora more similar to that of breastfed infants. It is not known, however, if this was due to peptides formed from α-lactalbumin. It is also conceivable that the shift in microflora, at least in part, was due to formation of peptides stimulating host-friendly bacteria. Kee et al. [11] have characterized peptide fractions from pepsin-hydrolyzed α-lactalbumin that stimulated the growth of bifidobacteria in vitro. The relative significance of these two types of peptides in vivo needs to be investigated further.

Lactoferrin

In principle, lactoferrin cannot be called a ‘novel’ milk protein fraction, as it was relatively early isolated from cow milk and marketed for a variety of potential biological effects. However, it was recently noted that previously marketed bovine lactoferrin contained significant concentrations of lipopolysaccharide (LPS), which possibly could have affected the bioactivity of lactoferrin. LPS forms strong complexes with lactoferrin and at a part of the molecule that is involved in several of its activities [12]. Similarly, purified bovine and human lactoferrin that was commercially available for laboratory scale experiments was contaminated with LPS; however, most investigators isolated their own preparations and may have been more successful in avoiding LPS contamination. This may potentially explain why many of the early clinical studies (~20 years ago) yielded very disappointing results, showing little effect on gut microflora, iron status and infections. It is therefore important to re-evaluate the effects of bovine lactoferrin prepared with better techniques and without any significant contamination with LPS.

Lactoferrin was first discovered to be an iron-binding protein [13]. It binds two atoms of ferric iron and its structure has similarities to that of transferrin. Lactoferrin, however, has a much stronger affinity towards iron (K_{in} ~10^{24}) than transferrin, and also holds on to iron to a considerably lower pH (~3). Both these properties are likely of significance with regard to some of its bioactivities. A major part of iron in breast milk is bound to lactoferrin, and a specific receptor for lactoferrin has been found in the infant small intestine [14]. This receptor will take up lactoferrin and iron into the enterocyte by clathrin-mediated endocytosis. Thus, this is a way for iron to be taken up by the apical membrane and enter the body, either to be stored in mucosal ferritin and eventually sloughed if iron status is satisfactory, or enter the systemic circulation if iron status is low. This may explain why the iron status of exclusively breastfed infants generally is satisfactory, in spite of the comparatively low iron concentration of human milk.
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In addition, lactoferrin can enter the cell and can thus affect cell signaling and gene transcription and, consequently, affect cell proliferation and immune function. A recent double-blind clinical trial by King et al. [15] showed that iron status (as evaluated by hematocrit) of infants fed infant formula with bovine lactoferrin was better than that of infants fed formula with the same amount of iron as ferrous sulfate, but with no lactoferrin. A recent study has shown that bovine lactoferrin can also bind to a recombinant human lactoferrin receptor [16], suggesting that some forms of bovine lactoferrin may also bind to the receptor in vivo. In our early studies, bovine lactoferrin did not bind to the lactoferrin receptor, but it is possible that at that time, the lactoferrin may have contained LPS (see above) and/or possibly have been more intensely heat-treated/processed.

Lactoferrin is also known to have antimicrobial activity. Certainly, LPS contamination of commercial lactoferrin may affect this activity. Lactoferrin was early shown to have bacteriostatic activity against iron-requiring pathogens, e.g. *E. coli* [13]. This activity was exerted only by apo-lactoferrin – addition of iron abolished the effect – and it was shown that lactoferrin effectively can compete with these bacteria for iron, due to its high affinity. Recently, lactoferrin was shown to inhibit the growth of *Enterobacter sakazakii*, a food-borne pathogen that can cause diarrhea in young infants [17]. This effect was also only observed by apo- and not holo-lactoferrin. Since human milk lactoferrin is primarily in the apo-form (only saturated to 6–9%), lactoferrin in breast milk is likely to contribute to the defense against infection. It was subsequently shown that lactoferrin also can have bactericidal activity and kill pathogens such as *Vibrio cholerae* and *Staphylococcus aureus* and this activity is not affected by the iron saturation of lactoferrin [18]. It is quite possible that this anti-bacterial activity is exerted by peptides formed during digestion of lactoferrin. Lactoferricin and lactoferrampin are two such peptides that are strongly cationic and have been shown to have strong antimicrobial activity in cell and animal models [19]. Lactoferrin has also been shown to have antiviral activity [20]. In a randomized controlled double-blind study on young Peruvian children hospitalized with acute diarrhea, we found that oral rehydration solution (ORS) with recombinant human lactoferrin and lysozyme resulted in significantly reduced diarrhea duration, diarrhea volume, and recurrence of diarrhea as compared to ORS without these proteins [21]. Since the ORS contained both lactoferrin and lysozyme, it cannot be ascertained that lactoferrin was responsible for the effect observed. Ellison and Giehl [22] have shown that lactoferrin and lysozyme can act synergistically to kill bacteria, which may have occurred in our study.

Recent clinical studies support that lactoferrin can prevent infections in children. Infants fed formula supplemented with bovine lactoferrin (0.85 g/l) had significantly fewer episodes of lower respiratory illness than the placebo group, but there was no difference in diarrhea. In a study on Japanese children [23], daily supplementation with bovine lactoferrin (100 mg) was shown
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to result in significantly lower frequency and duration of vomiting and diarrhoea than in the placebo group, although no difference in rotavirus infection was found. Finally, Ochoa et al. [24] have shown that Peruvian children given bovine lactoferrin had lower prevalence of colonization of Giardia than placebo controls.

Lactoferrin may also have a prebiotic function in the intestine. Peptides resulting from pepsin digestion of human lactoferrin have been shown to stimulate the growth of bifidobacteria [25] and it is thus possible that increased colonization by bifidobacteria may inhibit the growth of pathogens. Treatment of these peptides with pancreatic enzymes in vitro did not destroy the stimulatory effect on bifidobacteria, suggesting that these peptides, if formed in vivo, may survive further digestion and exert the prebiotic activity. It is thus possible that lactoferrin establishes a ‘beneficial’ microflora in newborn infants by both stimulating growth of bifidobacteria and inhibiting/killing pathogens. Such a scenario is supported by the recent study of Manzoni et al. [26] who found that very low-birthweight infants supplemented with bovine lactoferrin (100 mg/day) had a significantly lower incidence of late-onset sepsis than those receiving placebo, and that there was no significant difference between infants given lactoferrin with or without probiotics (*Lactobacillus rhamnosus* GG).

**Osteopontin**

Osteopontin is a multifunctional protein secreted by macrophages, T cells and epithelial cells and known to induce cell-mediated responses, chemotaxis of inflammatory cells, and anti-inflammatory responses [27]. It is an acidic, phosphorylated glycoprotein that was first discovered in mineralized bone matrix, but was later found in many tissues and physiological fluids, including human milk [28]. It contains an integrin-binding RGD (Arg-Gly-Asp) motif and is an important regulator of bone remodeling [27]. Human milk osteopontin has 36 phosphorylation sites and 5 O-glycosylation sites [28], whereas bovine milk osteopontin has 28 phosphorylated sites and 3 O-glycosylated sites [29]. It appears that osteopontin in milk is phosphorylated to a considerably higher extent than in bone and other tissues, making milk osteopontin unusually negatively charged. Osteopontin has been shown to form a strong electrostatic complex with lactoferrin [30], and each osteopontin molecule is capable of binding three molecules of lactoferrin. It is thus possible that osteopontin helps to protect lactoferrin against proteolysis, preserve its bioactivities in the gut, and aid its transport to specific sites in the intestinal mucosa.

The expression of osteopontin in human milk cells was found to be very high, and this high level of expression persists throughout the lactation period [31]. In a microarray analysis of 240 cytokine-related genes in human milk cells, osteopontin was most abundantly expressed [31]. The concentration of
osteopontin in human milk accounts for ~5–10% of total milk proteins, which nearly corresponds to the concentration of lactoferrin (see also above). The concentration of osteopontin in bovine milk is much lower than in human milk (<1/5th), but bovine osteopontin has recently become available commercially in larger quantities, making it possible to add this component to infant formula.

Osteopontin is considered as a key molecule inducing Th1 type immunity. Osteopontin-null mice exhibit a defective Th1 response and are more susceptible to infections than wild-type mice [32]. Growth of Mycobacterium bovis bacillus Calmette-Guerin (BCG) was more rapid in macrophages from osteopontin-null mice than from wild-type mice [33] and an inverse correlation was described between tissue osteopontin expression and disease progression [34]. Breastfed infants are known to have an increased response in T cell proliferation compared to formula-fed infants when vaccinated against BCG at birth, suggesting an effect of breastfeeding on Th1 stimulation [35, 36]. It is thus possible that breast milk osteopontin contributes to host resistance in infants by enhancing the Th1 response. Osteopontin-null mice were also found to be more susceptible to rotavirus infection. Since osteopontin can bind to various integrins via its RGD motif, it may prevent attachment of the virus RGD motif to integrins on target cells [37]. Both intact osteopontin and its proteolytic fragments are found in human milk throughout lactation, and one such fragment (close to the N-terminal) that contains the integrin binding motif has been found to induce IL-12 expression in macrophages [32]. Thus, osteopontin may increase host resistance against a variety of pathogens via both Th1 polarization and other mechanisms.

Milk Fat Globule Membrane Proteins

The lipids in milk are surrounded by a bi-layered membrane containing unique proteins, but also phospholipids and gangliosides (fig. 2). Although this MFGM protein fraction is quantitatively minor [40], it may be of significance in the protection against infections. Several of these proteins, such as lactadherin, butyrophilin, xanthine oxidase and alkaline phosphatase have been shown to have antimicrobial activity in vitro. For example, components of this fraction isolated from human milk were able to bind to some rotavirus strains and prevent their replication, and this activity was correlated with the protein lactadherin [39]. The bioactivity of lactadherin in human milk is supported by the observation that its content in breast milk was shown to be negatively correlated with symptomatic rotavirus infection in Mexican infants [33]. The phospholipids and gangliosides in the MFGM fraction may be important in providing building blocks for the brain and promoting neurodevelopment.

Many of the components of the MFGM fraction from human milk have been shown to also be present in the bovine MFGM fraction and also to have very
similar structure. Infant formula made from cow’s milk, however, is manufactured from skim milk powder and whey protein concentrate and consequently does not contain any MFGM. Recently, milk fractions enriched with MFGM have become available on a large scale commercially and they could therefore be added to infant formulas in the future, provided that support for their bioactivity can be obtained. Several proteins in the bovine MFGM have been shown to exert inhibitory activities against various pathogens, and a whey protein concentrate enriched with MFGM may therefore help to protect against diarrhea of both bacterial and viral origin [41]. The commercially available MFGM fraction contains several bioactive components including mucin (MUC1), lactadherin, lactoferrin, sialic acid, sphingomyelin, and gangliosides. A bovine milk fraction containing MUC1 has previously been shown to inhibit hemagglutination of *V. cholerae* and *E. coli* [42]. Further, mucin purified from MFGM was shown to decrease the adherence of *Yersinia enterolytica* to intestinal membranes [43]. The MFGM fraction has also been found to inhibit rotavirus in vitro [44]. Sphingolipids, particularly gangliosides, have been shown to inhibit enterotoxins both in vitro and in vivo [45]. In addition, infant formula with added sphingolipids (gangliosides) has been shown to reduce *E. coli* counts in infant feces, and to increase beneficial bifidobacteria [46].

We have tested the concept of MFGM protein fractions having an effect on infectious diseases in Peruvian infants [47]. The infants were given MFGM proteins (~6 g/day) in a milk-based meal twice daily for 6 months in a randomized controlled double-blind study. Prevalence of diarrhea was significantly lower in the group given MFGM than in the group given the same type of meal with skim milk protein instead of MFGM. Thus, it is quite possible...
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that addition of the MFGM fraction to infant formula may have an effect on infectious disease.

Conclusions

Several novel dairy fractions are now available, and have been shown to have biological activities. Further clinical studies are needed on infants fed formulas with these components incorporated.

References

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Lönnerdal

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Discussion

Dr. Hernell: I was thinking about the fragments of α-lactalbumin that you mentioned are formed during digestion. Not only is exocrine pancreas not fully developed at birth and at least in breastfed infants the secretion of digestive enzymes is low, but human milk also contains protease inhibitors. Given that, do you think that there is a difference in how much of these peptides are formed and how long they actually stay in the intestine before they are finally digested if you compare a breastfed infant receiving human α-lactalbumin and a formula-fed infant receiving bovine α-lactalbumin?

Dr. Lönnerdal: It's a very good point you bring up. Coming back to what we discussed earlier about breast milk composition, the protein composition changes a lot during lactation, but for infant formula you are stuck with one constant composition. When looking at protease inhibitors, they are in much higher concentration during early lactation. We have done studies on α1-antitrypsin, which is actually a major breast milk protein, and its digestive function. Pancreatic protease activity is lower in early infancy, but with increasing maturity there will be less inhibition of proteases, which means more effective digestion, and that will affect α-lactalbumin, lactoferrin, and many other components. I think you have a 'gradient of survival', and it would be interesting to follow the survival of these bioactive components with increasing maturity, possibly looking at both duodenal aspirates (which are not easy to obtain) and fecal output, and then by extrapolating what you see in the duodenum to the stool I think we will have a better idea how much of these proteins can persist in the intact form and for how long.

Dr. Gibson: I was wondering, can you help us put this into context? Some of the effects that you have seen from these trace fractions that you have been examining like lactoferrin and osteopontin and so forth, they have benefits and they have effects, but in well-nourished communities such as in the US or in Sweden the difference in the infection rate between the breast and the formula fed infant is relatively small. Is it going to make much difference if we put all these compounds into an infant formula and what sort of a difference are we expecting, and how important do you think it is?

Dr. Lönnerdal: We are touching socioeconomic and family factors here, but I would like to amplify on that. Kay Dewey did a study in Davis, where she looked at formula-fed infants and breastfed infants. In Davis, we have excellent sanitary conditions, mothers' average education is 16 years, they are of upper-to-middle class, and still there was a significant difference in illness, both in prevalence of illness and in duration. I think it matters if you are a parent, if you during a period of, for example,
3 months have three episodes of diarrhea, and you have to stay home from work to take care of your ill infant during 3 days instead of 1 day. We also know from studies in developing countries that each day of illness will have an effect on appetite and growth. I am not sure how to measure this, but the more ill you are, the more responsibilities for the parents in developed countries because they need to take time off from work, cost for the family and so on. Otherwise, I think that, in general, if we can prevent illness it will be beneficial for any infant, whether in an industrialized or in a developing country.

**Dr. Haschke:** You are studying very interesting bioactive molecules in formula. We are far away from putting them in infant formula because of regulatory aspects and costs. If you added the five bioactive molecules that you have presented in your talk to an infant formula, its price would be too high. Taking a stepwise approach, only the α-lactalbumin is realistic. I would see lactoferrin as the next step. Would you agree?

**Dr. Lönnerdal:** I am not in the commercial business, but it’s also going to be a question of demand and production – the greater the demand for an ingredient, the higher the production, greater the competition, and lower the prices. But I think with these findings for lactoferrin and osteopontin we may not need to copy everything in breast milk to make infant formula functionally more similar. The difficulty will be to do the kind of studies where you would add one component, another one, another one, and then add all of them; it’s very costly, requires a lot of subjects, and so on. We talked at previous workshops about the fact that federal agencies, the European Community, etc. don’t want to fund such studies because they basically say this is the responsibility of infant formula manufacturers. I respect that infant formula manufacturers cannot study all these permutations that I am talking about but that we as scientists would like to see. Therefore, we need to home in on some key components.

**Dr. Hernell:** Perhaps a comment to that; there are various groups of infants, for instance preterm infants, it may be a small fraction of all infants, for whom a benefit is greater while the cost less critical because the cost of care of a preterm is still quite high.

**Dr. Haschke:** It depends, the formula for premature infants which is delivered to hospitals usually is the most complex one; but the hospitals want to have discount prices, which slows down innovation in that important segment. Industry needs to prove safety and efficacy of any new bioactive molecule that is added to those formulas. Huge sample sizes are needed to prove a positive health outcome, and the likelihood that an industry-sponsored study is challenged is high.

**Dr. Lönnerdal:** Yes, I agree.

**Dr. Johansson:** You talked about the proteases and the release of bioactive peptides. I think we should not restrict our thinking to proteases in the milk as such but also have microbiota-produced proteases in mind, and how this could be beneficial or not depending on feeding.

**Dr. Lönnerdal:** That’s a very good point.

**Dr. Jongpiputvanich:** I would like to know whether α-lactalbumin in cow’s milk has a different amino acid sequence and function than α-lactalbumin in breast milk.

**Dr. Lönnerdal:** Certainly, it’s not identical when it comes to the amino acid sequence, but it seems like several of these structural motives that I talked about are similar and some smaller peptides are identical. For example, the calcium-binding site in bovine α-lactalbumin is identical or very similar to the one in human α-lactalbumin. Then, there may be stretches in the molecule where you have very different amino acid peptides, but they may not be related at all to these bioactivities that I talked about; however, the antibacterial peptides being formed were identical.

**Dr. Anderson:** You haven’t commented on obesity. Is there a difference in gastric emptying rate between formula and breast milk? That could in part be a determinant
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of excess caloric intake. I am puzzled why high-protein formulas don't associate with suppressed food intake rather than increased caloric intake and obesity.

*Dr. Lönnerdal:* You brought up several things here. With regard to implications for obesity, there are at least two scenarios. If you have hyperglycemia and hyperinsulinemia in infants because of high-protein formula, there is the possibility later on in life for diabetes and obesity. The other one is the recent studies by Jeff Gordon and his group with communication between the microbiota and the diet and the crosstalk with the intestinal mucosa, where they have shown a clear connection to energy metabolism. This is a very novel area, but it's exciting because there are some human studies now, following the mouse studies, showing that at least it's possible that it could affect things like obesity and energy metabolism. When it comes to gastric emptying, I think little has been done in human infants. We often use young rat pups as a model for digestion studies, and it's very obvious if you do sequential killings that there is much more rapid clearing of the stomach when you feed them breast milk than if you give them infant formula. My expectation would be that it's the same in the human infants, but I don't think we have firm data on that. When it comes to overall food intake though, we know that formula intake is much higher than the milk intake of breastfed infants, so it doesn't seem to have any suppressive effect; in contrast, it may be peptides of formula that stimulate the intake.

*Dr. Netrebenko:* Prof. Hernell mentioned in his lecture that breast milk composition varies greatly from one woman to another. Have you seen cases of low lactoferrin or other protective factors in the human, and does it result in worse immune function in infants?

*Dr. Lönnerdal:* I haven't seen any direct studies on it. I actually have thought about how such studies could be done. Even if the concentration is at the lower range of what is considered normal, there would still be enough to perform the biological function. This is something that has to be borne in mind when discussing nutrient recommendations. As we have heard, there is a large variation in the composition of breast milk from one woman to another, and there is a lot of tracking. Thus, if a mother has a low concentration of a nutrient at the beginning of lactation, this will remain so throughout lactation. The infants seem to be doing quite well, and then recommendations are set at the medium level, not at the lower level, even though the lower level intakes seem to be adequate. This would be interesting to study for both individual proteins, but also when it comes to how much protein breast milk should provide and therefore in its extension how much protein there should be in infant formula.

*Dr. Jongpiputvanich:* To mimic the protein composition of breast milk, why don't we make infant formula that has higher α-lactalbumin and has no β-lactoglobulin as in human milk?

*Dr. Lönnerdal:* There are formulas now that have increased levels of α-lactalbumin, so steps have been taken in that direction. We will have to ask Dr. Haschke, but there have also been attempts to remove β-lactoglobulin, but then the formula becomes too costly. Therefore, you may be able to make a very nice formula without β-lactoglobulin, but if the mother cannot afford it, you haven't gained that much. In addition, I haven't seen any evidence that it would eliminate or reduce the risk of allergy if you just take out β-lactoglobulin. If an infant develops an allergy against one cow's milk protein, it often develops an allergy against other components too, so just removing β-lactoglobulin may not be enough.

*Dr. Sankaranarayanan:* Can you comment on the role of bovine colostrum?

*Dr. Lönnerdal:* Bovine colostrum is a very interesting source of bioactive components, although I think the supply is relatively limited. Just like human colostrum is
higher in many components, bovine colostrum is high in immunoglobulins, lactoferrin, osteopontin, and many other things.

*Dr. Thorsdottir:* I read somewhere that amino acid cysteine was a stimulator for insulin-like growth factor, and you said that α-lactalbumin increases the amount of cysteine. Do you know anything about this, maybe this is wrong?

*Dr. Lönnerdal:* I was not aware of this. Maybe Dr. Martin who is going to speak tomorrow will have a chance to address that.

*Dr. Mouane:* I have a question about the milk fat globule membrane. I was expecting better neurological development or vision, and there is an anti-diarrhea effect, so what is the mechanism?

*Dr. Lönnerdal:* It’s a fairly complex mixture with several components. I wouldn’t be surprised if there was an effect on neurodevelopment, but no such studies have been done. When it comes to defense against infection, there may be other components.

*Dr. Melnik:* Concerning the insulinotropic effect of α-lactalbumin, it is known that hydrolyzed α-lactalbumin is a source of highly insulinotropic amino acids. α-Lactalbumin is especially enriched in tryptophan, an amino acid precursor of serotonin synthesis which stimulates pituitary growth hormone and prolactin release. This fits in experimental evidence observed in α-lactalbumin-enriched diets in humans and animals [1]. Both growth hormone and prolactin increase insulin secretion of pancreatic β-cells. In this regard, α-lactalbumin appears to be a most important signaling molecule of the mammalian lactation genome promoting insulinotropic effects of milk consumption.

**Reference**

Milk and Oral Health

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Abstract

Oral health includes freedom from disease in the gums, the mucosa and the teeth. There has been a striking reduction in dental caries and periodontitis in industrialized countries, although the proportion with severe disease has remained at 10–15%, and the prevalence increases in less developed countries. If left untreated, these diseases may lead to pain, and impaired quality of life and nutritional status. Prevention and treatment need, besides traditional implementation of proper oral hygiene, sugar restriction and use of fluoride, newer cost-effective strategies. Non-sweetened dairy products, which are proven non-cariogenic, or specific bioactive components from alike sources might prove to be part of such strategies. Thus, milk proteins, such as bovine and human caseins and lactoferrin, inhibit initial attachment of cariogenic mutans streptococci to hydroxyapatite coated with saliva or purified saliva host ligands. In contrast, both bovine and human milk coated on hydroxyapatite promotes attachment of commensal Actinomyces naeslundii and other streptococci in vitro, and phosphorylated milk-derived peptides promote maintenance of tooth minerals, as shown for the β-casein-derived caseino-phosphate peptide. Observational studies are promising, but randomized clinical trials are needed to reveal if dairy products could be a complementary treatment for oral health.

Oral Health in a Global Perspective

Dental caries and periodontitis are the most common infectious diseases worldwide. Though not life threatening, they may seriously affect quality of life and well-being due to pain, limitations in mastication and food selection, and reduced self-esteem. Other less prevalent oral diseases such as cancer and noma, may lead to untimely death. The global pattern of oral diseases has undergone a significant change in recent decades. There has been a striking reduction in dental caries in industrialized countries and a parallel increase in less developed countries. However, the proportion of
patients with severe caries has remained virtually unchanged at 10–15% in
the industrialized countries, and 90% of caries in low-income countries has
remained untreated. A similar pattern is seen for periodontal disease. In
spite of the widespread use of effective preventive measures, such as fluori-
dated toothpaste and improved oral hygiene, newer strategies are needed to
improve oral health. The present paper describes the potential role of milk
and dairy components in oral health promotion with special focus on dental
caries.

Determinants for Dental Caries

Dental caries is a chronic infectious disease with a slow progress of tooth
tissue destruction in most individuals. A permanent tooth crystal deminer-
alization results from alternating periods with low pH and neutral pH at the
tooth surface, during which demineralization and remineralization, respec-
tively, occur. Progression may be arrested if the local conditions are ben-
eficial, such as maintenance of neutral pH and supersaturation of calcium,
phosphate and/or fluoride in the fluid phase surrounding the hydroxyapatite
crystals.

Teeth, and other surfaces in the mouth, are covered with a biofilm of
microorganisms that are attached to host receptors or to each other [1].
In recent years, the ecosystem in the biofilm, rather than the presence of
any specific bacterial species, has been recognized as a determinant for
development of caries. Thus, enrichment of one or more bacterial species
tolerant to low pH and qualified acid producers from sugar metabolism
are held responsible for disease development [2]. Among such species are
the mutans streptococci, which are frequently isolated from caries sites
and induce caries development in sugar-fed animals. The mutans strepto-
cocci also have the ability to produce water-insoluble, extracellular glucans
that increase bacteria adhesion capacity in the biofilm. Other bacteria, such
as Streptococcus sanguinis and species of Actinomyces, are considered
‘protective’ against caries development. Several studies have confirmed
that low pH (not sugar per se) is a driving force for plaque ecology and
caries [3–5].

Bacterial colonization starts by the selective adhesion of a bacterium to
a suitable host receptor. In the mouth, these receptor sites are mainly pre-
sent on salivary proteins that adhere to the tooth tissues or epithelial cells
(fig. 1).

Earlier research on the role of saliva for caries development focused on its
role as a buffer and on the salivary proteins that limit bacterial growth and
metabolism, such as lactoferrin, and lysozyme. Today, research focuses on
biofilm ecology. Ligand-bacterial interaction sites are being mapped, and pro-
teolytically released peptides with biological activities that are several-fold
greater than the mother proteins are being studied. Externally derived proteins/peptides, such as from milk, may provide receptors for bacterial adhesion and may induce bacterial aggregation (clearance). Note that milk and saliva are exocrine secretions that contain an array of peptides/proteins and minerals that are the same and that confer similar biological functions. Other components are tissue specific, such as the lactose, fat, caseins and lactalbumins (only in milk), and the innate immunity protein gp340 (the major host receptor for mutans streptococci; only in saliva) [6]. These components confer different biological functions to the secretions. Note that some of the bacteria-binding/antimicrobial peptides with high affinity for the tooth tissues (statherin and histatins) are suggested to have evolved from an ancestral casein gene [7]. In addition, the family of saliva proline-rich proteins, which constitute >50% of the saliva protein content, share several structural similarities with the caseins.

Fig. 1. Oral biofilm formation. Components from both saliva and diet form a protein/peptide pellicle on oral tissues, and display adhesion sites for bacteria attachment. Such components may also bind to free-floating bacteria and clear them from the oral cavity. Initially colonizing bacteria, such as *Actinomyces* and *Streptococcus* spp., adhere to the pellicle or co-adhere to other bacteria.
Potential Anticariogenic Effects of Milk

Studies in rodents have shown that milk is non-cariogenic [8, 9], and have suggested that milk may have a protective effect against sugar when consumed together [9]. In vitro studies have found bioactive components in dairy products that block adhesion of cariogenic mutans streptococci, support adhesion of commensal Actinomyces and streptococci, reduce production of extracellular glucans, support hydroxyapatite remineralization, reduce acid production and buffer at low pH [10–13]. Several different milk components are involved in these actions, but in recent years, caseins and peptides thereof, such as caseino-phosphate peptide (CPP), have attracted special interest [for a review on the β-casein-derived CPP, see Azarpazhooh and Limeback 14].

Milk and Dental Health in Children

No randomized clinical trial on milk intake and caries development has been identified. However, several observational, cross-sectional studies find either lower caries frequency in children with milk consumption compared to those who do not drink milk, or significantly lower milk consumption in children with caries as compared to children without caries [15–19]. However, two prospective cohort studies failed to show those correlations [20, 21]. These contradictory results may be due to the fact that the studies were performed in different settings. Thus, Petti et al. [17], who studied 6- to 11-year-old non-fluoride users with poor oral hygiene, found that increasing milk consumption (0–650 ml per day) reduced caries, but only in children with high sucrose intake. Similarly, Leake et al. [19] studied small children with high caries development, i.e. 46% had severe early childhood tooth decay. The children who drank milk had fewer caries lesions after they began to walk (odds ratio 0.44, 90% CI 0.24–0.81). In contrast, the study by Öhlund et al. [20], which found no association with milk intake, was done in a low-caries prevalence area. However, Öhlund et al. [20] as well as Petridou et al. [22] found that intake of hard cheese was associated with less caries in a dose-response manner. The effect of hard cheese has been confirmed in an intervention study [23]. In a 2-year study in schoolchildren, an intervention group chewed one 5-gram piece of hard cheese daily, whereas no cheese was provided to controls. All children drank water with 0.3 ppm fluoride, brushed their teeth with fluoridated preparations and received fluoride drops. At the end of the intervention period, caries increment measured as decayed, missing and filled tooth surfaces (DMFS) was significantly lower in the cheese-eating group (0.65 DMFS) than in the control group (2.4 DMFS). Still, one prospective cohort study has found no association between reported cheese consumption and caries development [21].
Milk proteins, such as bovine and human caseins and lactoferrin have been shown to inhibit attachment (the first step in colonization) of cariogenic mutans streptococci to saliva host receptors coated on synthetic hydroxyapatite (a model for tooth tissue; fig. 2).

In contrast, milk, and specifically caseins, bound to hydroxyapatite do not mediate attachment of mutans streptococci, but bind commensal and other streptococci. The delineation of human milk components mediating attachment inhibition of strain Ingbritt to parotid saliva or purified host receptor (gp340) is illustrated in figure 3.

**Dairy Products and Oral Health in Adults**

Epidemiologic studies in adults and elderly people confirm that higher intake of dairy products is associated with less caries. Thus, elderly who ate cheese several times per week [24] or milk products [25] had significantly less root surface caries. A possible protective role of cheese is also supported by in situ studies of remineralization of enamel slabs worn in the mouth of dry mouth patients [26].
The association between periodontal conditions and intake of dairy products, such as milk, cheese, and lactic acid foods (yogurt and lactic acid drinks) has also been studied in adults [27, 28]. The former authors found that subjects who ate \( \geq 55 \text{ g} \) lactic acid foods per day had less periodontitis (odds ratio after adjustment for confounders, 0.40, 95% CI 0.23–0.70) than subjects not eating those foods. Similarly, Al-Zahrani [28] found periodontitis to be 41% less prevalent in individuals in the highest quintile of intake of dairy products compared with those in the lowest quintile. After standardization for known and suspected periodontitis risk factors (age, gender, race/ethnicity, cigarette smoking, education, diabetes, poverty index, vitamin use, body mass index, physical activity, time since the last dental visit, dental cal-

![Figure 3](image-url)
Milk and Oral Health

...culus, and gingival bleeding), it declined to 20%, but the trend remained statistically significant.

**Milk as a Carrier of Therapeutic Agents**

Combining dairy products and therapeutic components could theoretically lead to synergistic health effects. Such approaches have been used for prevention/treatment of dental caries using milk supplemented with fluoride on and probiotic bacteria. Thereby, both tooth mineralization (calcium-fluoride) and biofilm ecology (buffer effect, adhesion modification, and anti-metabolic effects) are influenced. The caries-protective effect of fluoridated milk has been evaluated in several Cochrane reviews, which all came to the conclusion that fluoridated milk is beneficial, but better quality clinical studies are needed to reach stronger conclusions on its efficacy [29].

Recently, increasing numbers of publications have studied the effect of probiotic bacteria in dairy products (mainly milk) on caries-associated factors. Several short-term studies support that probiotic lactobacilli both reduce caries risk markers and improve gingival conditions, at least on short term basis [30–33]. The mechanisms are not well understood, but possibly involve blocking of host receptors, such as saliva gp340 for mutans streptococci [34] and affecting bacteria metabolism [35]. The few published long-term studies support a caries-protective effect in preschool children. Thus, in one study a group of children who consumed milk with *Lactobacillus rhamnosus* for 7 months reduced caries development compared to a group of children who drank control milk [36], and a recent 21-month randomized clinical trial showed a 75% caries reduction by milk supplemented with *L. rhamnosus* and fluoride in a low caries population with organized dental care compared to a control group [37]. The latter study design did not allow an evaluation of the separate effects of fluoride and probiotic bacteria. These studies are promising, and future long-term studies will reveal if dairy products with natural or added probiotic bacteria could be a complementary treatment for oral health.

**Conclusion**

Oral health inequality is a serious reality both within industrialized countries and between industrialized and less developed countries. The relative importance of different disease-associated factors, and thus the efficacy of preventive strategies, will vary between cohorts. To overcome this, a multidimensional approach must be taken. The basis will be the traditional preventive measures that have proven successful, but newer cost-effective prevention/treatment strategies must be applied. Non-sweetened dairy
products or specific bioactive components from such sources might prove to be part of such strategies. However, properly designed clinical studies in target groups are needed. Such studies must include the effects of confounders and involve caries in various risk groups and types of disease, such as children and underprivileged groups in general, orthodontic patients, dry mouth groups, and coronary and root caries conditions to overcome the shortcomings of the present studies.

References

Milk and Oral Health


Discussion

Dr. Sankaranarayanan: Does dental caries mostly occur in milk teeth or in permanent teeth?

Dr. Johansson: Both, and these general aspects refer to both deciduous and permanent teeth.

Dr. Sankaranarayanan: We believe that multiple dental caries is likely to be due to very severe extra-epithelial manifestations of gastric refluxes. People who suffer from gastric reflux do very well particularly with anti-reflux measures and lifestyle modification, and multiple dental caries is commonly seen in neurologically impaired and mentally retarded children. Dental caries in milk tooth is a point of discussion at the differential diagnosis, and I would like to bring it to this forum, the milk-rich
feed may be a factor for constipation. I would like to have your comments about this because we think about one side of the coin, but I would like to see the other side of the coin as well.

Dr. Johansson: I assume that what you find in your reflux patients is dental erosion, and, as you describe, treatment involves antacids, modifications of toothbrushing behavior and intake of acidic products, and possibly covering of the damaged teeth. Many diseases and medications, including neurological impairment and mental retardation, as you mention, are associated with increased risk for caries. This may be due to impaired saliva secretion or muscle movements or improper oral hygiene and feeding. We find that appropriate prevention, involving oral hygiene with fluoridated toothpaste, restricted sugar intake and topical fluoride treatment, is effective. Your third question was on the risk of constipation by milk feed. The epidemiological studies supporting protective effects have not linked very high intakes of cheese or milk to caries protection, so from that point of view I do not think it would eliminate the possibility of a fiber-rich diet. I wanted to balance the not uncommon information on a risk of 'breastfeeding caries' given by some dental personnel, at least in European countries, to all parents. This is very unfortunate since human milk is not cariogenic per se. One might have to consider a risk in cases with ad libitum breastfeeding throughout the night after tooth eruption, and then 'bottle feeding caries' must be distinguished as this is a different issue.

Dr. Prentice: I wanted to thank you very much, you have introduced me to an area that I have not heard about before. My question is about the development of saliva. Many years ago, I was interested in the appearance of secretory IgA in saliva of the newborn and was interested just how long it took for concentrations to develop over a period of months. Given, as we heard today, that breast milk composition also changes, I wondered if you have any information on how long it takes the breastfed child to produce the adhesins for the bacteria – is it once the child's tooth has erupted and the child's own saliva produces those materials, or does breast milk contain some of the adhesins as well?

Dr. Johansson: Besides IgA, very few saliva components have been followed longitudinally from birth on, so I cannot give a specific description of changes in the concentrations. However, most components are detected at significant levels very soon after birth, including proteins with bacterial adhesion capacity and innate immunity functions. The low-volume saliva secreted in small babies also leads to concentrations that sometimes are higher than in the saliva of adults.

Dr. Savaiano: Could you comment on whether or not there is any evidence to believe that cheese vs. milk has differential effects?

Dr. Johansson: There are a couple of notable differences. First, there is no lactose in cheese, so there is no risk of microbial adaptation to lactose in frequent exposure. Then, there are higher concentrations of all components in cheese, and cheese contains an array of proteolytically cleaved peptides, some of which are biologically active, at least in vitro.

Dr. Garg: Until today, I had the impression that milk is cariogenic. In bottle baby syndrome, dental erosion of the front teeth occurs in children who are bottle fed. We have always thought that because milk remains in touch with the front teeth during the night, and that's what causes dental erosion. Has there been a change in the understanding of bottle baby syndrome?

Dr. Mouane: Just to join Dr. Garg, pediatricians often advise parents to avoid bottle feeding during the night.

Dr. Johansson: Bottle-feeding is one of well-documented risk factors for early childhood caries, and the advice not to use a bottle at night is correct. Often, night bottles contain sweet drinks but also the carbohydrates used in formulas are fermentable
by tooth-colonizing bacteria, and therefore should not be used ad libitum after tooth
eruption, and especially not at night when there is very low saliva production. Finally,
what I think you see in the bottle-fed babies you describe is dental caries and not
erosion, and prevention would consist in limiting exposure frequency/duration and
introducing gentle tooth cleaning as soon as the first teeth have erupted.

Dr. Haschke: Together with the University Dental Clinic in Zurich, the Nestlé
Research Centre did a lot of rat studies with glycomacropeptides because they are a
casein fraction with a strong anticariogenic effect. Our intention was to make a follow-
up formula that is anticariogenic. The problem was that so much of glycomacropep-
tides was needed to show an anticariogenic effect that the cost of such a formula
would have gone through the roof, and we sold the patent to Colgate. Are you aware
of this?

Dr. Johansson: Yes, I know these studies from what has been published.
Glycomacropeptides and casein phosphopeptides are peptides that have docu-
mented anti-caries effects in animals and the latter also in humans. The effects involve
prevention of adhesion of cariogenic mutans streptococci and enrichment of calcium
and phosphate in the tooth biofilm. Together with the studies by Bowen and coworkers,
these studies were the foundation for a concept that might be developed into a
valuable complement to fluoride.

Dr. Martin: Thank you very much for your very interesting talk. You call for high-
quality studies looking at breastfeeding vs. formula feeding. Do you have any comment
on the PROBIT results that were published in *Caries Research*? This was a trial with
17,000 mother-infant pairs, 8,000 randomized to the breastfeeding promotion inter-
vention, 8,000 to the control arm, with the intervention substantially increasing exclu-
sive and prolonged breastfeeding. In an analysis by intention to treat, removing any
effect of confounding, there was no difference in caries prevalence or caries experi-
ence in 6-year-old children between the two groups [1].

Dr. Johansson: The PROBIT study in Belarus is a powerful study that demon-
strates that in a population with a very high level of caries development no harmful
or beneficial effects are seen from breastfeeding in a long-term perspective. However,
information is also needed from other types of populations, for instance populations
with poor nutritional status and populations with the dual disease distribution that has
emerged in many countries today.

Dr. Martin: Just to follow up on Dr. Haschke's question about the GMP. You said
that you needed so much in the formula that it was not cost effective. How much
cheese do you need to have a protective effect?

Dr. Johansson: In the one intervention study that has been done, the children
were given 5 g a day.

Dr. Martin: Do you need to take it daily?

Dr. Johansson: Our results support that a daily intake is needed. Thus, we found
that children with one intake or more a day were caries free.

Dr. Haralappa: You said that to avoid caries one should use fluoride toothpaste.
Most of the small children swallow it. My question is, how much toothpaste can they
swallow? How much would be harmful and cause fluorosis?

Dr. Johansson: The main risk of developing fluorosis comes with the water that
children drink during tooth formation. A fluoride water content of 1 mg/l (1 ppm) is
considered optimal under most conditions. Anything above that level is associated
with an increasing risk for disturbance of tooth tissue formation. Under conditions
with high content of fluoride in the drinking water, one does not need fluoridated
toothpaste since the teeth are rinsed with fluoride at every intake. Tea is another
source that might be considered. In areas with a 'suboptimal' fluoride level, even small
children can be given fluoridated toothpaste in small amounts bearing in mind that
they swallow. In Scandinavia, we recommend that parents use regular toothpaste (1,000–1,500 ppm), but limit the portion to that of half a pea. At that level, there are no indications of harmful side effects.

Dr. Bodenstab: You mentioned cheese, but how about yogurt? And does it make a difference to have live bacteria in it or not?

Dr. Johansson: There is limited epidemiology for yogurt and caries, but my assumption is that the effect is closer to cheese than milk due to bacterial fermentation and lactose reduction and peptide production. Another positive thing with yogurt is that it has naturally or often even added probiotic bacteria. A positive effect of probiotic bacteria has been indicated by several short-term studies. It must, however, be observed that many yogurts on the market have added sugar, which changes a non-cariogenic into a caries-promoting product.

Reference

Milk during Childhood in Low- and High-Income Countries


Milk and Growth in Children: Effects of Whey and Casein

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Abstract

Consumption of cow’s milk is recommended in many countries. Observational and intervention studies show that cow’s milk most likely has a positive influence on growth in children. The strongest evidence comes from observational studies and intervention studies in low-income countries, but there are also observational studies from high-income countries showing positive associations between milk intake and growth. Milk seems thus to have a specific stimulating effect on linear growth, not only in developing countries with high rates of malnutrition, but also in industrialized countries. However, it is not known which components in milk stimulate growth. Possible components are proteins, minerals, vitamins or combinations of these. Cow’s milk proteins have a high protein quality, and whey has a slightly higher quality than casein, according to some indices based on amino acid composition. Studies, mainly from sport medicine, have suggested that whey protein also has the potential to increase muscle mass. Whether whey improves body composition to a larger extent than other milk proteins is not clear. The mechanism behind a possible growth-stimulating effect of milk and milk components is likely to be through a stimulation of insulin-like growth factor-I synthesis and maybe insulin secretion. In conclusion, there is strong evidence that milk stimulates linear growth. The mechanism is not yet clear, and more intervention studies are needed to understand which components in milk are responsible for the growth stimulation. The effects of milk on linear growth and adult height may have both positive and negative long-term implications.

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During the last half century, the consumption of cow’s milk has changed considerably worldwide. In some countries like China, there has been a huge increase in the intake of milk products [1]. Many countries recommend cow’s milk during childhood because it is believed that it is healthy and has a
positive influence on bone mineralization and growth. Due to the changes in milk consumption, there is a need to improve our understanding about both possible positive and negative effects of milk and milk products in different age groups.

Milk is designed by nature to stimulate and support growth in offspring, whether it is animals or humans. Milk is species specific and, for example, cow’s milk is designed by nature to support the high growth rate in calves. Cow’s milk is therefore also different from human milk which is designed to support the slower growth rate which is several times as high as the content in human breast milk [2]. Furthermore, the ratio between the milk proteins whey and casein is very different. Cow’s milk protein contains about 20% of whey and 80% of casein, while human milk contains about 60 and 40%, respectively. In infant formula based on cow’s milk, the amount of whey and casein have been adjusted to be close to the composition in human milk, because it is believed that the composition of human milk is optimal for healthy growth in infants. However, the type and content of specific proteins in the whey and casein fractions are also different in cow’s milk, compared to human milk. The principal fractions of caseins in cow’s milk are α-s1- and α-s2-caseins, β-casein and κ-casein [3]. These casein proteins are conjugated mainly with phosphate groups and bind large amounts of calcium phosphate.

Table 1. Content of energy and selected nutrients in cow’s milk, infant formula and breast milk (per 100 ml)

<table>
<thead>
<tr>
<th></th>
<th>Breast milk</th>
<th>Formula</th>
<th>Full-fat milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ</td>
<td>270–290</td>
<td>280–290</td>
<td>270</td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>65–70</td>
<td>67</td>
<td>65</td>
</tr>
<tr>
<td>Protein, g</td>
<td>0.9</td>
<td>1.2–1.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>6.7</td>
<td>7–8</td>
<td>4.4</td>
</tr>
<tr>
<td>Oligosaccharides, g</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fat, g</td>
<td>3.5</td>
<td>3.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>20–25</td>
<td>42</td>
<td>116</td>
</tr>
<tr>
<td>Phosphorus, mg</td>
<td>12–14</td>
<td>21</td>
<td>93</td>
</tr>
<tr>
<td>Sodium, mg</td>
<td>12–25</td>
<td>16</td>
<td>45</td>
</tr>
<tr>
<td>Potassium, mg</td>
<td>40–55</td>
<td>55</td>
<td>144</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>0.03–0.09</td>
<td>0.4–0.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>0.1–0.3</td>
<td>0.4</td>
<td>0.42</td>
</tr>
<tr>
<td>Vitamin A, µg</td>
<td>30–60</td>
<td>50</td>
<td>29</td>
</tr>
<tr>
<td>Vitamin C, µg</td>
<td>10</td>
<td>7–9</td>
<td>1.2</td>
</tr>
<tr>
<td>Vitamin D, µg</td>
<td>0.03</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin K, µg</td>
<td>0.2–0.5</td>
<td>2.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Folic acid, µg</td>
<td>80–140</td>
<td>6.5</td>
<td>11</td>
</tr>
</tbody>
</table>

From Michaelsen et al. [2].
Milk and Growth in Children: Effects of Whey and Casein

The major proteins in whey from cow’s milk are β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins and glycomacropeptide, while some of the minor proteins are lactoferrin, β-microglobulin, insulin-like growth factor (IGF) and γ-globulin [3]. β-Lactoglobulin is absent in human milk where α-lactalbumin is a major protein [4]. In addition to high-quality protein containing all essential amino acids, many other nutrients in milk may be related to growth (table 1) [2, 5, 6], such as calcium, magnesium, and phosphorus. Whole milk is also a good source of energy (266 kJ/100 g) and has a good balance between energy and protein, which is important for the optimal utilization of protein [4, 7].

Regulation of linear growth is complicated and not fully understood. The regulation may be different in different age groups as indicated by Karlberg’s Infancy, Childhood and Puberty Growth Model (fig. 1). It discriminates between infant growth, where growth hormone is less important than during childhood growth, and the puberty growth spurt with an additional effect of sex hormones [8]. The role of nutrients may also differ in different growth periods. Several studies have shown that in low-income countries addition of animal source foods to the diet in infancy, childhood and adolescence stimulates growth. The reason could be that animal foods add components such as micronutrients and quality protein to a food with a marginal content of these

Fig. 1. Karlberg’s Infancy, Childhood and Puberty Growth Model [8].
nutrients [5]. Milk contains many nutrients which may increase the nutritional value of the diet in a marginal situation [2, 5].

**Milk and Growth**

Several studies have shown that formula-fed infants have a higher growth velocity than breastfed infants especially during second half of infancy. But these studies are not randomized, and the mechanisms causing the higher growth velocity are not clear. One explanation could be the higher protein content in cow’s milk formula (1.2–1.8 g/100 ml) compared to breast milk (0.9 g/100 ml) [2].

In preschool children, only few studies are available. An intervention study from Guatemala found that energy intake and not protein intake was the most important factor for linear growth [9]. In well-nourished children, only few studies exist. In 2.5-year-old Danish children, a positive association was found between height and animal protein and milk intake [10].

There are more studies in school age children. One of the most famous is the Boyd Orr study conducted in Scotland about 80 years ago [11, 12]. The effect of whole milk, skinned milk and biscuits with the same energy content was studied in school children aged 5–6, 8–9 and 13–14 years by comparing them with control children receiving no intervention. The growth in the two milk groups was 20% higher during the 7-month intervention period compared to controls. The biscuit group did not grow more than the control group. Furthermore, those who got a supplement of milk for an additional year continued to grow at a higher rate compared to those who did not get extra milk. As the study was conducted in the 1920s, it is likely that the children had some degree of malnutrition.

In another study from New Guinea with 7- to 13-year-old children, many of them being stunted and having a low protein intake, Lampl et al. [13] also found an effect of skinned milk supplementation on linear growth compared to children getting margarine with similar energy content.

Older studies from the US reviewed by Hoppe et al. [5] from 1925 and 1945 also found an effect of extra milk on linear growth in deaf and blind children or children with growth failure. Similarly, a randomized intervention study with British schoolchildren aged 7–8 years also found an effect on height gain of 190 ml milk supplementation daily [14]. These children were classified as ‘disadvantaged’. In contrast, a number of other studies with school age children have found no effect of milk supplementation on height. Grillenberger et al. [15] found no difference in growth between schoolchildren supplemented with milk compared to meat in a Kenyan study. In a study with 6- to 7-year-old children conducted in England and Scotland, Cook et al. [16] found that children with free access to milk did not grow more than those with no access.
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Some intervention studies have examined the effect of dairy products on bone growth [17]. Cadogan et al. [18] found an effect on bone size of one pint of milk given for 18 months to 12-year-old girls with low habitual milk intake. However, no significant effect was found on height. Similarly, Chan et al. [19] found an effect on bone mineral density of dairy products given for 12 months. However, height gain was not different between the dairy group and the control group.

The association between dairy intake and height has also been examined in observational studies. Several but not all studies have shown that milk avoiders due to, e.g., milk allergy or lactose intolerance are shorter than children drinking milk [20]. Pastoralists in Africa and Asia are normally taller than agriculturalists. An explanation could be the higher intake of milk and milk products [5]. In a newly published cohort study with young girls aged 9–14 years at baseline from throughout the US, it was examined how dairy consumption influences growth in height. The girls were followed up to year 2003. When analyzing the effect of different types of macronutrients such as non-dairy animal protein, vegetable protein, dairy fat, non-dairy animal fat and vegetable fat, the growth in height was strongest associated with dairy protein [21]. However, the effect of milk on growth may not be the same in all age groups [22]. In a study using data from NHANES 1999–2002, milk consumption was a predictor of height in age group 12–18 years but not in age group 5–11 years [22]. It was not clear why there was no effect in the 5–11 years.

A recent review from our group summarizing observational and intervention studies concluded that cow’s milk most likely has a positive influence on growth in children [5]. The strongest evidence comes from observational studies and intervention studies in low-income countries, but there are also observational studies from high-income countries showing an association between milk intake and growth. Milk seems thus to have a specific stimulating effect on linear growth, not only in developing countries with high rates of malnutrition, but also in industrialized countries.

**Whey and Casein**

It is not known which components in milk may have the growth-stimulating effects. Possible components are proteins/peptides, minerals (like calcium [23], phosphor and magnesium), vitamins or combinations of these (table 1). Bioactive peptides may be present in milk or formed during digestion of the protein. Concerning protein, the quality of various proteins is defined based on the proteins’ ability to support maximal growth. Factors like amino acid profiles, digestibility of the protein and components with biologically active or inhibiting properties may be important [24]. In relation to protein and growth, several indexes for protein quality based on the amino acid composition have been used [24]. The preferred method in human nutrition is Protein
Digestibility-Corrected Amino Acid Score [7, 24]. This index reflects the first limiting essential amino acid in relation to a reference pattern of essential amino acids. According to most of these different indices, whey has a slightly higher quality than casein. But to our knowledge it is not known to what extent this in practice influences the growth-stimulating effects of whey and casein. In a study comparing short- and long-term effects of feeding hydrolyzed protein formulas, there was no difference in linear growth up to age 6 years between children fed with whey or casein-based formulas for the first 16 weeks of life [25]. Regarding the functions of whey or casein in children, attention has been paid to the effect on gastric emptying. In clinical nutrition, faster gastric emptying when giving high amounts of whey may reduce emesis [26]. It is not clear whether the intake of so called fast (whey) or slow (casein) proteins have a different influence on linear growth. The potential differences in stimulation of IGF-I are discussed below in the section on mechanism.

**Body Composition**

Studies have suggested that whey protein has the potential to increase muscle mass [24], which may be beneficial for a healthy body composition in children. One of the hypotheses is that whey contains amino acids with a pattern very similar to muscle proteins and especially a high amount of branched-chain amino acids, which may promote protein synthesis in the muscle. Whey seems to stimulate insulin and thereby protein synthesis. Furthermore, whey has high content of arginine and lysine, which both stimulate the anabolic hormone growth hormone [24]. It is, however, not clear whether whey, casein or whole milk have the strongest effect on body composition. Most studies are from sports medicine, and have only shown an effect if the protein is taken immediately in relation to endurance training [27]. A study comparing the net leucine balance over 7 h after intake of fast (whey) or slow (casein) protein found a higher leucine balance after casein, though whey intake resulted in a much higher but shorter increase in plasma amino acids [28]. In general, the fast protein whey results in a higher whole body protein synthesis than casein, while casein reduces the proteolysis, and the total result is a higher protein retention with casein compared to whey [27]. To our knowledge, no studies have compared the effects of whey and casein on body composition in children. The constituents in whey from cow’s milk also have a number of other potential beneficial functions, e.g. immune stimulation [24] which in some cases could be positive for growth.

**Mechanism**

The mechanism behind a possible growth-stimulating effect of milk is likely to be through a stimulation of IGF-I synthesis (fig. 2) [29, 30]. IGF-I has
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A central role in regulation of growth, and the secretion can be influenced by protein, energy, and certain micronutrients. The effects on insulin and IGF-I were investigated in an intervention study, where 8-year-old boys were randomized to a high intake of protein either in form of meat or skimmed milk for 1 week. It was shown that milk in contrast to meat stimulate IGF-I [29] and insulin secretion [31]. For children in this age group, stimulation of insulin secretion may be positive in relation to growth as insulin is an anabolic hormone inhibiting proteolysis. It has been shown that type 1 diabetic children aged 14 (±3) years increase their growth considerably when changed from conventional to more intensive insulin therapy [32]. To further examine which fractions in milk are responsible for stimulating these growth factors, 8-year-old boys were randomized to a diet with milk-based drinks containing either whey or casein and with a high or low content of minerals (P and Ca) for 1 week. The casein fraction resulted in a 15% increase in the IGF-I concentration, but there was no effect on fasting insulin, whereas the group receiving whey had 21% increase in fasting insulin concentration but no change in IGF-I concentration. There were no interactions with or independent effects of the milk minerals [33]. It seems therefore that the natural combination of whey and casein found in milk may be better to stimulate both IGF-I and insulin. The insulinotropic effect of whey has also been found in a test meal study with healthy young adults, where the postprandial insulin response was increased after a whey meal [34]. Likewise, supplementation of meals with a high glycemic index with whey resulted in higher postprandial insulin secretion than without whey in subjects with diet-treated type 2 diabetes in the age range 27–69 years [35].

**Fig. 2.** Possible associations between ingestion of milk, growth and health. Modified from Hoppe et al. [5].
In conclusion, there is strong evidence that milk stimulates linear growth. The mechanism is not yet clear, and more intervention studies are needed to understand which components in milk are responsible for the growth stimulation. The effects of milk on linear growth and adult height may have both positive and negative long-term effects, as discussed in other chapters of this book.

References

Milk and Growth in Children: Effects of Whey and Casein


Discussion

**Dr. De Beer:** You gave a very elegant presentation on the use of milk and its effect on height. I would like to hear your thought about milk consumption and weight because there are a couple of publications about milk consumption and overweight, especially in the preadolescent child. Perhaps you have some comments on that?

**Dr. Mølgaard:** I think there are some studies saying that a higher milk intake is related to a better body composition and thereby a lower risk of obesity, but still it’s a discussion at what time you consume it. There are studies that relate early protein intake and late increase in obesity to IGF-I stimulation. Still, I am not sure the evidence is so strong for this; this was discussed earlier, and there is also an intervention study from Koletzko’s group in Munich where they compared different levels of protein related to IGF-I but also related to body composition. In this study, they found a higher BMI after 2 years, but maybe we cannot translate BMI to obesity [1].

**Dr. Anderson:** As was presented on Friday, whey is not as insulinotropic as perhaps came out in your presentation. When you compared meat with milk, you had the milk in that study at 1.5 l, so that’s about 55 g of protein spread out over the day. Was meat intake spread out in the same way as milk intake?

**Dr. Mølgaard:** I agree that this is not only a question about protein, it’s also a question about other things in milk, for example calcium, but we decided that the protein content should be the same in the two. It was an intervention where we asked the
participants to drink 1.5 l of milk or to eat 250 g of meat, and the other part of the diet was free [2]. So, in this way it's not a strong control study, it's very difficult to make this in small children, I think.

Dr. Anderson: My point is that they would probably drink the milk with the meals over the day and maybe just eat the meat in the evening. That would make a huge difference in insulin, depending on when the samples were taken.

Dr. Molgaard: The samples were taken in the morning.

Dr. Giavi: Most of the hypoallergenic formulas are based on the whey protein hydrolysate. Why do we choose to exclude the casein fraction in most of the hydrolysate formulas, especially in the extensively hydrolyzed formulas?

Dr. Molgaard: I don’t know that, there could be technical reasons.

Dr. Haschke: The reason for choosing either whey or casein has to do with cow’s milk protein allergy. Allergic reactions against the casein and the whey fraction have been described. Therefore, some producers of high-degree hydrolysates have chosen to have either hydrolyzed casein or whey as the protein source.

Dr. Melnik: I think it’s very important for us to see that the insulinotropic effect of milk resides in the whey fraction. Have you gone a step further and looked into fractionation of whey to discriminate which proteins or which fractions within the whey fraction are most insulinotropic?

Dr. Molgaard: At the moment, we have no studies on this, but as I told you earlier we have another study with obese 12- to 15-year-old boys and girls where we compare the same amount of whey, casein, skimmed milk or water in a 3-month intervention study. We plan to include 200 children, and in this study we will look at the metabolic profile of lipids, insulin, etc.

Dr. Martin: The gene-environment interaction on the slide you showed is very interesting because if something is known about the function of those variants, that knowledge could provide insights into the biology of IGFs or IGF receptors. Can you provide insights into why there was an interaction?

Dr. Molgaard: I don’t know why there was an interaction, but I think IGF-II is important for early growth and also for fetal growth [3].

Dr. Martin: If that IGF-2 variant is associated with IGF-II, then that doesn’t explain the postnatal growth effects. It could be that there are other functional effects of that gene or that it has something to do with the receptor.

Dr. Molgaard: But also maybe it could be interesting to look at IGF-I receptors.

Dr. Martin: Yes.

Dr. Prentice: It is fascinating how the human body as it is growing manages to synchronize the growth of the lean tissue and the growth of the skeleton. Many years ago, Prof. MacIntyre in London postulated that amylin amide was cosecreted with insulin and was one of the factors that promoted the deposition of calcium into bone after a meal, such that after a meal, insulin would rise because of the energy and protein, and amylin amide would rise because of the bone-forming minerals [4]. I haven’t been able to find any information on that more recently, but I wondered if the high calcium content of whey may be part of the reason why you are seeing increases in insulin which may in fact be a proxy for other secretions that are happening at the same time?

Dr. Molgaard: But the calcium content of whey, is not so high, it’s much higher in casein.

Dr. Prentice: But it would be soluble casein?

Dr. Molgaard: I think it would be interesting to look more into the interaction between protein and minerals, especially calcium and phosphate.

Dr. Sankaranarayanan: Are there any comparative studies on cow’s milk and buffalo milk with reference to IGF and insulin response?

Dr. Molgaard: I have not seen these studies.
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Dr. Sankaranarayanan: Why shouldn’t we introduce a treatment with buffalo milk? It is supposed to be better than goat milk.

Dr. Mølgaard: It could be important to also make studies with milk from other species.

Dr. Klassen: Just a small comment on that, I do not know all published data, but I do know from internal analyses that there are some differences in the amino acid profile between buffalo and cow’s milk, but I am not sure that it would explain any differences in the effects compared to cow’s milk. As far as I know, it is a practice in India to mix cow’s milk with buffalo milk, so I wonder if consequently one would see any marked differences compared to cow’s milk.

Dr. Boukari: You said that milk with whey or casein showed an effect in children aged 12–18 years but not in children aged 5–11 years. Do you have an explanation for this?

Dr. Mølgaard: No, it was the study from the US, and I don’t think they themselves have any explanation. Still, it was interesting that the main determinant of height in this group was energy intake [5].

Dr. Boukari: Do you think we can extrapolate the conclusions of 1-week intervention studies, such as the one you did, to real life?

Dr. Mølgaard: I am not sure because to be honest it would be very nice if we had enough money and enough children who agree to drink a certain amount of milk for a longer period. In our new study now with 12-year-olds, we give these different milk products for 3 months and hope to see more lasting effects. Still, 3 months is a very short period in a whole life. I agree with you, I don’t think we can say that if you consume whey for a long period you will have very high insulin.

Dr. Michaelsen: The effect we saw on insulin with milk compared to meat might be a semi-acute effect because we don’t see it in cross-sectional studies when comparing milk and meat intake with fasting insulin. We did not want to give high amounts of milk for a long period for ethical reasons, but wanted to study the mechanisms short-term.

Dr. De Beer: My question relates to the previous speaker’s comment. You saw insulin’s trophic effect after consumption of high volumes of milk (1.5 l a day). In real life, we recommend maybe 2 glasses or 500 ml of milk a day to children. Do you expect to see the same trophic effect of insulin or some of the positive effects that you saw with the high consumption over a short period?

Dr. Mølgaard: I think that it could be an effect of a very high intake. I would not expect to see it with half a liter a day for a long period because I think there would be some mechanism to regulate it.

Dr. Sankaranarayanan: In the study you showed, you gave 1.5 l of milk per day or 250 g of meat to 8-year-old children. It’s a high intake of milk that never happens in real life.

Dr. Mølgaard: We wanted to test the biology behind this, and therefore we gave them a high dose of milk to see whether we could stimulate it. I know that it’s not a realistic intake in a normal everyday situation. It was a research study.

Dr. Sankaranarayanan: Are there any studies that state what milk consumption is optimal?

Dr. Mølgaard: I don’t think that there is a single answer for this. In our society, we recommend half a liter a day because we think that it’s a good source of calcium and also for protein, but we are also afraid that the children increase their intake to a much higher level because as we heard from Iceland the iron intake is too low and you can have anemia. We would not recommend a very high intake of milk, but in our society we recommend around half a liter a day. We don’t have strong scientific evidence that it should be exactly half a liter.
Dr. Melnik: I think, to answer this question, we have to differentiate between protein intake from dairy products with and without insulinotropic effects, and we also have to consider the frequent intake of yogurt as well. If you look at normal daily uptake of dairy products of young adults or adolescents, they have corn flakes in the morning with 300 ml of milk and may have a cacao drink with 100 or 200 ml, and they may consume 1 or 2 yogurts a day. So people of western civilization have an accumulated intake of insulinotropic milk proteins during the day. We observe a strong acne-promoting effect of insulinotropic milk products and high glycemic carbohydrates in adolescents, which is linked to the pathogenesis of acne [6–8].

Dr. Mølgaard: Our recommendation is half a liter, including all the other things.

Dr. Martin: I just wanted to go back to the comments about milk intake raising insulin levels and the effects of that in the long-term. Elwood et al. [9] published a systematic review of prospective studies looking at milk intake in relation to a number of outcomes. Milk intake in adulthood was inversely associated with metabolic syndrome in which insulin resistance is a component. Thus, the observational evidence suggests there is an inverse relationship in the long-term, not a positive relationship as your data would suggest.

References

Milk and Linear Growth: Programming of the IGF-I Axis and Implication for Health in Adulthood

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Abstract

There is increasing awareness that childhood circumstances influence disease risk in adulthood. As well as being strongly influenced by genes/genetic factors, stature acts as a marker for early-life exposures, such as diet, and is associated with risk of several chronic diseases in adulthood. Stature is also a marker for levels of insulin-like growth factor (IGF)-I in childhood. Levels of IGF-I are nutritionally regulated and are therefore modifiable. Milk intake in childhood and in adulthood is positively associated with higher levels of circulating IGF-I and, in children, higher circulating IGF-I promotes linear growth. Studies conducted by our team and others, however, indicate that the effect of milk is complicated because consumption in childhood appears to have long-term, programming effects which are opposite to the immediate effects of consuming milk. Specifically, studies suggest that the long-term effect of higher levels of milk intake in early childhood is opposite to the expected short-term effect, because milk intake in early-life is inversely associated with IGF-I levels throughout adult life. We hypothesize that this long-term programming effect is via a resetting of pituitary control in response to raised levels of IGF-I in childhood. Such a programming effect of milk intake in early life could potentially have implications for cancer and ischemic heart disease risk many years later.

Introduction

The opportunities to directly examine the relationship between early nutrition and diseases in adulthood are limited because few cohort studies have information from birth until old age. To date, therefore, most epidemiological
studies investigating early life origins of chronic diseases have used indirect markers of childhood nutritional exposures (for example, birthweight, height and leg length) and their relation with outcomes such as cancer, diabetes and ischemic heart disease [1]. Taller individuals generally have an increased risk of developing cancer [2] and a reduced risk of insulin resistance and ischemic heart disease [3]. There is some evidence that these associations may be specific to the leg length component of stature [2, 4]. Peak growth in leg length is prepubertal while peak growth in trunk is postpubertal [1]. This is demonstrated by changes in the trunk length:height ratio during growth. At birth, the ratio of trunk length to total height is approx 0.66, but by puberty it has declined to 0.52 [1]. From puberty, linear growth occurs equally in the trunk and legs. Stronger associations of adult chronic diseases with leg length have led to speculation that exposures which influence prepubertal long bone growth (e.g. early diet) may be more important in determining adult chronic disease risk [1, 5].

It has been hypothesized that influences of diet in childhood on the insulin-like growth factor (IGF) system may contribute to stature-chronic disease associations [6–9]. IGF-I in childhood is raised in response to some aspects of diet, particularly cow’s milk and dairy product intake [10], and raised childhood IGF-I in turn leads to greater subsequent growth in stature [11]. There is both experimental and observational evidence that raised IGF-I levels in early life subsequently program long-term modifications in the regulation of the IGF system. The most important evidence supporting the long-term programming of the IGF system comes from a randomized controlled trial of milk supplements provided to pregnant women and their offspring up to 5 years of age [9]. In a long-term follow-up of the offspring of the mothers originally recruited to the trial, circulating levels of IGF-I were measured at age 25 years. Those offspring who received milk supplements up to age 5 years had markedly lower serum IGF-I levels when measured 20 years later. The findings are opposite to the likely immediate responses to milk supplementation, which would have been to increase hepatic production of IGF-I [10]. We have hypothesized that a relatively high IGF-I level at the time of supplementation could cause a resetting of the pituitary due to greater feedback on the growth hormone (GH) axis from the prevailing circulating IGF-I during a sensitive period of life. This long-term resetting of the pituitary to raise the threshold for stimulating GH release would result in relatively lower hepatic IGF-I production and serum levels in later life. The reverse effect would occur in response to lower nutritional intake in early life (for example, in response to breastfeeding), which would be expected to lower IGF-I levels in early life but may program, via pituitary resetting, higher observed levels in later life [8].

In this chapter, we examine evidence supporting the hypothesis for nutritional programming of IGF-I levels in response to dietary exposures in childhood and the potential long-term implications of this. Our review draws on a number of studies that the authors have been appreciably involved in, includ-
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ing the Boyd Orr cohort [12], the Avon Longitudinal Study of Parents and Children (ALSPAC) [10], Barry-Caerphilly Growth Cohort study [9], Prostate testing for cancer and Treatment (ProtecT) study [13] and the Promotion of Breastfeeding Intervention trial (PROBIT) [14].

Childhood Stature and Health in Adulthood

Tall adults have an increased risk of cancer [2, 13] and a lower risk of cardiovascular disease [15, 16]. Stature in childhood and adulthood is dependent on genetic as well as environmental factors. However, childhood stature may better reflect prepubertal growth influencing exposures than adult height, which reflects a combination of childhood growth and also age and duration of pubertal maturation. Recent research is beginning to identify specific periods of early growth that are important to the risk of cancer [17, 18] and cardiovascular disease [4, 19]. For cancer, the most consistent associations with childhood growth have been found in relation to breast cancer. For example, faster pubertal growth (between 8–14 years) was positively associated with incident breast cancer risk independent of final height and explained the positive association of earlier age at menarche with breast cancer [17]. This finding has support from an ecological study in Japan where rapid increases in the heights of girls year on year between 1946–1966 were paralleled by large increases in breast cancer incidence among women after a 30 year time lag (i.e. 1976–1996) [20]. Growth-influencing exposures that changed rapidly after World War II include a 20-fold increase in milk and dairy product intake in Japan [21]. Associations with childhood stature have also been found for other cancer sites, in particular colorectal cancer, prostate cancer, endometrial cancer and hematopoietic cancers [22].

In those studies that investigated associations of the components of stature and cancer, leg length (a suggested marker of exposures influencing prepubertal growth, such as early socioeconomic circumstances, infection load and nutrition [1, 5]) was the component of height most often associated with increased adult chronic disease risk. For example, in the Boyd Orr cohort, a long-term follow-up of children surveyed between 1937 and 1939 and followed up in adulthood, we found that childhood stature was inversely associated with premature cardiovascular mortality (age <65 years) and self-reported ischemic heart disease, with leg length being the component with the strongest associations [4, 23]. Associations were explained by having been breastfed and childhood socioeconomic circumstances, confirming that leg length is a proxy for these prepubertal exposures.

A previous report from the Boyd Orr cohort (based on follow-up to 1995) showed a positive association between childhood leg length and mortality from cancers unrelated to smoking [24], most obvious for fatal sex hormone-dependent cancers (breast, uterus, ovary, prostate, other genital organs): the
risk of death increased by 126% for every unit increase in z score for leg length (approximately 3–4 mm). There was no evidence that trunk length was associated with cancer. A recent extended follow-up to 2004 suggested that associations were weaker than originally observed [18], although odds ratios (ORs) remained broadly consistent with a slight increase in risk with increasing childhood stature. Contrary to previously suggested stronger links with leg length [24], however, no single anthropometry measure was of particular importance in this longer-term follow-up, underlining the challenges of interpreting epidemiological data. The strongest associations were seen for breast cancer (OR per standard deviation increase in foot length: 1.16 (95% CI: 0.90–1.51); shoulder breadth: 1.16 (0.91–1.49); trunk: 1.26 (1.00–1.60)) and prostate cancer (OR for foot length: 1.22 (0.86–1.75)). Foot length is one of the first components to reach peak growth, while shoulder breadth is one of the last [25].

**Breast Milk, Cows Milk and Stature**

Analysis of the Boyd Orr cohort has shown that stature was a generalized marker for many aspects of diet, housing and socioeconomic position in the children [5, 26]. The individual components of stature most strongly associated with childhood environment were leg and foot length. More specifically, leg length (but not trunk length) in both childhood and adulthood has been associated with breastfeeding in infancy in analyses of the Boyd Orr cohort [27] and in the 43-year follow-up of the 1946 UK national birth cohort [28]. The specific association between breastfeeding and leg length, which persists after controlling for socioeconomic circumstances [27, 28], suggests that breastfeeding may be a biologically relevant early-life exposure, rather than a marker for a broader social class effect, underlining the observed associations of leg length with adult diseases. Positive, albeit weaker, associations of breastfeeding with childhood and early adult stature have also been observed in the 1958 British birth cohort (n = 10,953) [29], Brazil (n = 2,250) [30] and in the 6.5-year follow-up of 13,889 children cluster-randomized to a breastfeeding promotion intervention (43% exclusively breastfed at 3 months) vs. usual breastfeeding practices (6% exclusively breastfed at 3 months; PROBIT) [31]. The evidence from the PROBIT trial is particularly noteworthy since it provides experimental evidence based on an intention to treat analysis and so is unlikely to be explained by confounding or selection bias. In the Boyd Orr cohort, a mother’s leg length but not trunk length as a child was associated with her offspring’s birthweight, suggesting the intriguing possibility that early nutrition and growth may have transgenerational effects on later health [32]; however, the finding could also reflect that genetic influences on leg length are the same as those that determine birthweight.

There is a large literature on the effect of cow’s milk on stature [33], with intervention studies going as far back as Boyd Orr’s trial of food supplements
Programming of the IGF-I Axis and Implication for Health in Adulthood

Table 1. Association of family milk intake and height of children in the Boyd Orr cohort, 1937–1939 (mean age 7.5 years)

<table>
<thead>
<tr>
<th>Quartiles of milk intake</th>
<th>Quartiles of milk intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (low)</td>
<td>2</td>
</tr>
<tr>
<td>Median milk intake, g/day</td>
<td>89</td>
</tr>
<tr>
<td>Unadjusted OR</td>
<td>2.9 (2.3–3.7)</td>
</tr>
<tr>
<td>Adjusted1 OR</td>
<td>2.1 (1.6–2.6)</td>
</tr>
</tbody>
</table>

ORs are for the risk of having a below-average height for age and sex.
1 Adjusted for childhood socioeconomic position and food expenditure.

given to Scottish school children, published in 1928 [34] and 1929 [35]. We also observed a strong effect of family milk consumption on childhood stature in Boyd Orr’s later Carnegie Survey that was conducted in 16 survey centers across England and Scotland (1937–1939), and which formed the basis for the reconstruction of the Boyd Orr cohort (see table 1, with acknowledgments to Jolieke van der Pols). However, in a contemporary setting (Bristol, UK, children born between 1991–1992), we observed only a weak positive association of milk consumption in childhood with leg length amongst boys (not girls) at ages 7–8 years [10].

Milk and Health in Adulthood

Some dairy products such as whole milk, butter and cheese have a high content of saturated fatty acids and cholesterol, and consumption of these in adulthood has been thought to contribute to cardiovascular disease risk, though evidence for this is not consistent [36]. Intake of dairy products may affect a mixture of pathways associated with carcinogenesis (summarized by van der Pols [37], and including increased circulating IGF-I, modification of vitamin D status, increased intake of calcium, conjugated linolenic acid and exposure to contaminants such as polychlorinated biphenyls). Overviews of the evidence from observational studies, however, suggest a reduction in risk of ischemic heart disease, stroke, diabetes and colorectal cancer (but possibly an increased risk of prostate cancer) associated with relatively high consumption of milk [38, 39].

To date, most studies of the associations of dairy consumption with cancer and cardiovascular disease risk have been based on estimates of adult dairy intake. In children, the blood lipid profile at different stages of the life course is associated with the type of milk consumed. In comprehensive systematic reviews conducted by Owen et al. [40, 41], mean blood total cholesterol con-
centrations in breastfed infants, compared with those who were formula fed, were higher in infancy, similar in childhood and lower in adult life (the lower levels in adulthood being a possible example of nutritional programming). Children who consume low-fat milk have lower serum saturated fat fractions and higher polyunsaturated fat concentrations compared to those who drink whole milk [42]. Furthermore, there is evidence that high calcium intake, of which dairy products are an important dietary source, is associated with lower diastolic and systolic blood pressure in children [43].

However, there are few cohort studies investigating whether consumption of dairy products in childhood has long-lasting effects on cardiovascular disease or cancer risk in adulthood. Our 65-year follow-up studies based on the Boyd Orr cohort used per capita household intake estimates for dairy products and calcium as a proxy for individual intake [37, 44]. We found no convincing evidence of any increased risk of coronary heart disease or stroke in people who as children had the highest milk or dairy consumption [44]. While chance or residual confounding cannot be ruled out, childhood calcium intake was inversely associated with stroke mortality (multivariable adjusted hazard ratio (HR) for highest vs. lowest calcium group: 0.41; 95% CI 0.16–1.05; p for trend = 0.04), but not coronary heart disease mortality. All-cause mortality was lowest in those with the highest family dairy (HR = 0.77; 95% CI 0.61–0.98; p for trend = 0.04) and calcium intake (HR = 0.77, 95% CI 0.60–0.98; p for trend = 0.05). This latter finding could reflect socioeconomic confounding, and we concluded that: ‘replication in other study populations is needed to determine whether residual confounding explains part of these findings’ [44].

We also investigated associations of dairy product and calcium intake in childhood with cancer risk [37]. High childhood total dairy intake was associated with an almost tripling in the odds of colorectal cancer (multivariable OR: 2.90, 95% CI: 1.26–6.65) compared to low intake, independent of meat, fruit and vegetable intake and socioeconomic indicators. Milk intake showed a similar association with colorectal cancer risk. High milk intake was weakly inversely associated with prostate cancer risk (p for trend = 0.11). Childhood dairy intake was not associated with breast and stomach cancer risk, and a positive association with lung cancer risk was confounded by smoking behavior during adulthood. These findings appear to be in sharp contrast to the steadily increasing pool of evidence for a protective effect of dairy consumption on colorectal cancer risk in adult populations [39]. Our finding that high childhood intake of milk is associated with reduced prostate cancer risk is also contrary to findings in studies of adult intake [39], although the confidence intervals in our study indicate that this association was imprecisely estimated. We did not show any strong evidence of associations between childhood dairy consumption and breast cancer risk, although our point estimate (OR = 0.83, CI 0.41–1.69, in the highest vs. lowest quartile of childhood milk intake) is in line with the case-control findings by Michels et al. [45] (according to which milk intake in childhood was associated with reduced risk of breast cancer).
Insulin-Like Growth Factors, Nutrition and Adult Chronic Disease Risk

IGFs are multifunctional peptides that regulate cell proliferation, differentiation and apoptosis and play a fundamental role in somatic growth. Six circulating IGF-binding proteins (IGFBPs 1–6) modulate the availability of IGF-I to tissue. Over 90% of circulating IGF-I is bound to either a binary or ternary binding complex involving IGFBP-3. Circulating IGF-I is positively associated with growth in height in childhood [11, 46], suggesting that height may be acting as an anthropometric marker for levels of IGF-I [2]. Since childhood stature is a marker of the activity of IGF-I, modulation of the IGF system could provide a mechanism explaining associations of height with adult disease risk [2]. While adult height is not strongly associated with IGF-I in cross-sectional studies [47], stature may be a marker for this growth factor in childhood, and this may be the period during which it acts to increase disease risk in later life.

IGF-I is mainly secreted from the liver in response to GH and insulin, but dietary intake also influences IGF-I levels, with energy- and protein-deficient diets resulting in marked reductions in IGF [48]. An analysis based on the Boyd Orr cohort [49] found that a lower household calorie intake in childhood was associated with lower cancer risk in later life (relative hazard for all cancer mortality and cancers not related to smoking = 1.15 and 1.20 per MJ, respectively, in fully adjusted models; p = 0.001). In animals, calorie restriction reduces risk of cancer at least partly by reducing circulating concentrations of IGF-I [50]. It is therefore plausible that height-cancer associations may reflect an association between early diet and cancer risk that is mediated via the IGF system.

Studies have related specific aspects of diet to IGF-I levels in well-nourished humans [51–53]. For example, in cross-sectional analyses based on 1,037 healthy women in the Nurses Health Study, total energy and protein intake were positively associated with IGF-I levels when adjusted for covariates [51]. The association with protein intake was largely attributable to higher IGF-I levels among women who consumed higher amounts of milk. Of all the dietary determinants linked with circulating IGF-I in cross-sectional studies of adults and children, it is increasing milk intake that appears to be most consistently associated with higher levels [10, 33, 53]. In a cross-sectional analysis of dietary determinants of serum IGF-I in 7- to 8-year-old children (n = 538, diet assessed using a 3-day unweighed food record), cows milk and dairy intake were the components of diet most strongly and positively associated with IGF-I (in energy-adjusted models) [10]. Cow's milk was a major source of the child's protein (40% of total animal protein intake), and controlling for total protein or animal protein intake attenuated the association of IGF-I with cow's milk and dairy intake. Controlling for calcium intake, however, had no impact on the observed association. Evidence from experi-
mental studies, summarized by Hoppe et al. [33], suggests that the association of cow’s milk with IGF-I levels is likely to be causal.

Breastfed infants may be at reduced risk of early overnutrition and accelerated growth [54] compared with those who are formula fed, since formula-fed infants consume greater volumes of milk than breastfed infants and appear to have higher protein and energy intakes in infancy [55–58]. The lower energy and protein intakes of breastfed infants compared with those who are formula fed, may lower hepatic IGF-I production at the time of breastfeeding, which would be compatible with the slower growth rate of breastfed compared with formula-fed infants [54]. In a study based on 33 preterm infants (gestational age: 28–37 weeks) who were appropriate size for their gestational age, levels of IGF-I and IGFBP-3 were measured at 1 and 3 weeks after birth [59]. There were strong positive correlations of neonatal protein (r = 0.40; p < 0.01) and energy intakes (r = 0.45; p < 0.001) with IGF-I, suggesting that very early nutritional intake influences levels of these growth factors.

In a study of 942 appropriate weight for gestational age and term infants, IGF-I levels measured at age 3 months were lower in breastfed than formula-fed infants, independent of weight at 3 months [60]. Increasing exclusivity of breastfeeding was associated with lower levels of IGF-I in a dose-response pattern. Likewise, others have found that formula-fed infants have higher insulin levels compared with breastfed infants [61]. Thus, both changes in IGF-I and insulin could in part explain the higher initial postnatal growth rate in formula-fed vs. breastfed infants.

The associations of both childhood stature and milk intake with adult chronic disease risk have led to the speculation that a possible mechanism for these patterns is via the IGF system. Raised circulating IGF-I is positively associated with the development of premenopausal breast cancer, prostate cancer (particularly advanced prostate cancer [62], although confounding by the presence of benign prostatic hyperplasia has not been ruled out [63]), and colorectal cancer, the same sites for which height-cancer associations have been most frequently shown [6, 64]. In line with the inverse childhood height-cardiovascular disease associations, there is some evidence that raised circulating IGF-I is inversely associated with cardiovascular disease risk, perhaps because IGF-I enhances plaque stability [65]. Recent unpublished observations by our group suggest that men who gained a large amount of weight between childhood and adulthood had markedly lower circulating levels of IGFBP-2 in adulthood (irrespective of final weight) [Rowlands et al., submitted]. Lower IGFBP-2 levels indicate higher degrees of insulin resistance [66], which is in turn positively linked with cancer [67–69] and cardiovascular disease risk. Hyperinsulinemia may also increase IGF-I bioavailability by suppressing the hepatic production of IGFBP-1. Thus, IGFBP-2 may be a component of the IGF system that contributes to associations of weight increases over the life course with increased risk of cancer and cardiovascular disease.
Nutritional Programming of IGF-I

This section reviews the evidence linking early nutritional status with IGF-I levels in later life. Evidence from five long-term cohort studies to date supports the idea that the acute effects of greater nutritional intake, such as higher cow’s milk intake, which leads to higher levels of IGF-I in the short-term are opposite to the long-term effects of greater childhood nutrition, which seems to be associated with a lowering of IGF-I levels many years later. Similarly, the acute effects of lower nutritional intake (resulting in a lowering of circulating IGF-I) appear to be associated with higher IGF-I levels many years later. First, in an analysis of the ALSPAC cohort, we observed that having been breastfed in infancy was associated with increased IGF-I levels in children at age 7–8 years [8]. There was some evidence of a dose-response relationship: in age- and sex-adjusted analyses, for each increase in category of breastfeeding exclusivity (never, partial and exclusive), there was on average a 7.1 ng/ml increase in levels of IGF-I, attenuated to 3.6 ng/ml after controlling for socioeconomic and dietary variables. This positive association is in line with the positive association observed between breastfeeding and childhood stature seen in several settings [27–31], but contrasts with the data (reviewed above) indicating that circulating IGF-I levels are lower while babies are being breastfed [59, 60], possibly due to the lower protein and energy content of breast milk compared with formula milk.

Second, in the 65-year follow-up of the Boyd Orr cohort, higher milk intake in childhood (which will result in an acute increase IGF-I levels) was inversely associated with IGF-I levels in old age [7]. Third, data from 40 participants in the Copenhagen Cohort Study showed an inverse association between IGF-I levels at 9 months and IGF-I levels at 17 years of age (r = –0.39) [70]. A 1 ng/ml higher IGF-I concentration at 9 months was associated with a 0.95 ng/ml lower IGF-I concentration at 17 years. In line with this finding, infants who were fully breastfed (vs. those never breastfed) had lower levels of IGF-I in infancy (93 vs. 130 ng/ml, respectively) but higher levels in adolescence (328 vs. 292 ng/ml, respectively). These data are consistent with the above studies in suggesting that those with higher IGF in infancy will have lower IGF levels in adulthood (and vice versa).

Fourth, a natural experiment, based on 87 postmenopausal women living in Utrecht [71], provides evidence for an effect of early nutrition on long-term levels of IGF. In this study, the degree of childhood exposure to the 1944–1945 Dutch famine (when daily rations dropped from 1,500 kcal in September 1944 to below 700 kcal in January 1945 until liberation in May 1945) was associated in a dose-response manner with increased plasma levels of IGF-I and IGFBP3 at age 50–69. These data suggest that a relatively short period of caloric restriction was associated with increased long-term levels of IGF-I. No differences were found for c-peptide levels, a marker of insulin resistance.
The results are opposite to immediate responses seen under starvation, which would have caused a lowering of IGF-I.

Fifth, the Barry-Caerphilly trial, conducted in the 1970s, was a randomized controlled trial of milk supplements provided to pregnant women and their offspring up to 5 years of age [9]. The women in the supplemented group were provided with tokens that entitled them to free milk delivered by their milkman. In the long-term follow-up of the offspring of the mothers originally recruited to the trial (the Barry-Caerphilly Growth cohort), circulating levels of IGF-I and IGFBP-3 were measured in 663 subjects aged 25 years on average. Those individuals whose mothers were randomized during pregnancy to milk supplementation and who received milk supplements up to age 5 years had markedly lower serum IGF-I levels when measured 20 years later. The findings are opposite to the likely immediate responses to milk supplementation, which would have been to increase IGF-I [10]. These are important results because they provide experimental data, analyzed by intention to treat, on the role of early life programming of the IGF system, occurring either in the intrauterine or postnatal period. Such experimental data provide important evidence that long-term programming of IGF-I levels could be causally linked to nutritional intake earlier in life.

Altogether, these findings (summarized in table 2) from ALSPAC, Boyd Orr, Copenhagen, the Dutch famine and the Barry-Caerphilly Growth cohort, are compatible with increased nutritional intake in infancy or childhood, primarily protein intake, causing a direct increase in hepatic IGF-I production which then feeds back to suppress pituitary GH output with a long-term resetting of the pituitary resulting in lower IGF-I levels in the long-term [8].

**Implications**

Hepatic production of IGF-I is controlled not only by pituitary GH but also by insulin and by many nutrients. The data provided here suggest that there

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**Table 2.** Interrelationships between diet and IGF at various points in life course

<table>
<thead>
<tr>
<th>High milk/protein intake</th>
<th>IGF-I levels</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>infancy</td>
<td>childhood</td>
<td>adulthood</td>
</tr>
<tr>
<td>In infancy (bottle feeding)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>In childhood</td>
<td></td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>In adulthood</td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Positive association; – = inverse association.
may be critical or sensitive periods in childhood when nutritional exposures could result in long-term resetting of the pituitary, programming adult IGF-I levels.

There are several implications of these findings. A relatively low IGF-I level at the time of breastfeeding would be consistent with the slower infant growth rate at that time, and a consequent resetting of the pituitary, due to less feedback, could then result in a relatively high IGF-I level subsequently in later life. This would be compatible with our findings of greater height in later childhood and adulthood associated with breastfeeding [27]. Such ‘programming’ of the IGF-I system in later life provides a possible biological mechanism underlying the finding that exclusive breastfeeding was associated with greater childhood IQ in the experimental PROBIT trial [72], since IGF-I in childhood is positively associated with IQ [73].

The findings provide hints at possible sensitive periods in life when environmental exposures influence the future risk of various chronic diseases and suggest that the timing of these sensitive periods may differ for different outcomes. As discussed by van der Pols et al. [37], our observation that high intake of dairy foods in childhood is associated with an increased risk of colorectal cancer may indicate that it is the effect of childhood dairy intake on childhood (rather than adult) levels of IGF-I that is the important mediator of future risk, given the positive association between dairy intake and IGF-I concentrations in childhood [10] but inverse association between childhood dairy intake and adult IGF-I levels [7] and the positive link between adult IGF-I concentrations and colorectal cancer risk [64]. Our finding that a lower household calorie intake in childhood was associated with lower overall cancer risk in later life [49] could also reflect the importance of levels of IGF-I in childhood for carcinogenesis.

Our finding that childhood consumption of milk in the highest intake group is associated with reduced prostate cancer risk is contrary to findings in adult intake studies [74], but is in keeping with the possible long-term programming effect of childhood nutrition on adult IGF-I, and with the inverse association between childhood dairy intake and adult IGF-I levels observed in this study population [7]. This does assume, however, that for prostate cancer, it is levels of IGF-I in adulthood that are important.

These data fit in with the growth acceleration hypothesis proposed by Singhal and Lucas [75, 76]. In this case, dietary factors causing accelerated growth in infancy (such as nutrient-enriched formula milks) would be hypothesized to program lower levels of IGF-I in later life, a potential pathway linking rapid early growth with adult insulin resistance and cardiovascular disease.

Further studies, preferably experimental, should now be conducted to confirm or refute the hypothesis that variations in IGF-I explain associations of early-life environmental exposures with health in later life. Currently, robust data supporting the IGF-I programming hypothesis are provided by one randomized controlled trial (BCG cohort [9]) and one natural experiment.
[71]. The long-term follow-up of experiments (such as the PROBIT trial, in which infants were cluster randomized to a breastfeeding health promotion intervention vs. usual practice [14]) would provide further robust evidence on which to base inference about the causal nature of the observed long-term effects of early nutrition on the IGF system.

The complex interrelationships, which seem to vary depending on when exposure occurs during the life course, between dietary intake, IGF-I levels and adult disease risk indicate that there will need to be a careful appraisal of the overall health-balance sheet (benefits vs. adverse effects) of any potential preventative measures based on manipulating childhood dietary intakes that modify the IGF system in either or both the short and long-term.

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References

Programming of the IGF-I Axis and Implication for Health in Adulthood

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Discussion

Dr. Gibson: You raised the issue of epidemiologists being skeptical; biochemists are even more skeptical especially about the work of epidemiologists, which raises the issue to me about causation vs. association. In the early days of the cholesterol theory or hypothesis, there was a great deal made by the fact that the associations between cholesterol levels and coronary heart disease were not necessarily causative but were associative, and we are still debating the causation of cholesterol to this day. Are you really inferring from your data that IGF is somehow causative of all of these events
such as cancer and heart disease? How do we relate that to the fact that as nutritionists we are trying to improve growth all the time and then you are coming along and telling us that, I think if I understand it correctly, that increased growth is associated with IGF levels in some way and that's a cause of greater risk now.

Dr. Martin: You are absolutely right, most of what I have shown relates to associations. When we examine association data, we look for consistency and patterns in the results. We find consistent evidence from several studies [1–5] that circulating IGF-I levels are programmed from early life. We can’t assume that the acute effects of milk intake in childhood on IGF are going to reflect the pattern of IGF levels later in life. This is shown by the Barry-Caerphilly randomized controlled trial, which does allow causal assessment when analyzed by intention to treat [5]. So there is randomized evidence suggesting that the regulation of the IGF system is more complex than would appear from cross-sectional studies. We therefore need more prospective studies with long-term follow-up, such as the Copenhagen Cohort Study [3]. The relationship of IGF-I with cancer risk is all observational, and caution is required in interpreting observed results. For example, the positive relationship of IGF-I with prostate cancer could be explained by detection bias. If IGF-I causes benign prostatic hyperplasia and its symptoms, prompting men to seek a PSA test and be diagnosed with prostate cancer, this generates an artificial association of IGF-I with cancer. You are correct to be skeptical, and I think we should put more effort in obtaining experimental evidence looking at programming of the IGF system. For example, long-term follow-up of the randomized PROBIT trial, involving 17,000 children, is currently investigating, in an intention-to-treat analysis, associations of prolonged, exclusive breastfeeding with IGF-I at age 11.5 years [6]. This will provide unique experimental evidence on whether there is a real programming effect of early nutrition on later IGF-I levels.

Dr. Mølgaard: What about the relationship between one of the main killers, smoking, and the IGF-I levels? In your last paper, you said that you could not control for smoking, and smoking is related to lower milk intake as far as I remember.

Dr. Martin: In one paper, we investigated associations of dairy products and calcium intake in childhood with cancer risk [7]. We found that childhood dairy intake was positively associated with lung cancer risk, but felt this relationship was confounded by smoking behavior during adulthood.

Dr. Mølgaard: But smokers had lower IGF-I levels.

Dr. Martin: We don’t find a very strong relationship of IGF with smoking [8], although the smoking variable was relatively crudely measured. The association of IGF-I with milk intake is the strongest [9].

Dr. Prentice: As a biochemist working in pregnancy, I often wondered about the use of a concentration value in the circulation as a marker of hormone output or indeed of impact. I am assuming that the children who had high IGF-I levels in childhood were bigger as adults? If so, I just wondered, although I am sure this is not an original thought, if one was able to correct for body volume or at least blood volume, rather than relying on concentration, whether that would provide a true measure of the production of IGF-I during the day, and whether that would explain the associations you see?

Dr. Martin: You mean were the children taller as adults?

Dr. Prentice: The children given milk in childhood grew bigger, and I am making the assumption that they are bigger individuals as adults, so could blood concentration be confounded by body size is the question, I think.

Dr. Martin: I guess that’s possible. There is not a strong relationship of IGF-I with BMI and obesity, and controlling for that doesn’t make much of a difference [8].

Dr. Prentice: BMI is not a measure of size; it is a measure of adiposity or shape [10].
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**Dr. Martin:** It is the relationship of circulating levels with tissue levels that ultimately would determine any link of serum IGF-I with disease. Some of the changes in IGF-I associated with disease are actually quite small, although they increase over lifetime. Nevertheless, associations of circulating IGF-I with disease exist, so such levels are a proxy for something. Endocrinologists tell me that circulating levels are a reasonable proxy for what is going on at the tissue level. You need population-based studies to determine whether findings on tissues and in animal experiments actually translate into an impact at the population level.

**Dr. Melnik:** I would like to thank you for this very impressive talk, and I think we should also mention two very important epidemiological studies which presented epidemiological evidence for the association between milk and dairy protein consumption and elevated serum IGF-I levels [11, 12]. Moreover, Jacqueline Major from the Department of Family and Preventive Medicine at the University of California San Diego recently reported that serum IGF-I levels show a correlation with the overall cancer mortality in elderly men [13]. IGF-I is the strongest mitogen we know. It promotes cell proliferation and inhibits apoptosis. IGF-I signaling plays a serious role in cancer promotion [14].

**Dr. Martin:** I agree, the observational evidence is strong, providing similar data to the sort of data that we are providing, and the biochemistry makes perfect sense. As others point out, you do have to be skeptical. Ultimately, only randomized controlled trial evidence could robustly show if there is causation and a population health impact. Such trials may be difficult, but, for example, you could investigate men at high risk of prostate cancer progression and determine experimentally whether reducing milk intake in such men lowers IGF-I levels and ultimately leads to a reduction in risk of progression. I think we can do such trials, particularly in populations where the benefit to risk ratio allows equipoise.

**Dr. Clemens:** I appreciate Dr. Melnik’s comment about the signaling pathways. As we look at the data from yesterday, the comment about the β-lactoglobulins and α-lactalbumins in breast milk and then we look at the casein-dominant components in cow’s milk and obviously in cheese, would you expect to see that any of the peptides derived from these particular proteins may be part of the signaling pathways that may trigger some of these outcomes?

**Dr. Martin:** There was some evidence yesterday relating casein to raised IGF-I, but I don’t know the literature on those kind of peptides.

**Dr. Gibson:** You said there was this association between tallness and IGF. Is it also extended to obesity, that is weight as well?

**Dr. Martin:** The relationship between IGF-I and obesity is complex. We have shown in a very large study with 1,000 men an inverted U-shaped relationship, so that the highest levels of IGF-I are amongst those in the second and third quartiles of BMI [8]. There is a much stronger association of IGF-binding protein 2 (IGFBP-2) with obesity, such that obesity is associated with lower levels of IGFBP-2 [8], which at the cellular level may increase the bioavailability of IGF-I. So, IGFBP-2 is another component that might be involved in signaling mechanisms and progression of disease. The relationship of the IGF system with obesity has interested us as a possible mediator of reported associations of breastfeeding with obesity, although our systematic reviews [15, 16] and the PROBIT [17] did not find convincing evidence that there is a strong inverse association of breastfeeding with obesity.

**Dr. El Barbary:** How do you explain the finding that at the age 3 months the infants who were never breastfed had a higher level of IGF-I in comparison to those who were exclusively breastfed, and at the age of 7 to 8 years this situation was reversed?

**Dr. Martin:** Lower levels of IGF-I in those who were exclusively breastfed may be due to lower protein and energy intake at that time [18]. To explain the later switch
in the direction of associations at the age of 7 years, that is where we speculate that there may be a programming effect such that the lower IGF-I levels in infancy reset pituitary control, so that there are higher levels in later life [19].

Dr. Makrides: Apart from dietary factors, what other factors may program or influence IGF concentrations?

Dr. Martin: IGF-I is a nutritionally regulated peptide, so that is where we have concentrated most of our research efforts in programming. There are a myriad of exposures that could influence and regulate IGF-I, including mitogenic factors such as insulin, estrogen and antiproliferative agents such as vitamin D or retinoic acid. IGF-I is an important intermediate between upstream exposures and downstream signaling, integrating a whole host of exposures and signaling pathways.

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Programming of the IGF-I Axis and Implication for Health in Adulthood

Cow’s Milk in Treatment of Moderate and Severe Undernutrition in Low-Income Countries

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Abstract

Cow’s milk products have a central role in treatment of undernutrition, and the introduction of products with a high milk content (F-100 and ready to use therapeutic foods) has resulted in marked improvements in weight gain and reduction in mortality. Milk also has a specific effect on linear growth. Milk protein has a high quality score (PDCAAS) and contains many peptides and other bioactive factors, which might have special effects on recovery from undernutrition. Milk is an important source of minerals supporting growth (type II nutrients), such as potassium, magnesium, phosphorus and zinc, and the high lactose content also seems to support growth due to a prebiotic effect and improved absorption of minerals. The risk that the use of cow’s milk products suppresses breastfeeding should be prevented by supporting mothers in breastfeeding. There is consensus that children with severe undernutrition should be treated with products with high milk content, but because of the high cost of milk there is a need to perform more studies to determine the minimal amount of milk protein needed to make a clinically relevant difference in treating the 36 million children with moderate wasting. Such studies should not only focus on weight gain but also on linear growth, body composition, physical activity and cognitive development.

Introduction

Cow’s milk products have a central role in treatment of undernutrition both in industrialized and developing countries. In industrialized countries, almost all products used for enteral feeding of malnourished patients in a hospital...
setting, for both children and adults, are based on cow’s milk. Also, successful treatment of severe undernutrition in most developing country settings is based on products where protein comes from cow’s milk. It is not surprising that milk has such positive effects on recovery after a period of weight loss, as it has evolved as a food to support the offspring during a period of immaturity, vulnerability and high growth velocity. The aim of this review is to describe the effects of using cow’s milk in treating undernutrition in a developing country setting, with focus on children below 5 years. The potential effects of the most important constituents of milk, the type of milk products used in foods for treating undernutrition and current recommendations on using milk in these foods will be discussed. Although milk-based products seem to be very effective in treating and preventing undernutrition, the cost of cow’s milk is so high, compared to the resources available, that it is often not feasible to use products with high milk content for treating the many millions of children with undernutrition.

**Definition of Undernutrition**

Among children, wasting is defined as a weight-for-length/height (W/H) below –2 SD, based on the new WHO Growth Standards (www.who.int/childgrowth). Wasting can be divided into severe wasting (W/H < –3 SD) and moderate wasting (W/H between –2 SD and –3 SD). Stunting is defined as a height-for-age (H/A) below –2 SD and can also be divided into severe (H/A < –3 SD) and moderate (H/A between –2 SD and –3 SD). Underweight refers to children with a weight-for-age (W/A) below –2 SD. However, underweight is not a very useful classification, as low weight can be caused either by wasting, or by stunting or a combination of wasting and stunting. Children with bilateral pitting edema are always classified as severely undernourished, despite a W/H above –3 SD. The terms malnutrition and severe malnutrition was used previously, but since the term malnutrition in principle also includes obesity and other nutritional disorders, there has been consensus to use the term undernutrition for those with a low weight and/or height.

**Size of the Undernutrition Problem**

Undernutrition is widespread among children. According to recent estimates based on the new WHO Child Growth Standards, 178 (32%) of the 555 million children in the world below 5 years of age are stunted, while 112 million (20%) are underweight, and 55 million (10%) wasted [1]. Of the 55 million children with wasting, 19 million are severely and 36 million moderately wasted.
Cow's Milk and Undernutrition

Why Is Milk So Effective?

Maternal milk is the only food mammals receive during the early period of life when growth velocity is very high and the composition seems optimal to support a high rate of tissue synthesis, which is important for children recovering from undernutrition. The components of cow's milk that are especially important for growth are protein, minerals and lactose, while cow's milk fat is usually not used in the products for treating undernutrition. In comparison with plant-based foods, another important quality of cow's milk products is that they contain no antinutrients or fibers.

Protein

Milk protein has a very high quality. FAO/WHO have recommended the protein digestibility-corrected amino acid score (PDCAAS), as the method to evaluate protein quality. This score is calculated from the first limiting essential amino acid needed in children recovering from undernutrition, and milk is the food with the highest PDCAAS score, about 120% [2]. Furthermore, the protein fraction contains peptides and other bioactive factors, which might have specific effects on growth and recovery from undernutrition. These constituents are especially high in whey products. However, it has not been shown convincingly that products based on whey have advantages over dried skimmed milk (containing 20% whey and 80% casein) in treating malnourished children [3].

Lactose

The high lactose content in cow's milk may have a positive effect on growth. The concern for symptoms due to lactose intolerance is probably unjustified as undernourished children seem to tolerate products with a high lactose content well [4] and a meta-analysis showed that even children with mild acute diarrhea can safely continue with milk products [5]. Furthermore, Torun et al. [6] showed no further beneficial effect with regard to nutrient absorption during recovery from malnutrition when comparing cow's milk products with hydrolyzed lactose to products with lactose. The positive effects could be due to a prebiotic effect of lactose entering the large intestine, improved absorption of minerals and beneficial luminal effects [7, 8]. Furthermore, regular consumption of milk will upregulate the lactase content in the intestine and thereby facilitate digestion of lactose, as pointed out in the paper by Savaiano [9]. In some animal studies, the positive effect of whey on growth has been ascribed to the lactose fraction [10, 11].
The therapeutic milk diets F75 and F100, shown to be very successful in the treatment of severely malnourished children, contain 1.3 and 4.3 g lactose per 100 ml, respectively. For comparison, ordinary cow’s milk contains about 4.5 g per 100 ml, and the content in breast milk, which is the best food to give to a child with undernutrition and/or diarrhea is much higher, about 7 g per 100 ml.

Minerals

Milk is an important source of growth-supporting minerals, also referred to as type II nutrients, such as potassium, magnesium, phosphorus and zinc. These minerals are especially important during catch-up growth after a period of weight loss [12]. Since there is no body store of type II minerals, except in the functional tissue, these minerals will be lost as the tissue is broken down. During catch-up growth, all type II minerals will have to be available in balance to build up lean body mass. If one of these nutrients is deficient in the diet, the child will stop growing and instead gain fat mass, if sufficient energy is provided [12, 13]. Cow’s milk contains all the type II minerals, and the bioavailability of the minerals is high compared with plant sources. The high bioavailability of the milk minerals is especially important for the undernourished child since the acid secretion of the stomach, which promotes absorption, often is low or even absent [14]. Malnourished children often present with clinically relevant phosphate deficiency [15, 16], which has been shown to be related to the prognosis [17]. According to Golden [12], the high bioavailability of phosphorus in milk, compared to plant sources, is likely to be partially accountable for the success of milk in the treatment of malnutrition. Plant source foods are often high in phytate, which contains phosphate in a form that is not easily absorbed, and phytate also inhibits the absorption of some of the other type II minerals [2].

Effects of Milk on Nutritional Status

Milk has some of the advantages that are characteristic of animal source foods. These advantages are described in detail in the paper by Allen and Dror [18]. They conclude that the few randomized studies available from low-income countries investigating the effect of milk mainly have shown an effect on growth, especially in the younger children, while the effects on cognitive function and physical activity were more pronounced in children receiving meat. They also emphasize milk as an effective agent in increasing vitamin B₁₂ status in populations with a high prevalence of vitamin B₁₂ deficiency.

In studies of treatment of children with severe wasting, the use of milk-based products has been shown to improve recovery considerably, and in
some studies, mortality was reduced from about 30 to 5% [19, 20]. Although milk is a major ingredient in the tested products and is likely to play a major role, other nutritional aspects of these products are also important, especially the mineral composition, which is designed to cover the needs for tissue synthesis during periods with a very high weight gain [19].

A specific positive effect of milk on muscle mass would be valuable in undernourished children. Some studies suggest that drinking milk immediately after endurance training will increase muscle mass [21]. The evidence for such a specific effect on muscle mass in children with undernutrition is not available. However, very few studies have examined body composition during treatment of children with undernutrition.

In addition to the well-documented effects on weight gain, which has been a main outcome in most studies of treatment of children with undernutrition, there is strong evidence that milk also has a specific effect on linear growth. This is discussed in detail in the paper by Molgaard et al. [21] and in the review by Hoppe et al. [22].

**Cow’s Milk Products Used in Treatment of Undernutrition**

When cow’s milk is used in treatment of undernutrition, it is almost always as a powdered product used as an ingredient in a special food. The most commonly used product is skimmed milk powder (SMP), which is more widely available than whey products. Whole milk powder is seldom used because it is more expensive and has a short shelf life, since the fat easily turns rancid. Other alternatives are different types of whey. The most common whey products are sweet whey powder with 13% protein and 75% lactose, and whey protein concentrates which are produced with 34% (WPC34%) or 80% (WPC80%) of the content as protein. WPC34% has a lactose content of about 50%, the same as SMP, while WPC80% contains only 10% lactose. Compared to SMP, whey may have specific beneficial effects on the immune system and on muscle synthesis, but evidence is lacking in children with undernutrition [3].

The high cost of milk is an important limiting factor, because resources for treating children with undernutrition unfortunately are very limited. Prices for the most common milk products, expressed as price in USD per kg protein, were for the first quarter of 2010 about USD 7 for both SMP and WPC80 (www.dairyforglobalnutrition.org). WPC34% is slightly cheaper, about USD 6 per kg, but has previously been 20–25% cheaper than SMP. Although these prices are for bulk and cost for transport will be added, it is thought provoking that translating this into a cost per liter for the same protein content as cow’s milk, the price is only about 20 cents per liter (1 kg of WPC34% protein dissolved in 30 l of water with a final protein content about 35 g/l).
Products for Treating Undernutrition

Since the mid 1990s, F-100 has been the preferred product used for treatment of severe undernutrition [19, 23]. F-100 contains SMP and whey, vegetable oil, sugar, maltodextrin, and a mineral/vitamin mix. Thus, 100% of the protein comes from milk.

Another successful type of products for treatment of children with severe and moderate undernutrition is ready to use therapeutic foods, especially the milk and peanut butter-based product manufactured under the trade name PlumpyNut, in which about 50–60% of the protein content comes from SMP and whey [24, 25].

Corn Soy Blend (CSB) has been used for treatment of moderate undernutrition, but there is now consensus that this is not a suitable product for treatment, as the content of antinutrients and fiber is too high and it contains no animal protein [26]. An improved CSB, which has an 8% content of SMP, has been developed by WFP for children from 6–24 months [26]. It also has a higher oil and sugar content and thereby a higher energy density, and an improved vitamin-mineral blend, compared to the conventional CSB. However, the price of this improved CSB is also considerably higher than the price of the conventional CSB, and results from intervention studies are needed to document that the effectiveness of improved CSB can justify the higher price.

Potential Negative Effects of Using Cow’s Milk

If cow’s milk products are used in the treatment of infants and young children with undernutrition, there is a potential risk that intake of breast milk is reduced. In children with undernutrition, the positive effects of breastfeeding, especially in protection against infectious diseases are very important [27]. It is therefore crucial that mothers are supported and encouraged to breastfeed as much as possible. Guidance on how to support breastfeeding in these situations is given in the IMCI manual [28].

Another important issue is that powdered milk products should never be distributed to families with malnourished children as the risk of contamination during reconstitution in most settings is very high. If products like SMP are available in emergency settings, they can be used to fortify cereals or fortified blended foods, such as CSB used for preparing gruels, porridges or bread.

If liquid cow’s milk is available, it should only be used if it is full fat milk, as products with low fat content will have a relative protein content that is too high. Undiluted cow’s milk has a high content of protein and macrominerals, approximately three times as high as in breast milk, which results in a high renal solute load if given in large amounts, which can result in hypernatremia and dehydration. Liquid cow’s milk should therefore not be given undiluted to infants.
Conclusions and recommendations on milk in treatment of moderate malnutrition

Liquid milk and milk powder are good sources of high-quality protein and micronutrients important for growth.

The minimum amount of milk protein needed to improve growth in children with moderate malnutrition is not known, but a milk content providing 25–33% of the protein requirement is likely to have a positive effect on weight gain and linear growth. However, studies should be conducted to determine the amount that is cost-effective.

200–250 ml of milk or 15–20 g of milk powder or whey protein powder (SMP or whey protein concentrate 34%) per 1,000 kcal will provide 25–33% of the recommended protein intake (24–26 g/1,000 kcal).

Milk is likely to be more effective than meat in treating moderate stunting, as milk has a special effect on linear growth through stimulation of IGF-I production.

Powdered milk with reduced milk fat, such as SMP or whey protein, should never be used for preparing liquid milk, because of the high protein content and risk of infection if mixed with contaminated water, but it can be mixed with blended foods or other foods that are cooked or heated.

Whey contains peptides and proteins that have been suggested to have positive effects compared with SMP, but these effects have not been documented in children with moderate malnutrition.

The effects of using whey instead of SMP in the treatment of children with moderate malnutrition should be tested in intervention trials, both because whey protein concentrate is cheaper than SMP and because of the potential beneficial effects of whey.

Whole-milk powder should be used as a drink for children with moderate malnutrition only if it is prepared under strictly controlled and hygienic conditions.

If milk is the only animal source food given, sufficient iron should be provided in the diet.

Fermented milk products should be promoted, as they have advantages over other milk products.

Research recommendations

Research is needed to determine the amount of milk protein that has optimal cost-effectiveness in promoting growth.

Research is needed to determine if there are any advantages of using whey instead of SMP in the treatment of children with moderate malnutrition.

1 These conclusions and recommendations are from the review by Michaelsen et al. [2] prepared for the WHO/UNICEF/WFP/UNHCR consultation on the management of moderate malnutrition in children under 5 years of age held in Geneva 2008.
Recommendations on the Use of Milk in Treating Undernutrition

There is consensus that children with severe wasting should be treated with products with high milk content, such as F-100 or ready to use therapeutic foods, as these products have shown to be very effective in supporting weight gain and reducing mortality [29]. The need for milk-based products in treating the 36 million children with moderate wasting is not clear. At the WHO/UNICEF/WFP/UNHCR meeting 2008 in Geneva on management of moderate malnutrition, it was concluded that animal-source foods added to a plant-based diet promotes recovery, but also that there was a need to study to which degree plant-based foods with a high energy density, and low content of antinutrients and fibers can be effective in promoting recovery [30]. As the importance of minimizing the costs of foods for children with moderate malnutrition was underlined, one of the research recommendations was to identify the minimum quantities of animal-source foods needed in the diets of children with moderate undernutrition to make a significant impact on recovery. One of the background papers for the meeting provided a detailed description of foods and ingredients used for children with moderate undernutrition and also covered milk and milk products [2]. The conclusions and recommendations from the section of milk and milk products are given in table 1. A general recommendation from the meeting was that studies of recovery from undernutrition should not only focus on weight gain but also assess linear growth, body composition, physical activity, and cognitive development as well as immunocompetence, micronutrient status, renal concentrating ability, and sodium pump function [30]. Such studies will provide a better understanding of how to compose a diet for children with undernutrition which not only provides weight gain but also optimal development and health.

References

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Discussion

Dr. Johansson: I can give you one more argument for a change from maltodextrin to lactose. It relates to the pH drop in the tooth biofilm and risk of caries development. Maltodextrin gives as deep a pH fall as sucrose, whereas lactose does not cause a significant pH drop. So switching carbohydrate type would be tooth saving, and especially in malnourished children who are more prone to caries development.

Dr. Haschke: You presented products and product concepts. Maybe you can also elaborate on the outcome of trials which you have been conducting or you are aware of. What is the best practice in that segment, and what are the outcome variables that you consider clinically most relevant?

Dr. Michaelsen: There are many difficult problems within logistics, policies and economy when you distribute food and treat malnourished children. With limited resources, it is often necessary to choose between treating fewer children with an optimal food or more with a cheaper and less optimal food. Concerning outcomes, when you are talking about severe malnutrition the immediate and most important outcome is mortality, and we have now products that can reduce mortality to a very low level. Weight gain is used widely and is important because it is related to morbidity, but we should start to focus more on other outcomes like linear growth, body composition and functional outcomes. If weight gain is mainly body fat, it will not be advantageous in the long run. We also want to look at outcomes like physical activity and mental development. We would rather have them gaining a little less weight and running around playing than sitting in a corner being more fat.

Dr. Prentice: Over the years, there have been a number of preparations to add to these relief foods either to improve micronutrient status or digestibility, for example amylase and multimicronutrient mixes that are placed on food. Now there are these new improved fortified blends, would one use the older preparations, do they become redundant, or are they used with them as well?

Dr. Michaelsen: There is certainly a place for adding micronutrient supplements. But the mix used is being discussed and improved. There is also increased focus on the effects of antinutrients and fibers in the plant-based foods used. Therefore, enzymes like amylase and phytase added to the foods can have positive effects, but this is still being investigated. After the workshop in Geneva in 2008 on treatment of moderate malnutrition, there has been more focus on food technology and processing to make plant-based foods more acceptable and digestible for the young children who have a more vulnerable intestinal tract.

Dr. Garg: This is regarding the effect of lactose and reduction in NEC which has been shown in the preterm piglets study. The preterm formula which we use has 50% lactose, and 50% of the carbohydrate content consists of glucose polymers. Lactase activity in preterm babies is less, around 30% of a full-term baby, even at 34 weeks. How could the same information which is there in piglets, be clinically translated for preterm neonates as far as lactose content in the preterm baby formula is concerned?

Dr. Michaelsen: I am not a specialist in nutrition of preterm infants, and I think it’s a question about balance. If you have lactose instead of maltodextrin, you also have a
problem with higher osmolarity, so I think you have to balance these things; but I also think it’s important to have some lactose, but I couldn’t say at exactly which level it is acceptable. I think that many formulas used in clinical nutrition and in prevention of allergy contain no lactose because there has been an exaggerated fear of lactose intolerance.

Dr. Anderson: My questions relate to the composition of some of the products. First, I was surprised that whey protein isolate in your scale of cost was so much less than the dried skimmed milk, and maybe you can explain that. Second, what is the target level of protein in relation to calories in those supplements?

Dr. Michaelsen: In these products, you shouldn’t have much more than 10–11% of the energy as protein. You don’t want to go too high both because of the cost but also because there might be a metabolic disadvantage, and a high protein intake might have a negative effect on appetite. Concerning the price, there is a large difference between whey protein isolate, which is very expensive, and whey protein concentrate 94%, which is the cheapest when calculated according to protein content, and it’s actually cheaper than sweet whey. Whey protein concentrate is cheaper than skimmed milk powder because it’s a surplus product from cheese production. At present, it is 20–30% cheaper than dried skimmed milk, but world market prices are constantly fluctuating.

Dr. Sarwar Ferdaus: Malnourished patients always suffer from gastroenteritis and diarrheal disease, so their intestinal villi are already short. What we have seen is that if we give milk alone, it’s less tolerated than if we mix it with other food, especially with rice. Could you comment whether we should give milk alone or mix it with other food or make a formula in such a way that lactose is reduced to some extent to promote better absorption?

Dr. Michaelsen: In F75 there are cereals and the milk content is reduced considerably, so that’s what you would give in some settings in the first week. There, you would have considerably lower lactose. And I am not saying that lactose intolerance cannot be a problem in individual patients. I am just saying that the experience from using F100 in many treatment centers is that it works very well in the majority of these children. Some children might not tolerate the high levels of lactose from the beginning, but giving them small amounts of lactose has a beneficial effect on their lactase levels.

Dr. Bodenstab: I would like to have your views on what industry such as Nestlé could do for you. I am thinking of supply of products using the purchasing power, our manufacturing set up and distribution. I am also thinking of using our R&D system to codevelop products. Conceivably, these products could be nonbranded or branded in a way that would be different from our normal branding.

Dr. Michaelsen: There are many technological aspects that need to be developed further, and I am sure that you would have a lot of knowledge that could be used in these settings. Again, the cost of the products is crucial. We need low-cost products and we need products that can be locally produced. Issues like the optimal composition of micronutrient supplementation, the potential use of enzymes in fortified blended foods, and the effects of food processing are areas where large companies like Nestlé will have knowledge that could be used in developing these products further.

Dr. Boukari: Can you tell us about rice milk, what is its energy and protein content?

Dr. Michaelsen: The only thing I know is that the rice milk you buy in Denmark has extremely low protein content. The Danish National Board of Health have come up with a statement saying that it is dangerous to give rice milk to small children. In Denmark, we have seen a tendency that mothers use more soy milk and rice milk. It is a completely different issue to add rice as a cereal in foods for treating moderately
malnourished children. Rice has a high protein quality and a low content of antinutrients, but is also relatively expensive compared to other cereals.

Dr. Savaiano: Can I have a comment to that? For healthy adults and probably children, it’s the only carbohydrate that doesn’t cause fermentation in the colon. So, if the small bowel is working, none of the carbohydrate should get to the colon, that should be an advantage, I would think.

Dr. Haschke: You probably were referring to commercial products which should also be available in developed countries. I can say that one product based on hydrolyzed rice protein is safe (Risolac) but in the high-price segment.

Dr. Boukari: About rice, the problem with that product is that it’s not largely distributed, and we can’t have that product in some countries like Maghreb with medium undernutrition. We don’t need cheap products but products for that kind of undernutrition and perhaps with a medium price. In some hospitals in our country, we use very expensive products with highly hydrolyzed protein. To have a high density of energy like 1 cal/1 ml, we use a high concentration, so it becomes very expensive. Perhaps for that country we need to use a product with a reasonable price, and perhaps industries like Nestlé or others have a role to play in that kind of malnutrition.

Dr. Thorsdottir: I was thinking about the age of the children and infants. Were you only looking at very young infants or older children as well?

Dr. Michaelsen: When the WHO and the UNICEF approach the topic of malnutrition in children, they usually focus on children below 5 years, but the younger you get the bigger the problems are. So it’s especially from 6 months to 2 years that you have a vulnerability that is much greater than later on. But very malnourished 3- or 4-year-old children might have the same problems as younger children.

Dr. Adrianasolo: If I understand you well, this treatment is intended for children whose weight for height is below –3 z score. What about the children with kwashiorkor, with edema, whose weight for height might not necessarily be below –3 SD?

Dr. Michaelsen: I didn’t include that in my definition, but severe malnutrition includes any child with edema, so it’s a weight for height below –3 SD or edema. If you have edema, you are always severely malnourished according to the official recommendations, even if your weight for height is above –2 SD. But in my talk I also covered children with moderate malnutrition, which is defined as a weight for height between –3 SD and –2 SD.

Dr. Begum: You mentioned that you added vegetable oil to skim milk. My first question is, is vegetable fat better tolerated than animal fat by the malnourished child? And to my second question, you have mentioned that F75 and F100 are not available in some Asian countries; in that situation, which food should we add to the milk?

Dr. Michaelsen: Regarding the type of fat, the reason that in all these products you don’t have milk fat is because it would go rancid, so from a logistical point of view it’s much easier to export and transport products like dried skimmed milk and whey powder with no fats and then add the vegetable fat later. By adding vegetable oils like soybean oil, you will also get a quite fine balance of n-3 and n-6 fatty acids. Your second question, what to use when you do not have F-75 and F-100 available, is quite complex, and I have no short answers. You could either use clinical products for enteral nutrition, which are quite expensive or you can get some guidance in our review on which foods to use in treating moderate malnutrition [1].

Dr. Sankaranarayanan: The different milk-based recipes you showed are all simplified, but in practice we find it very difficult to match a particular diet to the needs of a child. It’s not only children with diarrhea that I’m talking about. It concerns most of the patients with edema and extreme protein malnutrition who are totally anorexic. They have extreme loss of appetite and don’t accept any food.
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Dr. Michaelsen: There are a lot of specific problems when you deal with an individual child, and if he/she has no appetite, you have to consider tube feeding. And there are a lot of details you have to consider, depending on how the child reacts to the treatment.

Dr. Sankaranarayanan: What we are faced with in these children is a triad of extreme risk factors, i.e. malnutrition, malabsorption and infection. But what is inevitable in these children is malabsorption plus malnutrition, so what is the recipe we can use?

Dr. Michaelsen: Malabsorption is almost always a problem in these children, and that’s part of the reason why milk is advantageous because many of the substances are easily absorbed. Still, you might have individual children with specific malnutrition problems that you have to deal with and you might have children that have milk allergy, and so there are a lot of specific problems when you are confronted with a specific child that I was not able to cover here.

Reference

Effects of Animal Source Foods, with Emphasis on Milk, in the Diet of Children in Low-Income Countries

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Abstract

This review evaluates evidence for benefits of including animal source foods (ASF) in the diets of children in developing countries. In observational studies, a higher usual intake of ASF in such countries is associated with better growth, status of some micronutrients, cognitive performance, motor development and activity. Only three randomized trials supplemented children with milk and compared outcomes with a nonintervention control group. Both height and weight growth were improved, although in Kenya height was increased only in younger schoolers who were stunted at baseline. Meat supplements have been evaluated in only two randomized controlled trials, in Kenya and Guatemala (mean baseline age 8 years and 1 year, respectively); growth was no better than in an equicaloric control group. Meat improved cognitive function and activity in Kenya; milk was less effective than meat for improving cognitive function and physical activity, perhaps due to its lower content of iron, zinc, or riboflavin. Meat and especially cow’s milk are excellent sources of vitamin B12, a micronutrient commonly deficient in populations which consume low amounts of ASF. Other micronutrients such as iron have been added to cow’s milk and resulted in improved nutritional outcomes for children.

Introduction

Animal source food (ASF) intake in developing countries is often very limited. According to FAO, ASF provide <5% of total energy intake in many countries of sub-Saharan Africa, 5–10% in most other African countries and southern Asia, 10–15% in eastern and northern Asia as well as Mexico, and ≥20% in the US, Canada, Europe and Australasia. Plant-based diets, high in
phytate and fiber, are often lower in energy density, provide poorer quality protein, and are low or completely lacking in some essential micronutrients [1, 2]. Per 100 g, whole cow’s milk provides 0.36 µg vitamin B₁₂, 138 IU vitamin A, 0.16 mg riboflavin, 5 µg folate, and 119 mg calcium [3]. Meat provides substantial amounts of available iron and zinc, riboflavin, and vitamin B₁₂ [4]. The provision of multiple micronutrients simultaneously can be especially advantageous; for example, vitamin A and riboflavin enhance iron mobilization and hemoglobin synthesis. Vitamin B₁₂ is found only in ASF, explaining the high global prevalence of deficiency in most developing countries [5]. The focus in this article is on demonstrated benefits of including ASF in the diets of children in developing countries. Here ‘milk’ refers to cow’s milk, and not to human milk or infant formulas, unless otherwise stated.

Interpretation of the literature on the importance of ASF in children’s diets is complicated by the many study designs used to investigate this question. These include: observational studies, with and without separation of milk from other ASF; interventions with: milk vs. nonintervention control; milk vs. an equal amount of energy; milk vs. meat, with and without a nonintervention control, and fortified milk vs. unfortified milk or a nonintervention control, vs. several other types of intervention.

**Observational Studies**

There is little doubt that infants and young children who receive more ASF in developing countries usually have better growth, cognitive performance and motor function. ASF are the only, or a major, source of many micronutrients that support children’s growth and development as well as the protein and other bioactive factors discussed elsewhere in this book.

Older studies of the adverse effects of macrobiotic diets on child development in The Netherlands are informative, because such diets are similar to – or arguably poorer than – those of some children in some developing countries (cereals, rice, vegetables, legumes, and small amounts of cooked fruit and fish, but no meat or dairy products). In the Dutch situation, there was also less confounding by illnesses resulting from poor local unsanitary conditions. Compared with omnivorous controls, the macrobiotic children’s intakes of protein, fat, calcium, riboflavin, vitamin B₁₂ and vitamin C were lower. Although birthweights were smaller (3,290 vs. 3,470 g), growth was normal until age 4 months, at which point it declined dramatically (13.2 cm/year compared with 16.7 cm/year in controls). It stabilized at 16 months, but there was no catch-up later [6]. Children from families who consumed dairy products three times a week grew better than those who consumed them rarely. The macrobiotic infants had a substantially higher prevalence of iron, riboflavin and vitamin B₁₂ deficiency, anemia and rickets [7]. They had delays in gross motor development, speech and language development. Vitamin B₁₂
Animal Source Foods and Milk for Children

status and cognitive function was still poorer than that of controls in early adolescence, although parents heeded advice to feed ASF, starting at age 6 years on average [8].

An analysis of the Demographic and Health Survey data from seven countries in Latin America revealed that milk intake was significantly associated with better length- or height-for-age z scores in all countries, while meat intake showed this association in only one country. However, it is possible that the range and amount of meat intake in the other countries was inadequate to reveal any associations with usual consumption [9]; in rural Kenyan schoolchildren, there was no association between ASF intake and adequacy of intake of micronutrients until additional amounts of ASF were added to the diets through supplementation [Allen et al., unpubl. data].

Usual ASF intake, expressed as percent of total energy, was the main predictor of many outcomes in the observational Nutrition Collaborative Research Support Program conducted in Egypt, Kenya and Mexico during the 1980s, including growth, affect, cognitive function, physical activity, and micronutrient status of children [10]. In an earlier study in Kenya, animal protein intake at 18–30 months of age was a stronger predictor of cognitive function 5 years later than was total protein intake [11]. Similar results were found amongst Egyptian children [12]. Associations with milk vs. meat were not analyzed separately. In Peru, linear growth between 12 and 15 months of age was positively associated with intake of ASF in children with a low intake of complementary foods, but again there was no separation of milk vs. meat in the analysis [13]. In Nepal, children with high meat or fish intake at age 13–24 months were significantly less likely to present with vitamin A deficiency-associated xerophthalmia at age 1–6 years [14].

**Intervention Trials**

A limited number of randomized, controlled intervention trials have been performed to determine the effects of adding ASF to the diets of young children. A review of the efficacy of different ASF interventions in improving child growth concluded that supplements containing at least some dried milk improved children's growth in twelve of fifteen studies [15]. Outcomes usually measured include growth, micronutrient status, cognitive performance, and/or level of physical activity. Studies are presented by type of intervention design, as far as possible (table 1).

**Interventions with Milk, Meat or Fish vs. a Nonintervention or Equicaloric Control Group**

Surprisingly few investigators in developing countries have fed milk to one group of children and compared outcomes with a nonintervention control group; only three such studies were identified. Some 40 years ago, the benefits
<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Subjects</th>
<th>Age at entry</th>
<th>Intervention</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>[16]</td>
<td>New Guinea</td>
<td>33</td>
<td>7–8 years</td>
<td>Daily for 13 weeks 1. 75 g skim milk + normal diet 2. Normal diet (high in taro and sweet potato)</td>
<td>Height and weight change in control group = twice that of controls</td>
</tr>
<tr>
<td>[17]</td>
<td>Bac Ninh province, Vietnam</td>
<td>454</td>
<td>7–8 years</td>
<td>6 months, school days only 1. 500 ml unfortified milk 2. 500 ml MMN fortified milk providing 5.5 mg Zn, 6.5 mg Fe, 6.7 µg vit. A, 165 mg vit. C, 13 mg vit. E/d 3. Control (no supplementation)</td>
<td>Weight-for-age and height-for-age z scores improved significantly in both milk groups compared with control Short-term memory scores significantly higher in children in milk groups, with superior scores in fortified milk group Parent reported health-related quality of life improved with milk intervention</td>
</tr>
<tr>
<td>[1, 19]</td>
<td>Embu district, Kenya</td>
<td>544</td>
<td>5–14 years</td>
<td>7 school terms (2.25 years) daily 1. Githeri (maize and bean-based porridge) with finely ground beef 2. Githeri with one glass UHT milk 3. Githeri with added vegetable oil 4. Control (no supplement)</td>
<td>Significant increase in plasma B₁₂ in meat and milk groups Significantly increased weight gain in all supplemented groups Significantly better performance on arithmetic tests and highest level of physical activity in meat group</td>
</tr>
<tr>
<td>[26]</td>
<td>Guatemala City</td>
<td>302</td>
<td>12 months</td>
<td>9 months, supervised feeding at 1 meal/day, 5 days/week for 9 months 1. Ground beef (72 g, 102 kcal/day, providing 0.56 µg B₁₂) 2. Fruit + vegetables (92 kcal/day, fortified with 0.86 µg B₁₂) 3. Fruit + vegetables, unfortified, (92 kcal/day)</td>
<td>No effects of beef or B₁₂ fortification on any child outcomes in a B₁₂-deficient population Cow's milk intake from usual diet positively predicted B₁₂ status, while breast milk intake was a negative predictor</td>
</tr>
<tr>
<td>Reference</td>
<td>Location</td>
<td>Participants</td>
<td>Age</td>
<td>Duration</td>
<td>Intervention Details</td>
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| [29]      | Mangochi district, Malawi | 630          | 2.5–7.5 years | 12 months dietary diversification strategy including 1. Increased consumption of ASF (especially whole dried fish) and orange-red fruits 2. Control (no intervention) | Significant improvement in mid-upper-arm circumference, z score and reduced prevalence of inadequate vit. B<sub>12</sub>, Ca, and Zn intake after intervention  
|           |                           |              |      |          |                                                                                      | Hb significantly higher, incidence of anemia and common infections significantly lower in intervention group than control |
| [30]      | Peri-urban Lima, Peru     | 137          | 12–17.9 years | 9 months 1. Community-based, behavioral and dietary intervention to increase heme iron, total iron and ascorbic acid intake 2. Nonintervention | Significant increase in total and heme iron intake (heme iron to 0.66 mg/day vs. 0.21 mg/day baseline)  
|           |                           |              |      |          |                                                                                      | No significant effect on anemia but prevented increase seen in control group |
| [27]      | Ghana                     | 208          | 6 months | 6 months, daily; local cereal from maize, soy, peanuts 1. Cereal + 20% whole fish powder 2. Cereal + MMN 3. Cereal 4. Cross-sectional nonintervention | No significant effects among intervention groups on weight or length gain, hematology, or iron, zinc or riboflavin status  
|           |                           |              |      |          |                                                                                      | Growth poorest in nonintervention group |
| [28]      | Northern Cape province, South Africa | 183 | 7–9 years | 6 months, school days only 1. 25 g bread spread containing marine fish flour (892 mg DHA/week) 2. 25 g bread spread without fish flour | Significantly higher EPA and DHA levels and cognitive function (verbal learning ability and memory) test scores in experimental group compared with control |

MMN = Multiple micronutrient; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.
Table 2. Fortified milk vs. unfortified milk supplementation trials in children

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Subjects</th>
<th>Age at entry</th>
<th>Intervention</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>[31]</td>
<td>Mexico City, Mexico</td>
<td>227</td>
<td>8–60 months</td>
<td>90-day supplementation with 500 ml fortified milk containing 22.5 mg Zn, 2.250 µg vit. A, 105 mg vit. C</td>
<td>Significant improvement in weight-for-height z scores, plasma levels of vit. B_{12}, folic acid, and Hb</td>
</tr>
<tr>
<td>[5, 33]</td>
<td>Central and eastern Mexico</td>
<td>567</td>
<td>12–30 months</td>
<td>12 months daily 1. 48 g fortified milk powder (5.3 mg Fe, 5.3 mg Zn, 48 mg vit. C), reconstituted to 400 ml 2. 48 g unfortified milk powder (0.2 mg Fe, 1.6 mg Zn, 6.8 mg vit. C), reconstituted to 400 ml</td>
<td>Prevalence of moderate anemia (Hb 90–100 g/l) and iron deficiency (serum ferritin &lt;12 µg/l) significantly lower in fortified milk group at 6 and 12 months (no other measures)</td>
</tr>
<tr>
<td>[32]</td>
<td>Puebla, Mexico</td>
<td>115</td>
<td>10–30 months</td>
<td>6 months daily 1. 48 g fortified milk powder (5.3 mg Fe, 5.3 mg Zn, 48 mg vit. C), reconstituted to 400 ml 2. 48 g unfortified milk powder (0.2 mg Fe, 1.9 mg Zn, 6.8 mg vit. C), reconstituted to 400 ml</td>
<td>Significant decline in prevalence of anemia (41.4–12.1%) in fortified milk group, no change in unfortified milk group Treatment with fortified milk significantly negatively associated with likelihood of anemia following intervention (no other measures)</td>
</tr>
<tr>
<td>[38]</td>
<td>New Delhi, India</td>
<td>633</td>
<td>1–3 years</td>
<td>1 year daily 1. MMN-fortified milk powder providing 9.6 mg Zn, 9.6 mg Fe, 6.6 µg Se, 0.3 mg Cu, 330 µg vit. A, 48.0 mg vit. C, 8.1 mg vit. E/d 2. Unfortified milk powder</td>
<td>Fortified milk significantly reduced incidence of diarrhea, acute lower respiratory tract infection, and overall days with severe illness during study period Differences in morbidity most pronounced in children &lt;2 years old</td>
</tr>
</tbody>
</table>
### Iron-deficient or anemic children in wealthier populations

<table>
<thead>
<tr>
<th>Study Location</th>
<th>Age</th>
<th>Incidence</th>
<th>Intervention</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dong Thap, Vietnam</td>
<td>6 years</td>
<td>1,080</td>
<td>143 nonconsecutive days over 529-day period</td>
<td>Small but significantly greater gain in weight and height in milk group compared with control over study period</td>
</tr>
<tr>
<td>Tupa, Brazil</td>
<td>6 months to 2 years</td>
<td>185</td>
<td>1 l/day Fe-fortified whole cow's milk (3 mg Fe) for 7.3 months</td>
<td>Hb normalized in 57% of subjects following intervention</td>
</tr>
<tr>
<td>Buenos Aires, Argentina</td>
<td>12–48 months with mild Fe deficiency, 65% anemia</td>
<td>17</td>
<td>4 months daily Fe-fortified fluid cow's milk (15 mg Fe/l), ad libitum</td>
<td>100% of children achieved normal values for Hct, Hb, serum Fe, and transferrin saturation</td>
</tr>
<tr>
<td>Santiago, Chile</td>
<td>3 months, percent anemia or iron deficiency not stated</td>
<td>554</td>
<td>12 months daily Fe with fortified milk powder</td>
<td>Change in Hct and Hb significantly greater in children anemic at study entry</td>
</tr>
</tbody>
</table>

1. 150 ml vit. A and D fortified milk + MMN-fortified biscuits, providing 424 µg vit A, 5 mg Fe, and 6 mg Zn
2. Control (no supplementation)
of adding 75 g skim milk daily to a control, normal diet (high in starch) were evaluated in New Guinea schoolchildren 7–8 years old [16]. Height increased by 2.3 cm during the 13-week intervention compared with 1.1 cm in the control group. In rural Vietnam, in a region where the prevalence of stunting was 50%, schoolers (age 7–8 years) were provided 500 ml of unfortified milk on school days for 6 months [17]. This milk increased energy intake by 278 kcal (20%) and protein intake by 13 g (40%). Weight gain was 0.5 kg greater and height gain 0.4 cm greater in the milk intervention group compared with the nonintervention controls, but these differences were not significant. The milk did significantly reduce underweight (by 13%, possibly due to the higher energy intake), stunting (by 2%) and the number of reported health problems. There was no effect on serum retinol or ferritin.

A well-designed study was conducted in rural Kenya, although participants were schoolers and not young children [18]. The area was known to be prone to food shortages and famines, with very low intakes of ASF (milk and meat combined providing only 1% of daily energy intake) and a high prevalence of stunting (30%) and multiple micronutrient deficiencies. Equicaloric (=250 kcal/day) supplementation of 544 children aged 5–14 years (median 7.4 years) with traditional maize- and bean-based porridge (githeri) containing additional oil, meat (60–80 g/day), or milk (1 cup/day) over seven consecutive school terms (2.25 years) significantly increased weight gain by about 10%, compared with a nonsupplemented control group [1]. Energy and protein intakes were adequate and similar in the three intervention groups. Milk supplementation had a significant effect (a 15% greater increase compared with the control group) on height gain in those children who were younger and already stunted, while meat supplementation was the only intervention that increased lean body mass. In an analysis taking into account the total dietary energy food intake of the children and controlling for sex, age, and socioeconomic status, growth was positively predicted by energy and nutrient intakes from milk or meat. Plasma vitamin B12 increased significantly in both the milk and meat intervention groups; prevalence of combined severe and moderate deficiency (plasma B12 <221 pM) fell from 80.7 to 64.1% in the meat group and from 71.6 to 45.1% in the milk group, after 2 years of intervention [19]. No improvement was found in other micronutrients in the same intervention groups, though changes may have been obscured by malaria and other infections [4]. Both height and weight gain were positively predicted by average daily energy intake from ASF, heme iron, preformed vitamin A, calcium, and vitamin B12, while gain in muscle mass was predicted by average daily energy intake from ASF, and vitamin B12 intake [20].

Cognitive status of the Kenyan schoolers was assessed repeatedly using a variety of tests that measured abstract reasoning, problem solving, arithmetic skills, verbal skills, and recall of number sequences. After 2.25 years of supplementation, the meat intervention group performed significantly better than the milk intervention and control groups on arithmetic tests, and showed
significantly greater gains in the Raven’s Colored Progressive Matrices (RPM) test, which evaluates abstract reasoning and problem solving skills. In contrast, the milk group had the lowest rate of increase in RPM scores. At the end of the intervention, there were no significant differences among groups in verbal skills or recall of numeric sequences, but the meat group showed significantly better improvements in end of term test scores. Differences in the effects of meat and milk interventions on cognitive performance may have been related to their iron content, with meat providing bioavailable heme iron that also enhances iron and zinc bioavailability from cereals, while milk may have impaired iron absorption due to its calcium, phosphorus and casein content [1, 21, 22]. Micronutrients provided in the meat supplement may have contributed to increased processing speed; longitudinal regression analysis showed significant relationships between iron, zinc, vitamin B₁₂ and riboflavin intake and improved cognitive test scores, after controlling for confounders [21, 23]. Activity level was assessed by observing schoolyard behaviors during unstructured play using the same methods as in the NCSRP. The meat group spent the greatest percentage of time in high levels of physical activity and had the largest decrease in percent time spent in low levels of physical activity. The milk group had less activity than in all but the control group [1, 24].

Vitamin B₁₂ is found only in ASF where diets are not fortified with this nutrient, and evidence has accumulated during the past two decades to indicate that this deficiency is highly prevalent in developing countries, affecting population subgroups with a low intake of animal products. This vitamin deficiency is highly prevalent in Guatemalan women, and their infants and children [25]. In collaboration with the Institute of Nutrition of Central America and Panama we conducted an intervention study to investigate the feasibility and efficacy of supervised equicaloric supplementation of children with meat vs. fruit and vegetables containing added B₁₂, vs. fruit and vegetables alone, 5 days a week from 12 months of age [26]. At the end of the study, there were no significant differences in growth, hemoglobin, ferritin, or plasma vitamin B₁₂ among the groups, or in cognitive or motor function, or any of several other measures of child development. At baseline (12 months of age) and after intervention, vitamin B₁₂ deficiency was associated with poorer cognitive function and slower motor development. The same children tended to be B₁₂ deficient at the beginning and end of the intervention. Breast milk at 12 months postpartum was seriously inadequate in vitamin B₁₂ with none detectable in about half of the samples. The infants’ usual intake of cow’s milk – usually reconstituted, unfortified dry milk – was a positive predictor of the infant’s vitamin B₁₂ status, while breast milk intake was a negative predictor. In the Kenyan trial that provided meat or milk to schoolers over a 2-year period, vitamin B₁₂ status was significantly improved but still did not reach normal values in many children [19].

A few trials tested the benefit of adding fish to children’s diets. The addition of 20% by weight of dried, whole fish to the local weaning food product
produced for complementary feeding in Ghana (based on maize, soybeans and peanuts) did not improve growth or micronutrient status compared with an unfortified group [27]. South African children aged 7–9 years were assigned to a fish flour spread or a placebo spread for 6 months to explore the benefits of a higher intake of n-3 polyunsaturated fatty acids for cognitive function. There were significant increases in eicosapentaenoic and docosahexaenoic acid concentrations in the intervention group and improved performance on learning and reading tests [28].

Dietary diversification and modification strategies have attempted to increase consumption of ASF. A trial to increase ASF (especially dried fish with bones) intake and reduce dietary phytate in Malawian children aged 2.5–7.5 years significantly improved z scores for mid-upper-arm circumference and arm muscle area after 12 months. There was no effect on weight or height gain compared with controls. The authors hypothesized that lack of ponderal or linear growth change in the intervention group may have been due to insufficient duration of the intervention, the age range of the children, or intergenerational effects of malnutrition [29]. However, the intervention reduced the prevalence of inadequate intakes of vitamin B12, calcium, and bioavailable zinc. Additionally, mean hemoglobin concentration was significantly higher, and incidence of anemia lower, in the intervention group compared with the controls.

A community-based, randomized, behavioral and dietary intervention trial in Peruvian adolescent girls increased heme iron and ascorbic acid intakes considerably, but had no effect on hemoglobin or iron status although it prevented the fall in these indicators in the control group [30].

**Interventions with Micronutrient-Fortified Cow’s Milk**

In recognition of the fact that cow’s milk lacks substantial amounts of some micronutrients, but at the same time it is a relatively nutritious, affordable, available and well-liked food for children, considerable efforts have been made to evaluate the benefits of adding micronutrients to dry or liquid cow’s milk. Milk is well recognized to be especially low in iron, and impede absorption of other sources of dietary iron due to its high levels of calcium, phosphorus and casein, so most attention has been paid to improving its iron content.

Daily consumption of 500 ml/day of multimicronutrient-fortified whole milk for 90 days by 227 children aged 8–60 months in Mexico City significantly improved weight-for-height z scores and plasma concentrations of vitamin B12, folic acid and hemoglobin. Per 500 ml, the fortified milk provided 1.5 mg iron, 3 µg vitamin B12, 2 mg folic acid, and 22.5 mg zinc in addition to other micronutrients. There was no control group. The fortified milk was well tolerated and widely accepted [31]. A significant reduction in anemia prevalence, from 41 to 12%, resulted when Mexican children aged 10–30 months were given 220 g milk powder reconstituted to 400 ml and fortified with 5.3 mg iron, 5.3 mg zinc and 48 mg vitamin C, daily for 12 months. The control group received unfortified milk and showed no reduction in anemia [32].
Mexico has a large-scale program in which micronutrient-fortified milk is distributed to low-income children. An evaluation of the program showed the children consumed approximately 600 ml/month. While it is not possible to separate the effects of individual nutrients on outcomes, the provision of MMN-fortified vs. unfortified milk caused weakly significantly greater reductions in prevalence of anemia and iron deficiency [33]. Because the program included other interventions, it was not possible to separate the beneficial effects of milk from other benefits of the program.

Iron-fortified cow’s milk also improves iron status of iron-deficient and/or anemic children in wealthier populations. In a study of 17 mildly iron-deficient Argentinian children aged 12–48 months, mothers were instructed to replace regular cow’s milk with an iron-fortified fluid whole cow’s milk containing 15 mg Fe/l. After 4 months of intervention with an average daily milk intake of 877 ± 310 ml, all children achieved normal values for hematocrit, hemoglobin, serum iron and transferrin saturation. There were no problems with tolerance or acceptance of the fortified milk [34]. The results agreed with those from earlier studies of iron-fortified milk products (powders and acidified milk) used to prevent or treat iron deficiency in infants and young children. In those studies, ascorbic acid was used to enhance the bioavailability of iron in fortified milks [35–37].

In a peri-urban area of northern India, 633 children aged 1–3 years were randomly assigned to receive multimicronutrient fortified or control milk reconstituted from powder three times per day for 1 year. The micronutrient-fortified milk was designed to deliver daily doses of 9.6 mg iron, 2.7 µg vitamin B₁₂, 9.6 mg zinc, and 330 µg vitamin A. Compared with control milk, consumption of fortified milk significantly reduced the incidence of diarrhea, acute lower respiratory tract infection, and overall days with severe illness across age groups, although differences were most pronounced in children under the age of 2 years. Fortified milk was well accepted by the study population [38].

Milk from Other Animals

In some developing countries, goats, sheep or buffalo are more accessible as household livestock than cows, and their milk may be perceived as more suitable for children. For example, children of the Gursum community in Ethiopia are often given goat milk as a supplement to breast milk [39]. Aside from containing more medium-chain fatty acids, the macronutrient composition of goat’s milk is similar to cow’s milk, and it has been used successfully as an alternative to cow’s milk in short-term rehabilitation of undernourished children in Madagascar [40–41]. However, goat’s milk is known to be deficient in folate (1 µg/100 ml compared with 5 µg in human and cow milk), and infants fed exclusively with goat’s milk can present with megaloblastic anemia.
of folate deficiency [42]. The milk of sheep and buffalo has about 25% more protein and twice as much fat compared to cow's milk.

Unresolved Questions Concerning Cow’s Milk and Children’s Health in Developing Countries

Despite the numerous benefits of cow’s milk, there are some concerns regarding its use in the diets of children. These include its introduction at too young an age, iron depletion, an excessive renal solute load, and potential programming for metabolic imbalances later in life. Excessive cow’s milk consumption has been implicated in energy displacement and deficiencies of micronutrients lacking in milk, especially iron. Occult intestinal blood loss is estimated to occur in 40% of normal infants less than 12 months of age during feeding of cow’s milk [42], which could exacerbate the higher risk of iron deficiency in developing countries. The low DHA content of cow’s milk vs. breast milk could also be an issue where usual intake of this nutrient is low. Compared with breast milk, cow’s milk conveys a high renal solute load due to its protein and mineral content. Immaturity of the renal system in concentrating urine, in conjunction with low fluid intake or extrarenal water losses, can lead to severe dehydration in infants fed cow’s milk. In developing countries, the prevalence of low birthweight and preterm delivery is substantially higher, and such infants have a less mature renal system. Milk is one of the most common food allergens in children, and little is known about its allergenicity in populations with compromised immune function due to undernutrition. There has been relatively little attention to these issues in developing countries, except for concern about its early introduction displacing breastfeeding. In most countries, including the US, introduction of unmodified cow’s milk is discouraged until after the 1st year of life due to limitations in its composition and potential adverse effects on infant health.

Conclusions

While a higher usual intake of ASF by children is associated with a range of more positive outcomes, this review, limited to studies in developing countries, reveals that there have been few well-designed trials of the efficacy or effectiveness of increasing children’s intake of ASF. Only three trials evaluated the benefits of unfortified cow’s milk compared with a control. They all revealed improved gain in weight and in height – at least in stunted children. In the two trials in Kenya that supplied meat, it did not increase gain in weight but did improve cognitive function more than milk. Meat, and especially milk, is an important source of vitamin B12 for children, and longer-term supplementation with ASF can improve B12 status of children. This micronutrient...
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is deficient in many populations consuming low amounts of ASF, and inadequate amounts are obtained from breast milk of deficient mothers. Benefits of adding fish to children's diets have been little evaluated, but there was no effect on growth or micronutrient status when it was added to a weaning cereal in Ghana. No trials evaluated the benefits of increasing egg intake. Cow's milk can be a good fortification vehicle for micronutrients, such as iron, supporting its positive effects on child growth. Potentially problematic issues concerning the use of cow's milk in children's diets have been poorly explored in the developing country context.

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Discussion

Dr. Bhattacharya: I am very interested in the topic you have presented with regard to the population effect of borderline malnutrition. Your findings are very nice, especially the qualitative measures that you included like parents reporting fewer health side effects or teachers saying that the children are getting more active; I think they are very important issues to research in this part of the world. However, you showed how difficult it was to make the children eat beef. I think there must be another composite model, and I would be very interested to talk to you about this because I think there are certain solutions that we can exchange.

Dr. Allen: Thank you, I appreciate your comments. It actually took us months to get the recipe right so these little kids in Guatemala would eat this beef. Once we did, they loved it. The point you are making of course is that if we are talking about micronutrients and perhaps fatty acids and so on, how to do this, that of course is part of the rationale for all of these studies with lipid-based nutrient spreads. The idea is, you just take a teaspoon, or perhaps you take two teaspoons for older children and pregnant women and mix it with the normal food, and theoretically they are going to eat this happily for the rest of their life. It's going to be very interesting to see this, but the idea is to give the concentrated source of these nutrients without large amounts of energy.

Dr. Bhattacharya: My point, being a developmental pediatrician, is how to get the food to them so that they accept it. I think we can work out a composite model which can be researched.

Dr. De Beer: I would like to find out more about the milk that you used in these trials; was it fresh milk? Did you ever consider powdered milk which in most cases is iron fortified? And have you ever considered the use of fermented milk in any of these trials?

Dr. Allen: In the Kenya trial, it was just fresh UHT milk. In Vietnam, it was also fresh milk in cartons. In New Guinea it was dried skim milk powder, I believe, normally fresh milk. I don't believe that anybody has shown that adding extra micronutrients to milk improves child development, but this is the basis, for example, of large national studies. For example, in Mexico the government is providing essentially dried milk fortified with micronutrients to the population, and that has been shown to improve growth of children and linear growth in the population. So you are right, when you read these papers, everybody is trying something different.

Dr. De Beer: And fermented milk?

Dr. Allen: I don't know of any experience with that; there are no good intervention studies.

Dr. Mouane: I have a question about meat. Studies often talk about beef meat, but what about chicken meat? It's more affordable.

Dr. Allen: Yes, I realize there are a lot of cultural aspects to these studies. Chicken meat, as far as I know, should be virtually the same in terms of its nutrient content. There have been education interventions in Peru and in Malawi. In Malawi, the participants were advised to increase fish intake and reduce phytate and so on, and in Peru
it was all sorts of animal source foods in a low-income community. In both of those, you saw improvements in iron status, but there were no other demonstrated benefits of the intervention. It's very hard, unless you are doing a real intervention study giving this every single day, to see impacts of giving most foods. They are slow to show effects, and so the hope is that this would be somehow available for the usual diet of the population.

Dr. Haschke: I have a question concerning your reference values of vitamin B₁₂. If we consider the American or the European child as the reference, the Indian child will be substantially below that reference intake. But this does not necessarily indicate deficiency. In India, a substantial segment of the population consists of vegetarians. Shouldn’t we have reference values for the vegetarian child population, with no signs of B₁₂ deficiency?

Dr. Allen: That’s a very complex question and that’s of course exactly why I am trying to do the intervention studies to prove that it matters. If I compare the plasma levels of the Guatemalan infants with those from studies where the children have been clinically diagnosed as having delays and hypotonia and all the other symptoms of B₁₂ deficiency, certainly the ones in the deficient group are the same as those children in industrialized countries who have been revealed to have these different problems. So that’s part of my answer. Also I think that we don’t know all of the adverse effects of B₁₂ yet. The hematology is one of the last things to change, it’s not the right outcome to be looking at. Certainly in India, there is elevated methylmalonic acid showing metabolic insufficiency of B₁₂ in both the marginal and the deficient groups, and then there are all the concerns about epigenetics, methylation in pregnancy and risk of chronic disease and obesity in later life and so on. So vitamin B₁₂ is one of the more undiscovered nutrients, and you are absolutely right, we have to prove that these low levels are not healthy, and investigate the associations that they have with maternal depression, poor motor development and poor school performance, both in Kenya and in Guatemala.

Dr. Haschke: I would like to add something. My question was related to a nonmalnourished population with vegetarian eating habits. There is a debate whether vegetarian eating habits are associated with longer lifespan and better health. Therefore, being a vegetarian could be an advantage rather than a disadvantage.

Dr. Allen: Well, it depends on what age group you are talking about. I say it’s very clear that it is a disadvantage for young children; it may not be for older persons. It’s very important with B₁₂ to know that usual intake parallels serum concentrations. You don’t have to be a vegetarian, you can just avoid meat and be relatively deficient. So it’s a continuum, but regarding young children, the Dutch macrobiotic studies where some fish was consumed by children showed very greatly impaired development of those children. I think it might be a different case if you are talking about adult diseases and reducing risk of those diseases compared to having too much meat; moderation is the answer here.

Dr. Fewtrell: I just wanted to ask you about the acceptability, affordability and availability of the meat; for example, in your Kenyan population would they actually have access to meat beyond the period of the study, and would it normally be consumed by that population? Secondly, I wondered if you have any thoughts about the use of liver as a source of meat.

Dr. Allen: Very important issue. In that supplement of the Journal of Nutrition which I described to you, there are actually several papers talking about the feasibility of producing animals for meat and the effect on the economic status of the household. It doesn’t have to be beef, it can be chicken and so on. In these populations, this project actually was involved in increasing the production of dual purpose goats, for example, which was very successful, provided a living for the women, and providing
more meat for the community. This won’t be possible everywhere and absolutely, I am not saying it is, but it is much more feasible than one might imagine. Growing chickens is another strategy which has been worked with local veterinarians to reduce poultry diseases. We tried that in Nepal and it was really quite successful in terms of increasing meat intake of children. It’s not something we should just discount out of hand as being impossible, and you should read the literature on how this can be a terrific boon for women in the communities in terms of increased income as well.

Dr. Mohan: In India, a majority of vegetarians drink milk; in fact, a lot of vegetarians eat eggs too. So, at least in my experience, we have not seen a lot of patients who have been taking milk to be B12 deficient, but yes we do come across B12-deficient children who do not take milk for reasons of taste. My question is, this 80% which has been looked into, which population was it, maybe a population that was not even taking milk?

Dr. Allen: These are results of Helga Refsum and Ranjan Yajnik, so probably around the Pune area in particular, but I think it was a mixture of children in Pune and the surrounding location.

Dr. Mohan: I would only say that that’s not a true representation of the country because we don’t get to see that, most of them do take milk.

Dr. Adrianasolo: My question is related to the methodology. Apart from meals given at school, did you also have controlled data on food eaten at home each day by each child?

Dr. Allen: There were 24 days of food intake for each child, actually 48 over the 2 years. So, yes, we had an enormous amount of data on that. There was a very slight increase in energy intake in the meat and milk group, but the calculation showed that that probably was expended in higher activity levels of those children. All of these results still remained controlling for usual intake, and the intake at home. The paper on that by Murphy is in that supplement to the Journal of Nutrition.

Dr. Dehbi: I would like to take the opportunity to make comments about nutritional problems. Research is very pertinent and is essential for us to improve our practice, but in developing countries milk and meat are very expensive and we cannot afford them. It’s not sufficient to continue to think about just how to resolve nutritional problems clinically and by research. We need to think about this problem more deeply than this. Without a global approach, we can’t resolve our nutritional problems, so if we need to use milk and meat for our children in developing countries, we need to improve family income. In parallel to research, in parallel to our medical practice we need to develop other strategies to take care of the real determinants of these nutritional problems. Without this approach we can’t improve the nutritional status of our children.

Dr. Allen: I see this is a sort of a hierarchy of alternatives, often using more than one together. For example, where a country could afford to provide school milk for children this would seem like a very good thing to do if the situation requires that the children be provided with better nutrition, and the economic argument would be that building human capital in this way is a very good investment for a country. The second level is perhaps providing the micronutrients in a much cheaper way. With the kind of milks that Dr. Michaelsen is talking about, that are much cheaper and governments could support and subsidize and could be produced locally. The third one is providing the micronutrients in a variety of different ways, and the fourth would just be a micronutrient supplement. These are a continuum of interventions. And food fortification, fortification of staples might provide most of these nutrients in a country where there are certain staples that are providing a large part of the children’s nutrients. So the right mix depends on the situation, and we don’t know all the answers. But certainly you can look at the nutrient intake of children and see which
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nutrients are missing. There is never any substitute for getting some good food intake data and that is not that difficult, and then saying what the gaps are, and then how do we meet those by different strategies, starting with the most affordable and the most coverage for that kind of intervention.

Dr. Michaelsen: Some of the effects that you have shown could most likely also be seen after eating some underutilized animal foods like small fish, organs like intestines and kidneys, snails, as we have seen in the market here in Marrakesh, and insects, like mopane worms eaten in southern Africa. These underutilized foods are often cheap and should be promoted where culturally acceptable.

Dr. Allen: I think that's true in theory, and the farmers will be very happy if you are eating all their insects. But when you figure out how much it probably takes, it's probably not enough. I didn't answer the question about liver. One of WHO's recommendations is for young children to eat liver, because it's one of the few ways you can get enough available iron and zinc. The trouble is, if you eat the amounts recommended to supply your needed iron and zinc, you are probably at toxic levels of vitamin A, and there are not enough chicken livers in the world. But in Guatemala, we see intestines and so on as the main source of meat for some poor families, and they do correlate at least with B12 status that.

Dr. Ashish: Is the gut flora an important source of vitamin B12? The reason I am asking is that it is widely believed that our previous generations in India did not become significantly vitamin B12 deficient because there was an overgrowth of gut flora possibly due to poor quality drinking water, and as time goes by and water becomes more and more affordable we would have less amount of gut flora and vitamin B12 deficiency will become more obvious in the population. Do you have any comment on that?

Dr. Allen: I am familiar with the proponents of that theory, but I would maintain that really B12 status has always been an issue in populations with a low source of animal foods, and this is not a new phenomenon. I actually disbelieved completely this theory until actually Ranjan Yajnik talked about a study he had done. He measured the B12 on fruits and vegetables before and after they have been sitting on the counter a whole night and actually showed there was an increase, but still the amounts were extremely low and unlikely to support adequate nutrition. So, if you do those calculations, it doesn't adapt to meeting even close to requirements.

Dr. Boukari: In this intervention study, have you looked at other micronutrients like zinc, and could you make some comments on the data on the zinc status in these populations?

Dr. Allen: In Kenya or Guatemala, we did not see an effect of any of the interventions on serum zinc. Serum zinc is very hard to shift. There were also a lot of infections, there was a high prevalence of malaria, and all of these things were possible confounders. Even though we corrected for C-reactive protein and all these things, there was still a tremendous amount of noise among children, and you could see it depended on whether they had malaria recently or not. So, we really haven't seen any effects yet. I am not sure that any food intervention study has shown an improvement in serum zinc, I don't think so. It's hard enough with fortified flour to see the improvement. Supplements work, but there is only one study showing an effect of zinc fortification of flour on zinc status.

Dr. Boukari: Do you think zinc plays a role in infant nutrition?

Dr. Allen: Probably yes. Certainly, zinc deficiency is associated with stunting, but it has to be fairly severe deficiency and very low plasma zinc, and probably that's why meat was more effective at restoring activity and muscle mass and so on than milk, but that's a conjecture at this point.
Evidence for Acne-Promoting Effects of Milk and Other Insulinotropic Dairy Products

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Abstract
Acne vulgaris, the most common skin disease of western civilization, has evolved to an epidemic affecting more than 85% of adolescents. Acne can be regarded as an indicator disease of exaggerated insulinotropic western nutrition. Especially milk and whey protein-based products contribute to elevations of postprandial insulin and basal insulin-like growth factor-I (IGF-I) plasma levels. It is the evolutionary principle of mammalian milk to promote growth and support anabolic conditions for the neonate during the nursing period. Whey proteins are most potent inducers of glucose-dependent insulinotropic polypeptide secreted by enteroendocrine K cells which in concert with hydrolyzed whey protein-derived essential amino acids stimulate insulin secretion of pancreatic β-cells. Increased insulin/IGF-I signaling activates the phosphoinositide-3 kinase/Akt pathway, thereby reducing the nuclear content of the transcription factor FoxO1, the key nutrigenomic regulator of acne target genes. Nuclear FoxO1 deficiency has been linked to all major factors of acne pathogenesis, i.e. androgen receptor transactivation, comedogenesis, increased sebaceous lipogenesis, and follicular inflammation. The elimination of the whey protein-based insulinotropic mechanisms of milk will be the most important future challenge for nutrition research. Both, restriction of milk consumption or generation of less insulinotropic milk will have an enormous impact on the prevention of epidemic western diseases like obesity, diabetes mellitus, cancer, neurodegenerative diseases and acne.

Acne vulgaris, the most common dermatological disease, has evolved to an epidemic in western countries, affecting more than 85% of adolescents and often persisting into adulthood [1]. Adults in the US within the age range of 20–29 years, exhibit acne prevalence rates of 50.9% in women and
42.5% in men, respectively [2]. In contrast, acne is absent in non-westernized populations such as the Inuit, Okinawa islanders, Ache hunter-gatherers and Kitavan islanders [3] which do not consume milk and dairy products and ingest less carbohydrates with high glycemic index (GI) [3]. Thus, epidemiological observations clearly point to nutritional factors in the etiology of acne.

Until 2005, cross-sectional, case-control, cohort, and clinical intervention studies designed to address the relationship between diet and acne failed to incorporate adequate controls, objective measures, and appropriate statistical analyses. However, well-designed prospective studies published since 2005 provided evidence that components of western diets, particularly milk and dairy products and diets enriched in carbohydrates with high GI and glycemic load (GL) are associated with acne [4]. It has been recognized that acne pathogenesis is closely related to the consumption of insulinemic foods providing increased growth factor signaling of insulin and insulin-like growth factor-I (IGF-I) [5, 6]. Insulinotropic food, especially refined sugars and grains, potatoes, milk and dairy products are ubiquitous elements in western diet and comprise nearly 50% of the per capita energy intake [7].

**Epidemiological Evidence for the Relation between Milk Consumption and Acne**

Retrospective evaluation of the data of the Nurses Health Study II of 47,355 women who completed questionnaires on their high school diet and physician-diagnosed severe teenage acne showed a positive association with acne for intake of total milk and skim milk [8]. Prospective cohort studies in the US (Growing Up Today Study) in 4,273 teenage boys and 6,094 teenage girls confirmed a correlation between milk consumption and acne [9, 10]. In the study of boys, the strongest association has been found between intake of skim milk and acne [10] which already points to the acne-promoting activity of the protein fraction of milk.

Increased insulin/IGF-I signaling plays a most important role in acne pathogenesis [5, 6]. A correlation between increased facial sebum secretion and IGF-I serum levels has been reported in acne patients [11], whereas isotretinoin treatment, the most effective sebum suppressive anti-acne agent, decreased IGF-I serum levels [12].

In 2,109 European women, a positive correlation between dairy consumption and IGF-I serum levels has been observed [13]. A recent systematic meta-analysis confirmed the relationship between milk consumption and IGF-I serum levels [14]. Most convincingly, the multicenter study group of the European Prospective Investigation into Cancer and Nutrition has presented data for a significant correlation between milk protein consumption and IGF-I serum levels in 4,731 male and female participants [15].

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Combinations of Milk and Carbohydrates Potentiate Insulinotropic Effects

It has been demonstrated in a randomized, investigator-masked, controlled trial that a low GL diet resulted in clinical improvement of acne and reduced sebum secretion [16]. A great part of processed food products of western diet are combinations of milk, milk protein, carbohydrates and sugar. A typical western breakfast of adolescents consists of milk and cornflakes, a combination of insulinotropic milk protein and hyperglycemic carbohydrates. The addition of an ordinary amount of 200 ml milk to a meal with a low GI increased the insulin response by 300% to a level typically seen from a meal with a very high GI like white bread [17]. A lot of food products of industrialized countries are combinations of milk and carbohydrates and sugar like chocolate and ice cream. A most critical development is the abuse of highly insulinotropic whey protein concentrates – provided in 5-kg buckets – in the body building and fitness environment [18]. The daily intake of 40 to 80 g of concentrated whey protein is a usual procedure during muscle training periods. As 1 liter of milk contains approximately 6.6 g whey proteins, the whey abuse corresponds to a daily milk intake between 6 l and 12 l. Moreover, insulinotropic whey protein concentrates are often combined with synthetic growth hormone (GH), insulin, IGF-I as well as androgens, which all potentiate growth factor signaling [18].

Milk Consumption Elevates Postprandial Insulinemia, GH and IGF-I Plasma Levels

After a month of drinking 710 ml of UHT vitamin D-fortified whole milk daily, Mongolian children, prior not used to milk consumption, had a higher mean plasma level of IGF-I, higher IGF-I/IGFBP-3, and 75th percentile of GH levels [19]. The mean plasma IGF-I levels were significantly raised in the children after 4 weeks of milk consumption by 23.4% from mean pretreatment values of 290.93 ± 93.98 to 358.34 ± 125.62 ng/ml [19].

Fermented and nonfermented milk products give rise to hyperinsulinemic responses far exceeding what could be expected from their low GIs. Despite a low GI of 15–30, milk products exhibit 3- to 6-fold higher insulinemic indices (II) of 90–98 [20]. A large and similar dissociation of the GI and II exists for both whole milk (GI: 42 ± 5; II: 148 ±14) and skim milk (GI: 37 ± 9; II: 140 ± 13) [21]. It has already been suggested that some factor within the protein fraction of milk is responsible for milk’s insulinotropic effect [21]. Skim milk has been identified as a potent insulin secretagogue in type 2 diabetic patients [20]. Except for cheese with an II of 45, milk and all dairy products including yoghurt, ice cream, cottage cheese, and fermented milk products have potent insulinotropic properties [22]. In a 1-week intervention study of
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24 prepubertal 8-year-old boys, the effect of daily intake of 53 g of either lean meat or skim milk (1.5 l per day) was studied with regard to insulin and IGF-I responses. In the skim milk group, insulin significantly increased by 105% (from 22 to 45 pM) and IGF-I significantly increased by 19% (from 209 to 249 ng/ml) [23]. Remarkably, there was no significant increase in either insulin or IGF-I in the meat group [23].

**Whey Proteins: The Major Inducers of Postprandial Hyperinsulinemia**

The major protein fraction of cow’s milk is casein (80%), and the remaining 20% are whey proteins. Both, whey and casein contain specific proteins and peptides that have growth-stimulating effects. The effect of whey and casein fractions of milk on fasting concentrations of insulin and IGF-I has been examined in 57 8-year-old boys who received over 7 days either whey protein or casein fractions with protein amounts of whey or casein similar to the content of 1.5 l skim milk. In the whey group, fasting insulin increased by 21%, with no change in IGF-I. In the casein group, serum IGF-I increased by 15%, whereas there was no change in fasting insulin [24]. The insulin response to a whey meal has been reported to be higher than that of a milk meal. This differential response suggests that the insulinotropic component of milk resides predominantly within the whey fraction of soluble milk proteins, whereas casein has a stronger IGF-I stimulating effect than whey [24]. It has been shown that specific whey proteins or their enzymatically cleaved peptides function as secretagogues for the release of the intestinal incretin glucose-dependent insulinotropic polypeptide (GIP) [25]. GIP is a 42-amino acid hormone that is produced by enteroendocrine K cells and released into the circulation in response to nutrient stimulation [26] (fig. 1).

**A Possible Role of Bovine Betacellulin in Acne Pathogenesis**

The bovine whey protein fraction contains several active growth factors, in particular high concentrations of betacellulin (BTC) [27], a ligand of epidermal growth factor receptor (EGFR). Remarkably, EGFR faces the gut contents rather than the bloodstream pointing to a special biological function of luminal EGFR expression. In rat duodenum, EGFR has been located at the luminal apical surface of enterocytes in the brush border [28], whereas in humans EGFR was detected at the basolateral membrane of enterocytes [29]. It has been hypothesized by Cordain [30] that milk-derived BTC is taken up and internalized by luminal EGFRs and released by a transcellular EGFR route into the lymphatic circulation and blood plasma, where BTC stimulates EGFR expressed on keratinocytes and sebocytes. The human and bovine BTC
Acne-Promoting Effects of Milk and Dairy

Precursors share 88% sequence identity and it is generally expected that BTC is absorbed through the small intestine. Of great importance is the mitogenic effect of BTC on β-cell stimulation and insulin secretion. BTC plays an important role in β-cell mass regulation and growth. BTC binding to EGFR results in the activation of the phosphoinositide-3 kinase (PI3K)/Akt pathway leading to reduced nuclear levels of FoxO1 [31]. Thus, milk-derived bovine BTC may directly stimulate sebocyte and keratinocyte proliferation via binding to EGFRs and may induce an indirect stimulation of the cells of the pilosebaceous unit by BTC-induced upregulation of insulin secretion (fig. 1).

Thus, an accumulated body of evidence points to the most critical role of the whey protein/whey growth factor signaling network in the elevation of postprandial insulin plasma levels. The concomitant increase in the more slowly reacting basal IGF-I plasma levels after prolonged consumption of milk and dairy protein can be explained by increased insulin-mediated hepatic IGF-I synthesis as well as intestinal IGF-I absorption from the casein fraction. Thus, overactivation of the whey protein/BTC enteroinsular axis by consumption of whey proteins and BTC results in overstimulated GIP and most likely EGFR signaling which both contribute to milk-induced hyperinsulinemia and increased IGF-I plasma levels. Elevated insulin/IGF-I signaling has been

![Fig. 1. Impact of milk protein consumption on enteroinsular-pilosebaceous signaling network. AA = Essential amino acids; IR = insulin receptor.](image-url)
related to nutrigenomic dysregulation of the metabolic sensor and transcription factor FoxO1 which controls important target genes and nuclear receptors involved in acne pathogenesis [5, 6] (fig. 2).

**Evidence for a Milk-Entero-Pituitary Axis?**

The pathophysiology of acne appears to be closely linked to the enteroin-sular axis. GIP is rapidly released from K cells by nutrient stimulation with glucose and whey protein. GIP is an important incretin which stimulates insulin secretion of pancreatic β-cells. GIP itself exerts growth-promoting and anabolic effects on various other organ systems like adipose tissue by binding to GIP receptors (GIP-Rs). Most intriguingly, GIP-Rs have been found in the pituitary [32], where GIP may activate GH secretion as observed in acromegalic patients [33]. GIP-stimulated GH release from the pituitary may be a fundamental signaling mechanism of whey proteins during the nursing period.
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(fig. 1). In fact, Mongolian children not used to milk consumption exhibited markedly higher GH levels after 1 month of drinking milk [19]. Thus, whey-induced GIP stimulation not only activates pancreatic insulin secretion, but most likely stimulates the secretion of pituitary GH which is the strongest inducer of hepatic IGF-I synthesis. Moreover, GH has been shown in dogs to enhance the insulin secretory response to GIP.

Existence of a Milk-Pituitary-Adrenal-Sebocyte Axis?

GIP enhances pituitary ACTH release and is involved in the regulation of the hypothalamus-pituitary-adrenal axis [34]. ACTH is a ligand of melanocortin-5 receptor [35] which is upregulated in differentiated sebocytes stimulating lipogenesis [36]. GIP-Rs are also present in the zona fasciculata reticularis of normal adrenals and are particularly abundant in some types of adrenocortical adenomas and adrenal hyperplasia. This interaction may be of importance in acne pathogenesis, as increasing DHEAS plasma levels are associated with hormonal changes of prepuberty and the development of acne comedonica. Furthermore, in the sebaceous follicle, DHEAS is converted in an intracrine manner to testosterone and the more potent dihydrotestosterone. The activity of the converting enzyme, 5-α-reductase, is IGF-I dependent.

Nutrigenomic Effects of Milk on FoxO1 Signaling in Acne Pathogenesis

Sebocytes and follicular keratinocytes express androgen receptor (AR), EGFR, FGFR2, insulin receptor, IGF-I receptor (IGFIR) and GHR. The IGF-I/IGFIR-mediated activation of the PI3K/Akt pathway has been shown to play a crucial role in upregulation of sterol response element binding protein-1 expression associated with increased sebaceous lipogenesis [37].

Five main factors play a pivotal role in the pathogenesis of acne: androgen dependence, follicular retention hyperkeratosis, increased sebum production, increased colonization with *Propionibacterium acnes*, and inflammatory follicular events (fig. 2). Puberty is a period of transient insulin resistance with increased GH secretion and elevated hepatic IGF-I synthesis. Milk consumption mimics the endocrine signaling of puberty and increases GH and IGF-I plasma levels [19]. In this regard, physiological GH/insulin/IGF-I signaling of puberty is superimposed by nonphysiological GH/insulin/IGF-I signaling due to milk consumption, the potent growth factor signaling network of bovine milk.

The resulting net effect of elevated growth factor signaling during puberty and persistent growth factor signals of western diet due to increased dairy consumption finally overstimulate the export of nuclear FoxO1 into the cytoplasm via overactivation of the PI3K/Akt pathway [38]. In the absence of growth fac-
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tors, nuclear FoxO1 suppresses nuclear receptors (AR, PPAR\(\gamma\)) and key genes and transcription factors of cell proliferation (cyclin D2), matrix modulation (matrix metalloproteinases), lipid biosynthesis (sterol response element binding protein-1) and inflammatory signaling (NF\(\kappa\)B) [5]. With increased growth factor signaling, however, genes and nuclear receptors involved in acne are de-repressed, leading to increased AR-mediated signal transduction [39], increased cell proliferation of androgen-dependent cells, induction of sebaceous lipogenesis [37], upregulation of Toll-like-receptor-2-dependent inflammatory cytokines and decreased synthesis of antimicrobial peptides.

In contrast, retinoids, antibiotics and dietary intervention may increase the nuclear content of FoxO1 [5], thereby normalizing increased transcription of acne target genes upregulated by increased diet-mediated growth factor signaling. Various receptor-mediated growth factor signals and especially whey proteins and carbohydrates induce elevations of the insulin/IGF-I signal transduction pathways, which are finally integrated at the level of PI3K/Akt activation resulting in a nuclear FoxO1 deficiency, the underlying nutrigenomic mechanism in the pathogenesis of acne [5].

**Acne-Cancer Relationship**

There is now considerable evidence that insulin/IGF-I signal transduction networks play an important role in neoplasia [40]. Acne is often associated with disorders of increased growth factor signaling and insulin resistance, like polycystic ovary syndrome, acromegaly and Apert syndrome with a gain of function mutation of fibroblast growth factor receptor-2 [6]. All these diseases are associated with an increased incidence of cancer. Polycystic ovary syndrome is associated with an increased risk for endometrial cancer and diabetes mellitus. Patients with acromegaly have an increased prevalence of colorectal cancer, breast cancer and prostatic malignancies. Severe acne in males has been associated with an increased risk of prostate carcinoma [41]. Intriguingly, a recent meta-analysis confirmed that a high intake of dairy products is related to an increased risk of prostate cancer [42]. It is conceivable that elevated insulin/IGF-I signal transduction may stimulate proliferation of androgen-dependent cells of the prostate by FoxO1-mediated upregulation of AR transactivation and subsequent androgen-dependent growth factor signaling [39].

In the field of oncology, recent efforts are undertaken to reduce increased insulin/IGF-I signaling by the administration of insulin-sensitizing agents like metformin or IGFIR and EGFR antagonists [40, 43]. However, all these pharmacological efforts affect the ‘too late’ efferent site of western nutrition and lifestyle. It would be more prudent to control the afferent, causative mechanisms of acne and cancer promotion associated with increased growth factor signaling of insulinotropic western diet.
### Table 1. Proposed impact of milk-induced GIP/insulin/IGF-I oversignaling in the pathogenesis of acne and other chronic western diseases [44]

<table>
<thead>
<tr>
<th>Stage</th>
<th>Increased GIP, insulin and IGF-I plasma levels</th>
<th>Disturbed</th>
<th>Allergy, atopy, autoimmune diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-natal</td>
<td>Increased insulin-IGF-I signaling in the thymus</td>
<td>Disturbed T cell maturation, impaired T cell apoptosis</td>
<td>Fetal macrosomia, disposition for obesity</td>
</tr>
<tr>
<td></td>
<td>Increased placental growth and maternal glucose transport</td>
<td>Disturbed</td>
<td>Fetal macro somia, disposition for obesity</td>
</tr>
<tr>
<td></td>
<td>Post-natal</td>
<td>Increased GIP, insulin and IGF-I plasma levels</td>
<td>Disturbed neonatal programming of the somatotropic IGF-I axis</td>
</tr>
<tr>
<td>Pre-natal</td>
<td>Overstimulation of physiological growth factor signaling of puberty, nuclear FoxO1 deficiency</td>
<td>Promotion of acne, increased androgen signaling, increased sebogenesis, comedogenesis</td>
<td>Epidemic acne, persistence of acne in adulthood</td>
</tr>
<tr>
<td>Adolescence</td>
<td>Overstimulation of physiological growth factor signaling of puberty, nuclear FoxO1 deficiency</td>
<td>Overstimulation of the pancreatic β-cells</td>
<td>Early onset of replicative β-cell senescence</td>
</tr>
<tr>
<td>Adulthood</td>
<td>Overstimulation of pancreatic β-cells</td>
<td>Promotion of atheroma formation</td>
<td>Coronary heart disease, stroke</td>
</tr>
<tr>
<td>Adulthood</td>
<td>Overstimulation of endothelial and smooth muscle cells and increased lipogenesis</td>
<td>Overstimulation of the insulin/IGF-I signaling network</td>
<td>Stimulation of the oncogenic PI3K/Akt pathway</td>
</tr>
<tr>
<td>Adulthood</td>
<td>Overstimulation of neuronal cells</td>
<td>Overstimulation of adipocyte differentiation and lipogenesis</td>
<td>Imbalance between protein synthesis and degradation, diabetes of the brain</td>
</tr>
<tr>
<td>Adulthood</td>
<td>Overstimulation of neuronal cells</td>
<td>Overstimulation of the insulin/IGF-I signaling network</td>
<td>Early onset of neurodegenerative diseases</td>
</tr>
</tbody>
</table>

**Note:** This table summarizes the proposed impact of milk-induced GIP/insulin/IGF-I oversignaling in the pathogenesis of acne and other chronic western diseases.
Conclusion

Acne, an epidemic skin disease in countries with western nutrition, is promoted by increased consumption of insulinotropic food, especially milk, dairy products as well as high loads of carbohydrates with high GI. Acne can be regarded as the visible metabolic syndrome of skin displaying the daily adverse effects of exaggerated insulinotropic nutrition. Therefore, acne is a useful clinical and epidemiological indicator of optimized healthy human nutrition.

Nuclear FoxO1 deficiency induced by increased insulin/IGF-I signaling upregulates all major mechanisms of acne pathogenesis [5]. Whey proteins are most important essential signaling proteins promoting increased insulin/IGF-I signaling for growth and survival of mammalian neonates. By million years of evolution, this signaling system of mammalian milk is exclusively and physiologically provided to the newborn only during the nursing period. The chronic ‘abuse’ of this mammalian postnatal signaling system by widespread cow milk and dairy consumption in humans of industrialized societies has been proposed to be the major cause of the acne epidemic and the more serious chronic western diseases [44].

There are two solutions to this problem: the restriction of milk protein consumption or the elimination of the insulinotropic effectors of milk. The attenuation of the whey protein- and whey growth factor-based insulinotropic signaling mechanisms of milk will be the most important future challenge for an interdisciplinary cooperation between medicine, molecular biology, nutrition research and milk processing biotechnology. The goal will be to reduce the II of milk to a level corresponding only to its carbohydrate moiety. The generation of less insulinotropic milk and milk products with an II below 45 will have an enormous impact on the prevention of epidemic western diseases like obesity, diabetes mellitus, cancer, neurodegenerative diseases and acne (table 1) [44].

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Discussion

Dr. Hernell: Do you intend to do a blinded study? You could use encapsulated whey protein powder to see whether there is an effect on acne or not. I think that would be useful. The second question is, how organ specific is the effect on lipogenesis? Do you see it also in other organs? With high IGF-I level, do you for instance see a change in plasma lipoproteins with increased VLDL secretion by the liver?

Dr. Melnik: The sebaceous gland is not the only target exhibiting stimulated lipogenesis by increased IGF-I signaling. The differentiation of preadipocytes to adipocytes is also IGF-I dependent [1]. It is well known that insulin and IGF-I regulate hepatic VLDL synthesis via nuclear extrusion of the transcription factor FoxO1. However, in the liver VLDL synthesis and secretion are downregulated by insulin and IGF-I signaling. Intriguingly, milk consumption, especially whey protein has been shown to stimulate the secretion of the intestinal incretin glucose-dependent insulinotropic polypeptide (GIP), which has strong adipogenic effects in adipocytes that express the GIP receptor. It will be important to intensify studies focusing on whey protein-mediated GIP responses [2]. Formula-fed infants gain weight more easily than breastfed infants. Whey-based formula-fed infants exhibited high postprandial serum levels of GIP and insulin. Especially hydrolyzed α-lactalbumin provokes a strong GIP response with increased postprandial GIP serum concentrations [3, 4]. The transcription factor FoxO1 plays a crucial role in adipogenesis and is regulated by insulin and IGF-I [5, 6]. Insulin and IGF-I-mediated regulation of the transcription factor FoxO1 plays also an important role in insulin secretion of pancreatic β-cells [7–9]. Thus, the insulinotropic effect of milk with increased insulin and IGF-I signal transduction modifies the transcription factor FoxO1 in various cell systems, the sebocyte, the adipocyte...
Acne-Promoting Effects of Milk and Dairy

and the pancreatic β-cell, explaining the observed insulinotropic, lipogenic and adipogenic effects of milk consumption.

Dr. Rock: As you move into the cancer arena here, I have to add a cautionary note and comment on a few things. Although IGF-I is certainly involved in the whole signaling and cell proliferation and differentiation regulation, it’s really only a player, and in fact I would argue that emerging evidence suggests that it’s simply eating a calorically dense diet that has the effect rather than milk per se. The primary problem is insulin resistance, probably triggered by an inflammatory response in relation to obesity and excess caloric intake. Inflammatory markers trigger the insulin resistance, and hyperinsulinemia has this effect of stimulating the synthesis of IGF-I. And then the other part that I don’t see on your graphics is the effect of insulin on synthesis of sex hormone-binding globulin, so although you mention androgens as being a dated idea, I would argue that the reproductive hormones are a part of this pathway too, so it’s reductive to focus only on IGF-I.

Dr. Melnik: I agree, increased IGF-I is only one important player of the cancer-promoting effect of milk. There is also evidence that milk consumption induces basal serum insulin levels as well as insulin resistance [10]. Insulin and IGF-I via modulation of the nuclear content of FoxO1 also have an impact on androgen metabolism. FoxO1 is a nuclear corepressor of the androgen receptor. Thus, increased insulin and IGF-I signaling results in increased androgen receptor transactivation [11]. Milk-driven insulinotropic signaling via insulin/IGF-I-mediated nuclear exclusion of FoxO1 may stimulate androgen-mediated cancerogenesis in the prostate, a possible link confirmed by epidemiological evidence [12]. There is more experimental evidence for the cancer-promoting effect of milk consumption in anthracene-induced mammary cancers in rats fed a cow’s milk diet in comparison with rats without cow’s milk in their diet [13]. Milk-fed rats doubled their increase in tumor volume and tumor mass. With regard to insulin/IGF-I/FoxO1 signaling, it is remarkable that the anthracene-induced tumor system is phosphoinositol-3 kinase (PI3K)/Akt dependent. A further milk-mediated upregulation of PI3K/Akt stimulation may explain the observed milk-stimulated tumor progression in anthracene-induced mammary tumors in rats [14].

Dr. Martin: Thank you very much for a very nice talk. The signaling information was very convincing. How do you tie all that in with systematic reviews showing that increased milk intake is associated with a reduced risk of colorectal cancer, breast cancer, cardiovascular disease, diabetes and stroke, such that overall, in absolute numbers, milk seems to have an overall survival advantage [15]?

Dr. Melnik: Epidemiological studies of the past concerning milk and dairy consumption and their relation to chronic Western diseases gave conflicting results most likely because they did not differentiate between strong insulinotropic and less insulinotropic milk products. Studies which mixed dairy products with variable insulinemic index are thus not suitable to present a clear picture of the situation.

Dr. De Beer: You mentioned that in your clinical practice you advise your patients to reduce the milk intake and go on a low glycemic index diet. My question to you is, if we consider that acne is a multifactorial disease, how big is the role that milk or a low glycemic index diet might play, and if you then divide the two, which one is the most important, the milk or the low glycemic index diet?

Dr. Melnik: Both milk and insulinotropic milk products and hyperglycemic carbohydrates play a role in the induction or aggravation of acne [16–18]. At present, studies comparing the effects of diets with milk vs. high glycemic carbohydrates or combinations of both on the course and activity of acne are missing. It has to be expected that the combination of milk with high glycemic carbohydrates will provoke the strongest effect on acne because it has been shown that the addition of milk to a meal with a low glycemic index significantly increases postprandial insulin response [19].
Dr. Saldanha: Concerning the drug isotretinoin, do you know studies correlating the use of this drug and the final height of adolescents? I mean are we treating acne and getting shorter adults?

Dr. Melnik: Yes, it is possible with high isotretinoin doses like those given to patients with ichthyosis and other generalized disorders of keratinization. It has recently been demonstrated that isotretinoin treatment of acne patients lowered serum IGF-I levels and growth hormone [20]. Growth hormone/IGF-I signaling of puberty is superimposed by insulinotropic signaling of western diet. I could recently provide indirect evidence for isotretinoin-mediated increase in nuclear FoxO1 [21].

Dr. Jongpiputvanich: You suggested that acne is more related to IGF-I than androgen level. I wonder what would happen if we conducted a randomized-controlled trial in elderly persons and included one group with a high amount of milk intake and another with no milk intake.

Dr. Melnik: Indeed, milk consumption raises serum IGF-I levels and via IGF-I-mediated extrusion of FoxO1 from the nucleus increases androgen receptor transactivation. IGF-I also increases the free androgen index by downregulating sex hormone-binding globulin. IGF-I also stimulates 5α-reductase activity and adrenal DHEAS and gonadal androgen synthesis. Thus, IGF-I has a strong influence on androgen-mediated signal transduction [22, 23].

References

Acne-Promoting Effects of Milk and Dairy


Milk Proteins in the Regulation of Body Weight, Satiety, Food Intake and Glycemia

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Abstract

Consumption of dairy products and their milk proteins increase satiety and reduce food intake and blood glucose response when consumed alone or with carbohydrate. Dairy proteins are of interest because proteins are more satiating than either carbohydrate or fat, and they regulate food intake and metabolic functions by the combined actions of the intact protein, encrypted peptides and amino acids on gastrointestinal and central pathways. As shown in this review, milk proteins have physiologic functions that contribute to the maintenance of a healthy body weight and control of factors associated with the metabolic syndrome through their effects on mechanisms regulating food intake and blood glucose. More recent reports show that these benefits can be achieved within the range of usual consumption of dairy. In addition, recent research points to an intrinsic value of small amounts of milk protein or dairy consumed shortly before a meal to reduce the glycemic response to carbohydrate and that this is not at the cost of increased demand for insulin.

Introduction

A role for dairy consumption and its physiologic functionality beyond the provision of nutrients in the management of obesity and metabolic syndrome is of interest because epidemiological studies have reported strong associations between higher dairy consumption and lower body weight [1]. Additionally, experimental studies show that consumption of dairy products and their milk proteins enhance satiety and reduce short-term food intake and blood glucose response when consumed alone or with carbohydrate.
Dairy proteins are of interest because proteins are more satiating than either carbohydrate or fat [2]. Milk proteins have received considerable investigation and have been shown to regulate food intake and metabolic functions through gastrointestinal and central pathways by the combined actions of the intact protein, encrypted bioactive peptides (BAPs) and amino acids [1, 3].

The following provides a review of the role of dairy in the regulation of body weight and the role of cow's milk proteins in the regulation of satiety, food intake, blood glucose and their mechanisms of action.

**Dairy Consumption, Body Weight and the Metabolic Syndrome**

In recent years, numerous studies have associated milk and dairy consumption with favorable effects on body weight and metabolic control. Several epidemiological studies of adults have reported an inverse association between frequent dairy intake and adiposity as measured by the body mass index [1, 4]. In addition, a dietary pattern that included increased consumption of whole fruit and milk (skimmed and partly skimmed) were the two food patterns among 51 foods that were associated with the prevention of body weight increase in adults followed for 6 years in the Quebec Family Study [5].

Dietary patterns characterized by increased dairy consumption have also demonstrated a strong inverse association with insulin resistance, risk of type 2 diabetes and cardiovascular disease among overweight adults [1, 4, 6]. In the Health Professionals Follow-up Study, male participants with no history of diabetes, cardiovascular disease or cancer at baseline were followed for 12 years. Each serving per day increase in total dairy intake was associated with a 9% lower risk of type 2 diabetes and a corresponding relative risk of 0.88 for low-fat dairy intake [4, 7].

In a 10-year prospective study of dairy intake and the risk of type 2 diabetes in 37,183 middle-aged or older women, a dietary pattern that incorporated higher low-fat dairy products was inversely associated with the risk of type 2 diabetes [8]. Overall, daily consumption of dairy was associated with a 21% lower likelihood of the presence of the insulin resistance syndrome both in Blacks and Whites or men and women [6].

**Characteristics of Cow’s Milk Proteins**

Although the reasons for the observed benefits of increased dairy consumption and lower prevalence of obesity and chronic diseases remain unclear, the physiologic actions of their proteins, beyond providing essential amino acids for protein synthesis, has been offered as an explanation [1, 3, 9].
Physiologic Functions of Milk Proteins

Casein and whey proteins make up 80 and 20% of cow’s milk proteins, respectively, and each are made up of complex proteins of different characteristics [10]. Bovine casein consists of αs1-, αs2-, β-, and κ-caseins, which represent 37, 10, 35 and 12% of whole casein, respectively. Bovine whey protein contains approximately 50% β-lactoglobulin, 20% α-lactalbumin, 10% albumin and lactoferrin, and lactoperoxidase making up the remainder [10]. Both proteins have intense application as value-added ingredients in a wide range of food products [11].

The differences in the physical and chemical properties of casein and whey are attributed to their physiological effects when ingested [1, 3]. Whey protein is rapidly digested (a fast protein), whereas casein is more slowly digested (a slow protein). The classification of casein and whey as ‘slow’ and ‘fast’ proteins is based on their contribution to protein synthesis and their effect on plasma amino acid concentrations [12]. In humans, consumption of whey (0.45 g/kg body weight) resulted in a fast, but short and transient, increase in plasma amino acids that peaked in 40 min to 2 h after its ingestion and returned to baseline values after 3–4 h. Casein, in contrast, and consistent with its slow gastric emptying, results in plasma amino acid concentrations that rose more slowly and are lower, but had sustained a plateau lasting for 7 h.

Amino acids released from milk proteins have a multitude of effects beyond their role in protein synthesis in the body. For example, they are a significant factor accounting for the insulinotropic effect of milk protein. Healthy subjects that ingested a mixture of branched-chain amino acids (BCAAs) including leucine, isoleucine, valine, lysine and threonine had glycemic and insulinemic responses similar to those with intact whey [13]. The high level of BCAAs, specifically the leucine content of milk proteins, may contribute to increased fat mobilization from adipose tissue observed in young obese subjects on high-dairy energy-restricted diet [14].

In addition to providing a high-quality source of amino acids, milk proteins are precursors of BAPs which are released either due to the separate or combined action of digestive, microbial and milk proteases during gastrointestinal digestion or dairy processing. Therefore, BAPs are generated in vivo after the ingestion of products containing milk proteins or externally, in the fermented dairy products such as yogurt, sour milk and cheese. At least 26 BAPs are encrypted in the primary structure of milk proteins and many of them have been isolated from dairy products, including sour milk, yogurts, and cheeses [15]. During digestion, the caseins produce numerous BAPs. The casokinins originate from major subunits of casein, αs1- and β-caseins and inhibit angiotensin I-converting enzyme (ACE), reducing blood pressure [16]. Opioid peptides known as β-casomorphins are also derived from casein and are involved in the regulation of various physiological processes, including food intake regulation, gastrointestinal motility, and plasma insulin concentrations [17]. During cheese-making, κ-casein is hydrolyzed by chymosin into para-κ-casein
and caseinomacropeptide (CMP), which includes the glycosylated form of CMP called glycomacropeptide (GMP). As a result, CMP becomes an ingredient of commercially available whey protein products with its content between 12 and 28% [18].

Peptides derived from whey have physiological functions including the modulation of blood pressure, inflammatory processes, blood glucose and systems regulating food intake. Whey proteins (α-lactalbumin and β-lactoglobulin) are precursors of ACE-inhibitory peptides called lactokinins [16], which have antihypertensive activities.

Many BAPs from milk proteins possess numerous physiologic activities and are considered to be multifunctional peptides. For example, whey-derived α-lactorphin and casein-derived β-casomorphin-7 have both opioid and ACE-inhibitory activities [16].

Milk Proteins, Satiety and Food Intake

Many studies show that milk proteins increase satiety, but not necessarily later food intake. However, the variability in results can be explained in part by the protein source, quantity and time of measurements used in the studies [19]. For example, a randomized, single-blind study of 25 healthy adults found that a breakfast with 25% of energy from casein was more satiating than a breakfast with 10% of energy from casein to lunch (3–4 h later) [20]. However, there were no differences in energy intake at lunch. Similarly, whey has been shown to suppress appetite in a number of short-term studies, but the effects on energy intake are inconsistent.

Most often, dairy proteins are consumed in food form. Satiety has been reported after consumption of dairy foods such as nonfat milk [21], chocolate milk [22], high protein cheese [23], and low-fat yogurt [24]. While increased satiety translated into reduced ad libitum energy intake in some of these studies [21]; it failed to do so in others [22, 24], but did not result in higher energy intake compared to control conditions [23]. These observations can be attributed to the amount of preload, protein content and time of measurement of food intake. Consumption of 600 ml of milk (21 g protein) at breakfast prior to lunch 4 h later resulted in reduced food intake at lunch [21], and a cheese snack (22 g protein) containing either casein or a mixture of casein and whey proteins 1 h before lunch resulted in partial energy compensation at lunch and full energy compensation over 24 h [23]. However, a study investigating the effects of 200-kcal preloads consisting of semisolid yogurt (17.1 g protein), liquid yogurt (17.1 g protein), dairy fruit beverage (2.6 g protein) and fruit drink (0 g protein) prior to a lunch 90 min later did not lead to energy compensation [24]. Similarly, 500 ml of a chocolate milk drink compared with 500 ml of a sugar-sweetened carbonated beverage did not result in a reduction in ad libitum food intake 30 min later [22].
Physiologic Functions of Milk Proteins

Of the milk proteins, whey protein preloads of 45–50 g have been shown to decrease food intake more than casein at meals consumed 30–90 min later [2], while casein reduced food intake more at 180 min [25]. These early studies used treatment doses well above that which may be obtained from usual serving sizes, and thus provided little evidence that dairy consumed in usual amounts contribute to food intake regulation. However, more recent studies indicate that protein intake from milk in the range of usual serving sizes, defined as 250 ml (9 g protein), but often consumed in larger amounts of 360–500 ml (18 g protein), is of functional significance for food intake control through the combination of the effects of whey in early, and casein in later, satiety. The consumption of 600 ml (21 g protein) of milk, compared with a fruit-based beverage, and served with a toast and jam breakfast reduced measures of hunger and increased fullness over 4 h to lunch time and reduced food intake [21]. A decrease in subjective appetite ratings was found after consumption of approximately 15 g of whey providing 10% of the energy content of breakfast, compared with casein or soy protein [20]. Although the lowest dose of whey protein (15% GMP) found to suppress food intake when consumed 30 min prior to a meal was shown to be 20 g based on a sample size of 16 healthy individuals (fig. 1) [26], the reduction of 78 kcal at the meal following 10 g whey protein was predicted to be statistically significant in a sample size of 40 subjects.

![Caloric compensation (%)](image)

**Fig. 1.** Food intake 30 min following consumption of whey protein drinks. Food intake (mean ± SEM) was calculated based on energy consumed in an ad libitum pizza meal 30 min following consumption of whey protein in water and a water control (300 ml). Caloric compensation was calculated as kcal consumed at the meal after the water control – kcal consumed at the meal after the whey protein drinks/kcal in the whey protein drinks × 100. Different superscripts are significantly different (p < 0.05, n = 16).
Milk Proteins and Satiety Mechanisms

To explain protein-induced satiety, several mechanisms have been proposed based on their release of amino acids, encrypted BAPs and satiety hormones [3].

There is a long history of the study of intake regulation based on amino acid-sensing systems in the brain [27]. Imbalances in the essential amino acid content of dietary proteins are readily sensed by the brain and lead to reduced food intake. Other amino acids including tryptophan and tyrosine provide precursor control of serotonergic and catecholaminergic systems which also are involved in food intake regulation. Finally, the brain uses BCAAs as fuel, which may also be a satiety signal [28]. Milk proteins are high in BCAAs and neurotransmitter precursors [29].

The role of BAPs in control mechanisms regulating food intake and metabolism has received relatively little investigation. However, the importance of BAPs in food intake regulation is suggested based on the following observations: (1) BAPs from casein reduce food intake via peripheral opioid and cholecystokinin (CCK)-A receptors in the gut in rats [30] and stimulate glucagon-like peptide-1 (GLP-1) release [31]; (2) A mixture of free amino acids fails to stimulate the effect of intact whey on incretin secretion in the gut hormones [13]; (3) The ingestion of milk proteins stimulates the release of the gut hormones including CCK, GLP-1, glucagon inhibitory peptide (GIP), peptide tyrosine tyrosine (PYY) and of insulin, while reducing ghrelin [1, 3], all known to be involved in appetite and food intake regulation. Although whey protein, compared to casein, has a stronger effect on these hormones and contains a higher content of BAPs, the results reported may be due also to the duration of measurement which may not have been extended long enough to show the complete effect of the more slowly digested casein [19]; (4) GMP stimulates satiety hormones (e.g. CCK) and has been suggested to be a factor accounting for the effects of milk proteins or commercial whey proteins on satiety. Unfortunately, much confusion on the efficacy of GMP arises from the failure of studies to report the GMP content of whey protein [3]. For example, GMP (0.8 g) was not found to be of importance in pre-meal whey-induced satiety or later food intake [32]. However, in 25 healthy adults, ad libitum energy intake was 10% lower 3 h later at lunch after a breakfast containing both 10% (15 g protein) or 25% (37.5 g of protein) of energy from whey protein containing GMP compared to a breakfast with whey protein without GMP [33]. Although the GMP content of the treatments was not described, it is possible that the whey protein with GMP contained 15% GMP [3]. If so, the amounts of GMP consumed would have been substantially more than that provided in the experiments of Burton-Freeman [32]. A dose-response study with defined quantities of GMP compared with GMP-free whey is required to resolve this issue.
Milk Proteins and Glycemic Control

Short-term experimental studies support the hypotheses that dairy consumption before or as part of a meal offers benefits in metabolic control. Milk consumption decreases the glycemic response to carbohydrate ingestion [34, 35]. Lower glycemic responses have been found after high milk breakfasts compared with high fat breakfasts or high fiber breakfasts in both healthy participants and individuals with type 2 diabetes [36]. When milk or whey were added to either high or low glycemic index carbohydrate meals, there were markedly lower glycemic responses [34, 35, 37]. An insulinotropic effect of milk is seen with both whole and skim milk indicating that insulin release is due to its protein content [4, 34].

There are many reports of the effect of whey protein, consumed alone, in beverage form or when consumed with carbohydrate on increasing plasma insulin as the mechanism by which the reduction in blood glucose occurs [13, 37, 38]. Addition of whey (total 18 g) to a meal containing rapidly digested and absorbed carbohydrates, stimulated greater plasma insulin concentrations (+57% after lunch) and reduced postprandial blood glucose (~21% over 120 min response) in subjects with type 2 diabetes [38]. Whey protein has been suggested to be the primary insulinotropic factor because a 50% greater increase in insulin response was found after preloads of 25 g carbohydrate with 18 g of whey protein than with the same amount of protein from milk or cheese [37]. Although it has been suggested that the insulinotropic effect of whey protein [1, 3, 9] arises from its rapid digestion and high content of BCAAs [37], the comparison of its effect with milk or cheese high in casein may be attributed as well to food form and rate of stomach emptying and digestion.

More recent evidence suggests that the explanation for the effect of whey protein on glycemic control is not solely dependent on its insulinotropic effects. A BCAA mixture was found to mimic the glycemic and insulinotropic responses after whey protein but did not reproduce the effect of the intact whey protein on gut hormones in humans [13], suggesting that postprandial glucose reduction occurs by mechanisms in addition to insulin action. Furthermore, a recent study has shown that a lower blood glucose following a meal of fixed size occurs without an overall increase in insulin requirement in response to the 10–40 g of whey preloads consumed 30 min prior to a meal of fixed size given to healthy young men and women [26]. A lower post-meal blood glucose with increasing doses of whey protein (15% GMP) was achieved in the presence of a lower, not higher, post-meal insulin AUC and a similar cumulative (0–170 min) insulin AUC (table 1). When the cumulative AUC for blood glucose was divided by the cumulative AUC for insulin to evaluate the efficacy of insulin action, the ratio was decreased, in a dose-dependent manner, to 50% of the control after pre-meal consumption of intact whey protein of 40 g (fig. 2). In contrast to 10 g of intact protein, 10 g
of whey protein hydrolysate did not result in a lower cumulative blood glucose than the control even though it increased the post-meal and cumulative insulin AUC similarly, suggesting that noninsulinotropic mechanisms require stimulation arising from the digestion of intact proteins, perhaps due to the release of encrypted BAPs.

Although the mechanism by which pre-meal whey protein brings about improved post-meal glucose control with a lower requirement for insulin is unclear, the most probable explanation for the insulin-independent actions of pre-meal consumption of whey protein on blood glucose control resides in the

### Table 1. Pre- and post-meal blood glucose and insulin responses after the doses of whey protein and whey protein hydrolysate (WPH)

<table>
<thead>
<tr>
<th>Preload</th>
<th>Blood glucose, mmol×min/l</th>
<th>Insulin, µIU×min/ml</th>
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<tbody>
<tr>
<td><strong>Pre-meal AUC (0–30 min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>–1.6 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0 ± 4.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whey protein, 5 g</td>
<td>1.5 ± 1.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>48.5 ± 11.0&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>WPH, 10 g</td>
<td>5.6 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.1 ± 11.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whey protein, 10 g</td>
<td>–1.4 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>107.9 ± 12.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Whey protein, 20 g</td>
<td>4.3 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>185.5 ± 25.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Whey protein, 40 g</td>
<td>4.2 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>217.5 ± 32.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>p</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Post-meal AUC (30–170 min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>232.9 ± 21.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,593.4 ± 510.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Whey protein, 5 g</td>
<td>207.1 ± 17.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2,720.2 ± 313.6&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>WPH, 10 g</td>
<td>196.6 ± 17.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2,173.3 ± 272.2&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>1,995.8 ± 271.3&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>1,502.6 ± 253.5&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Whey protein, 40 g</td>
<td>82.2 ± 13.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1,578.1 ± 303.7&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td><strong>p</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Cumulative AUC (0–170 min)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>214.6 ± 22.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,593.7 ± 507.0</td>
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<td>Whey protein, 5 g</td>
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<td>3,221.6 ± 354.4</td>
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<td>WPH, 10 g</td>
<td>234.4 ± 24.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Whey protein, 10 g</td>
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<td>Whey protein, 40 g</td>
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<tr>
<td><strong>p</strong></td>
<td>&lt;0.0001</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Mean ± SEM blood glucose AUC (mmol × min/l) and insulin AUC (µIU × min/ml) after the whey protein and WPH preload consumption were calculated for pre-meal (0–30 min) and post-meal (30–170 min) and cumulative (0–170 min; n = 21). One-factor repeated-measures ANOVA followed by Tukey’s post-hoc was used to compare the effect of preloads (means with different superscripts at pre- and post-meal and cumulative AUC are different, p < 0.0001).
effect of protein on gastric emptying [26]. Even a modest change in gastric emptying affects the magnitude and timing of postprandial blood glucose and insulin increase and is decreased by protein ingestion consumed either with carbohydrate or alone or when consumed before a meal. Furthermore, slower stomach emptying would be expected because whey protein and other proteins release CCK, GLP-1, glucagon inhibitory peptide and peptide tyrosine tyrosine from the intestinal enteroendocrine cells [1].

**Fig. 2.** Ratio of cumulative blood glucose/insulin AUCs following consumption of whey protein (a) Ratio (mean ± SEM) of cumulative blood glucose/insulin AUCs after whey protein consumption (n = 21). One-factor repeated-measures ANOVA followed by Tukey’s post-hoc was used to compare the effect of preloads (means with different superscripts are different, p < 0.05). (b) Association (r = –0.33, p < 0.001) between whey protein doses and ratio of cumulative (0–170 min) blood glucose/insulin AUC after consumption (Pearson’s correlation coefficient).
Conclusions

Milk proteins have physiologic functions that contribute to the maintenance of a healthy body weight and control of factors associated with the metabolic syndrome through their effects on mechanisms regulating food intake and blood glucose. Recent reports show that these benefits can be achieved within the range of usual consumption of dairy. In addition, more recent research points to an intrinsic value of small amounts of milk protein or dairy consumed shortly before a meal to reduce the glycemic response to carbohydrate and that this is not explained by its insulinotropic effect alone.

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Physiological Functions of Milk Proteins


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Discussion

Dr. Gibson: You talked about studies in which casein and whey fractions are being compared, and I often read in the literature this sort of thing. Is there a comparison...
between whey proteins and non-bovine milk proteins such as soy proteins or other animal proteins? Are there comparative studies?

_Dr. Anderson_: In the first slide I showed, soy protein did not suppress food intake as much as whey. Also, it was surprising that egg albumin didn't have any effect at all. This may be due to its low solubility over that time and slow release from the stomach. Margriet Westerterp and her group in Holland have done a lot of studies looking for biomarkers of satiety with different proteins. They reported different effects on PYY, CCK, insulin, and other hormones, but found that outcome depends on dose and meal timing, and what the meal consists of. These biomarkers generally fail to associate with subjective measures of satiety. I guess the only thing I could offer is that generally whey keeps coming out as more efficacious in short-term studies.

_Dr. Melnik_: I would like to know whether you have also looked at ghrelin response after whey feeding. After milk ingestion, we observed a strong insulin response and a decrease in ghrelin levels.

_Dr. Anderson_: Ghrelin goes down after whey consumption, also in rats, not just in humans.

_Dr. Clemens_: Would you like to speculate on the impact some of these hydrolytic enzymes that are used in food processes might have on satiety and stimulation of IGF-I?

_Dr. Anderson_: Let's just step back. If you compare soy-based diet with a milk-based diet, antioxidant capacity is improved and blood lipid oxidation is decreased with the milk-based diet. If you hydrolyze proteins, then of course depending on which enzymes are used you will get different peptide fractions. It's very hard to determine if they reflect normal digestive processes or, even if they don't, what lengths of the peptides are left in the process. Some will expose proteins to digestive enzymes for 30 min and then separate the peptides, and others let the process go for much longer. Unfortunately, authors often fail to give details about how the hydrolysates were prepared or their composition. I am not aware of reports of their effects on IGF-I, but I am sure that would depend on the degree of hydrolysis. Perhaps Dr. Haschke would like to comment?

_Dr. Haschke_: I can only comment on infant formulas. We have formulas with partial or extensive protein hydrolysis. Producers of those formulas have to specify the enzymes that are used and the exact profile of peptides in the hydrolysate. The reason for that is that the molecular size of the peptide fractions is very important for allergy prevention or treatment. As already mentioned, at 6 years of age growth (weight, height, BMI) of children who were fed three different hydrolysates (Nutramigen, Pepti-Junior, NAN-HA) during infancy was similar to that of children who were exclusively breastfed during infancy [1]. However, one cannot conclude that long-term growth of children who were fed other hydrolysates would be similar.

_Dr. Anderson_: I think that the point that I want to make, going back to the formulas, is that we don't have any metabolic measures on infants being fed different formulas to the extent that you could make the comparison, and to the extent that that would be possible I think it would be worthwhile doing.

_Dr. Haschke_: We have very limited data, I agree with you. For example, we have data which are published on one partially hydrolyzed formula in terms of IGF-I secretion and insulin secretion, and the reference is again the breastfed infant. The data have been shown earlier during the meeting, and it's clear that it's the amount of protein that determines insulin secretion and IGF-I levels -- the higher the protein the higher the IGF-I levels. But I agree with you, we don't have data comparing extensively hydrolyzed and partially hydrolyzed intake formulas; as far as I know, this cannot be found in the literature.
Physiologic Functions of Milk Proteins

Dr. Anderson: One of the points going back to the question of bodybuilders and some of the adverse effects suggested to be due to the huge amounts of whey they consume, my concern is that we might throw the baby over the bath water. I am trying to give the message that dairy products in the amounts that are normally consumed and within our dietary guidance of three servings a day provide whey in amounts much below the large amounts consumed by bodybuilders. There is no evidence that normal consumption levels are a problem.

Dr. Lönnerdal: I would like to emphasize what you discussed regarding extensively hydrolyzed infant formulas. Dr. Hernell and I did a clinical study in which we compared regular infant formula, breastfeeding and extensively hydrolyzed formula based on either 100% casein or 100% whey, starting from 1 month of age up to 6 months of age, but it was not sized to look at growth. As you may know, the daily volume consumed by formula-fed infants is higher than that of breastfed infants. The intakes of the extensively hydrolyzed formulas were even higher, so I wonder what this did to appetite control once these peptides had broken down to a very small size. I am coming back to Dr. Haschke’s comment that we have to be a bit cautious about growth studies on infants fed especially extensively hydrolyzed formulas because very often they are given to compromised infants with allergies that always have poor growth and need to catch up. In this Swedish study, we compared healthy normal infants with regular formula controls and breastfed infants.

Dr. Anderson: I agree. If you take the branched-chain amino acids alone or hydrolyzed whey and compare them with intact whey you get a very different endocrine and food intake responses. There is good evidence that you need the intact protein to get the full benefits from gut and metabolic hormones. But I just want to make another point that arose from Dr. Melnik’s presentation, where he provided an example of feeding adults with infant formula. We know little about how this compares to cow’s milk or soy beverage.

Dr. Dehbi: I wonder if all these findings may be applied to diabetes in children; maybe this will prepare for a formula for children with diabetes because it’s difficult to control diabetes in childhood.

Dr. Anderson: Yes, absolutely. In Canada, as in the US, we are now into bariatric surgery for teenagers and young children, and of course we see diabetes in children. The question is which is the chicken and which is the egg in the etiology of energy imbalance and diabetes? Has the obese child lost the physiologic signals or is the problem all environmental, or can you still make use of the physiological signals in order to help them lose weight? If so, then the high-satiety foods may be of value for appetite control. That’s why we are studying milk because we think milk and whey are highly satiating. However, I also showed that whey consumed before a meal by adults results in blood glucose control through mechanisms in addition to insulin. If so, then a practical approach to glycemic controls may be to encourage a child with obesity and diabetes to have a glass of milk or a beverage with 5–10 g whey before eating. This possibility is being investigated.

Reference

Lactose Intolerance: An Unnecessary Risk for Low Bone Density

Dennis Savaiano

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Abstract

The potential for lactose intolerance causes 25–50 million Americans and an unknown number of people around the world to avoid milk. Milk avoidance is a significant risk factor for low bone density. Individuals who avoid milk, due to intolerance or learned aversion, consume significantly less calcium and have poorer bone health and probable higher risk of osteoporosis. Lactose intolerance is easily managed by: (1) regular consumption of milk that adapts the colon bacteria and facilitates digestion of lactose; (2) consumption of yogurts and cheeses and other dairy foods low in lactose; (3) consumption of dairy foods with meals to slow transit and maximize digestion, and (4) use of lactose-digestive aids. As dairying spreads around the world to new markets and dairy foods become the dominant source of calcium in these markets, the potential for lactose intolerance will grow. Management of lactose intolerance globally will require both education and product development.
Lactose intolerance can cause moderate and acute symptoms of excessive flatulence, stomach discomfort and diarrhea. Typically, initial symptoms include initial stomach distension and discomfort followed by flatulence. If maldigestion is severe, diarrhea can quickly follow. The occurrence of symptoms depends on several variables. Those variables include dose, gastrointestinal motility, individual digestibility, and microflora profile.

**Dose:** Typically, one cup of milk (containing 12 g of lactose) or lactose in water served alone or with a meal is well tolerated by maldigesters, even those claiming severe intolerance (table 1). As the results below demonstrate in a double-blinded, randomized protocol, physiological relevant symptoms occur only when more than 12 g of lactose is consumed.

If milk (or in the example below, food supplement containing lactose) is consumed with breakfast, it remains well tolerated (fig. 1). Dairy sources vary considerably in lactose content. Lactose is water soluble and thus found in the whey portion when curds and whey are separated during cheese production. Thus, hard cheeses have minimal lactose and soft cheeses are intermediate in lactose content. Yogurts are well tolerated due to microbial β-galactosidase that is active in vivo during digestion, supplementing the body’s own lactase activity. The primary source of lactose in the diet is fluid milk.

**Gastrointestinal Motility:** The rate at which the lactose passes into the intestine is a function of stomach emptying and meal feeding. Lactose tolerance is significantly improved when lactose is fed with a meal. The effect is more difficult to demonstrate with individual foods such as whole milk compared to fat-free milk.

**Digestibility:** The residual lactase activity in the small intestine presumably varies among individuals and likely influences tolerance. However, this variance is not well understood or evaluated relative to tolerance.

**Table 1.** Intolerance symptoms from lactose in water consumed on an empty stomach for breakfast [1]

<table>
<thead>
<tr>
<th>Lactose dose</th>
<th>Flatus frequency (over 5 h)</th>
<th>Flatus ratings</th>
<th>Abdominal pain ratings</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 g</td>
<td>4.0 ± 1.3a</td>
<td>3.4 ± 1.0ab</td>
<td>1.7 ± 0.8a</td>
</tr>
<tr>
<td>2 g</td>
<td>4.3 ± 1.8a</td>
<td>3.8 ± 1.4b</td>
<td>1.7 ± 0.9a</td>
</tr>
<tr>
<td>6 g</td>
<td>5.1 ± 0.6a</td>
<td>1.9 ± 0.9b</td>
<td>1.2 ± 0.5a</td>
</tr>
<tr>
<td>12 g</td>
<td>4.6 ± 1.1a</td>
<td>3.5 ± 1.3b</td>
<td>3.4 ± 0.8b</td>
</tr>
<tr>
<td>20 g</td>
<td>9.0 ± 2.6b</td>
<td>6.6 ± 1.8c</td>
<td>5.3 ± 1.8b</td>
</tr>
</tbody>
</table>

a,b,c Treatments not sharing the same letter are significantly different (p < 0.05); ratings of symptoms of hours 1–8.
Lactose Intolerance

**Microflora:** The ability of the large intestine bacteria to compensate for maldigestion depends on how well the microflora are adapted to metabolize lactose.

Regular consumption of lactose in both double-blinded and free living studies suggests that diet history and adaptation are major factors in determining tolerance. In the example below (fig. 2), subjects were adapted to either dextrose or lactose over a 10-day period and then challenged with a breath hydrogen test.

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**Fig. 1.** Severity of lactose intolerance symptoms following consumption of the test meal. Ranking of severity of symptoms: 0 = no symptoms; 1 = mild gas and/or borborygmi; 2 = excessive gas; 3 = severe gas and/or cramps; 4 = loose stools; 5 = severe diarrhea. *p < 0.001, significance vs. aqueous lactose. **p < 0.001, significance improved with supplement.

**Fig. 2.** Breath hydrogen response (increase above fasting concentration) to a lactose challenge.
Table 2. Intolerance symptom ratings to the lactose challenges after the dextrose and lactose feeding periods [2]

<table>
<thead>
<tr>
<th></th>
<th>After dextrose</th>
<th>After lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flatus ratings (n = 20)</td>
<td>8.1 ± 1.6</td>
<td>4.5 ± 1.0(^a)</td>
</tr>
<tr>
<td>Flatus frequency (n = 6)</td>
<td>23.0 ± 2.8</td>
<td>11.0 ± 2.6(^b)</td>
</tr>
</tbody>
</table>

Data are the sum ratings for hours 1–8 after the lactose challenge (mean ± SEM).
\(^a\) \(p = 0.025\), \(^b\) \(p = 0.028\), significantly different from after dextrose.

Table 3. Impact of lactose intolerance and lactose maldigestion on bone density

<table>
<thead>
<tr>
<th>Population</th>
<th>Sample size</th>
<th>Age years</th>
<th>Summary</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmenopausal women</td>
<td>58</td>
<td>57 ± 7</td>
<td>No significant difference between LI and non-LI women</td>
<td>Corazza et al. [3]</td>
</tr>
<tr>
<td>Young adults</td>
<td>103; includes 55 with LI</td>
<td>28 ± 2</td>
<td>↓ BMD with LI</td>
<td>Di Sefano et al. [4]</td>
</tr>
<tr>
<td>Adults</td>
<td>66 with LI</td>
<td>20 – 78</td>
<td>↓ BMD and ↑ bone turnover with LI</td>
<td>Segal et al. [5]</td>
</tr>
<tr>
<td>Adults</td>
<td>218; 115 with LI; 103 controls</td>
<td>58 ± 11</td>
<td>No significant difference in BMD; ↑ risk of vertebral fracture among LI subjects</td>
<td>Kudlacek et al. [6]</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>33 with idiopathic osteoporosis; 33 controls</td>
<td>54 (31–65)</td>
<td>↓ lactose absorption among osteoporotic subjects</td>
<td>Finkenstedt et al. [7]</td>
</tr>
<tr>
<td>Adults</td>
<td>745</td>
<td>≥60</td>
<td>↑ dairy → ↑ BMD (hip) in males, not females</td>
<td>McCabe et al. [8]</td>
</tr>
<tr>
<td>Young women</td>
<td>291; 100 with LI</td>
<td>10–13</td>
<td>Perceived LI (even at 10 years) → ↓ BMD reflecting low milk intake</td>
<td>Matlik et al. [9]</td>
</tr>
<tr>
<td>Children</td>
<td>19</td>
<td>9.6 ± 1.9</td>
<td>Low lactose intake → low Ca intake and low BMD among LI children</td>
<td>Stallings et al. [10]</td>
</tr>
</tbody>
</table>

BMD = Bone mineral density; LI = lactose intolerance.
The amount of gas and gastrointestinal symptoms were dramatically reduced when subjects were adapted to lactose (table 2).

The primary clinical issue of concern related to lactose maldigestion and intolerance is reduced calcium intake, leading to low bone density and thus increasing the risk for osteoporosis. Studies that typify these concerns are summarized in table 3 and illustrated in figure 3.

The preponderance of evidence among those with perceived and diagnosed lactose intolerance indicates that there is an increased risk of decreased bone mineral density. This clinical presentation increases the risk of bone fractures, such as vertebral bone. The poor bone health status reflects avoidance by consumers to eat dairy products, even among children, vs. the evidence that those with lactose intolerance can consume and tolerate at least 8 oz (240 ml) of milk or related products. This is consistent with the numerous studies that indicate a strong relationship between dietary calcium and vitamin D with bone fragility, particularly the spine [11].

The incidence of symptoms resulting from intolerance to milk and dairy products in various populations has been well documented [12–15]. The FDA’s Consumer Health Information website [16] states that NIH ‘estimates that 30–50 million Americans are lactose intolerant’. The NIH’s website contains a substantial amount of information on this condition [17]. Numbers of individuals who avoid milk worldwide are not known. But, it is likely that this number will grow dramatically in the years ahead as dairying is expanded in global markets such as China and the Middle East, and worldwide dietary patterns evolve.

Dairy foods account for 73% of the calcium available in the US food supply [18] and 51% of the total calcium intake [19]. Calcium intakes of most Americans are far below recommendations [20–22]. Fleming and Heimbach [23] provide specific data of the amount of calcium intake in the US by sex, age, ethnic group, region, and food group. Further, it is difficult to get ade-
Savaiano

quate calcium in a western diet without dairy foods [23–25]. It is hypothesized that adequate calcium not only helps reduce the risk of osteoporosis and hypertension [26, 27], but also possibly reduces the risk for several cancers [9, 26].

An estimated 75% of the world’s population and 25% of Americans are maldigesters of lactose. Like all other mammals, these maldigesters lose 90%+ of their infantile levels of lactase during early childhood development. Thus, they have limited ability to digest lactose into its component sugars (galactose and glucose) in the small intestine. The NIH estimate of 30–50 million milk avoiders is supported by a survey by Elbon et al. [28] demonstrating that 17% of Whites and 35% of Blacks indicated a perceived milk intolerance. The National Dairy Council African American Lactose Intolerance Study [28] reported that 24% of respondents considered themselves lactose intolerant, and 49% reported some physical discomfort at some time following dairy food consumption, of which 27% said they experience discomfort all the time. Evidence suggests that all maldigesters have a similar potential for intolerance [29, 30]. If the conservative estimate of 24% of the entire African-American population is used for extrapolation (i.e. 35% of African-American maldigesters are estimated to be intolerant since only 75% of African-Americans are maldigesters) to the general US population, at least 25 million (1/3 of 75 million) Americans are avoiding dairy foods due to lactose intolerance. If the 17% figure from Elbon et al. [28] is used, 50 million Americans are avoiding dairy foods.

As the dairy industry continues to develop worldwide, and dairy foods become a dominant global source of calcium in the diet, the potential for symptoms of lactose intolerance among maldigesters will continue to grow. Education and new product development can address this issue.

References

Lactose Intolerance

16 http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm094550.htm.

Discussion

Dr. Anderson: I was really impressed by your point that if you give lactose and you change the microbiology in the large intestine then you may have an impact on obesity, appetite control and food intake regulation. Maybe people should be recommended to take lactose to change their gut population and combat obesity. There was a discussion earlier that obese people have different microflora than lean people. One
Savaiano would argue you don’t know which is the chicken and which is the egg, so perhaps it is not a simple solution. I think your point that you can change the bacteria quite quickly depending on the type of diet is a very important observation. I think it’s the same thing with dietary fiber, and as you know many people say they cannot tolerate fiber when it fact it is due to healthy activity in the colon. However, with constant application they do adapt. Do both amount and type of bacteria change?

Dr. Savaiano: We have a paper on soy, a soy isolated product that’s high in stachyose and raffinose showing adaptation increase in the bifidus. My graduate student just had it accepted. I have a colleague who is a microbiologist, we are also in the middle of the study trying to do the similar kind of work with lactose. It’s clear that it looks like bifidus will change, from everything we know about bifidus it will rather go up. But the difference between change in the number of bacteria, the species of bacteria and the metabolic capacity of bacteria hasn’t been given enough attention because one might not change the nature of the bacteria in terms of their species, but one might induce an enzyme activity or metabolic pathway as we have shown with lactose, where you can increase hydrogen utilization and increase β-galactosidase. But the numbers of bacteria do not change at all, there may simply be an induction or you might see an increase in certain populations, or both. The molecular tools we are now developing I think will start to allow us to answer some of those interesting questions, but they are not easy questions to answer.

Dr. Haschke: We have discussed that gene polymorphisms are associated with lactose intolerance. At least in European countries, screening to detect those polymorphisms is done in many hospitals when pediatric or adult patients are suspected to have lactose intolerance. The diagnosis is then confirmed by positive hydrogen breath testing. Could you elaborate on this and on polymorphisms which have been detected in populations other than the Caucasian?

Dr. Savaiano: We thought there were three genetic polymorphisms for persistence, one originated out of northern Europe, one originated out of the Middle East and one originated out of central Africa. It now looks like there are at least seven or eight polymorphisms, most of them in Africa. There is new evidence of finding six polymorphisms in parts of Africa where we have persistence. So this move to persistence is a very easy change that happens in a number of environments for a number of reasons, and it’s probably different in those seven or eight different populations. How that difference influences tolerance, we don’t have any idea. Whether that actually influences tolerance, we don’t know. The genetic testing that’s going on is highly correlated with the hydrogen test, it seems to work quite effectively, but the hydrogen testing in a clinical setting is a challenge. In a research setting it’s actually quite easy. But yes, the new tools are growing, and I think clinicians will find their use probably a lot easier. Will they tell us something different than what we already know, I am not sure.

Dr. Prentice: Over coffee, we were talking about the change in the microbiota that occurs between a milk-based diet and a rice-based diet. Given what you have just told us about inducing lactase in the microbiota, is the ability to digest lactose going to depend on the type of carbohydrate that is generally the staple in the population?

Dr. Savaiano: Presumably, you would need a galactose-glucose bond, the β-galactoaldic bond, in order to induce the pathway. So in theory, one would find compounds that have that bond potentially doing the same thing. I think that’s probably all I have to say on that given what we are doing commercially, but certainly I do believe that if you took stachyose or raffinose and you fed it, you would get β-galactosidase induction at least, you might secondarily get some of that but clearly you get changes in the microbiota. I live in a place where there are lots of soybean farmers, and the soybean farmers tell me that if they eat soy for a couple of weeks they are fine, they don’t have any symptoms of flatulence or intestinal distress, so my soybean farmer friends tell
Microbiota are incredibly adaptable. Many of us who traveled over the world probably have had some changes in our microbiota with the change in diet, some of us for the better and some for the worse, and as we go back we go back to those environments again, I think it's a huge area of research.

**Dr. Prentice:** I was thinking more about inducing tolerance to lactose if your carbohydrate background was different.

**Dr. Savaiano:** I think that the microbiota are incredibly responsive to whatever you feed them; so if you feed them something, they are going to adapt to use it to continue to grow and prosper in their microenvironment. Any carbohydrate you feed them, they will adapt to that carbohydrate. Bread, for example, has about 10% nondigestible starch, that's probably another place where there is some potential for adaptation.

**Dr. Hernell:** If you analyze small intestinal biopsies from patients with congenital lactase deficiency, the residual activity is extremely low, it is virtually absent. But if you do the same on individuals with hypolactasia, you always find residual activity which, as you showed, varies considerably. What is your opinion, which fraction of these individuals do lose 90% or more of their activity, because probably 10% residual activity is what most people would need to remain lactose tolerant.

**Dr. Savaiano:** There is an interesting study by Levett in the US that was done in the 1970s I believe, where he demonstrated that in nonpersistent individuals about half of lactose was still digested in the small bowel. So the residual lactase is very important. And, in fact, if you feed lactulose which is completely nondigested vs. lactose, you have to feed about twice much lactose to get the same hydrogen curves. That would be indirect evidence that would suggest that this residual lactase also is important. Now the variation in that residual lactase I think is interesting, I would love to do that study. I am not quite sure how to do it because of the difficulties with biopsy in human subjects and so forth, but that would be a wonderful study to determine if there are populations where there is variation that has relevance to digestion. It's a very good question. I wish I had a better answer.

**Dr. El Barbary:** Does lactose intolerance increase with age in the same individual?

**Dr. Savaiano:** It probably increases with age between the ages of 3 and 5, that's not your question I understand, but that's what the evidence would suggest. There are some indications in the elderly that small bowel overgrowth can have an influence on lactose intolerance, and if you treat the small bowel with antibiotics you get some recovery. So that's a microbiota issue though, not a lactase issue. We have done a study with Asian American young and elderly and found absolutely no difference in their nonpersistence in terms of symptoms and maldigestion. I believe that a lot of the incidence we see of individuals who claim as they get older to have a symptom of intolerance has to do in fact with infection, malnutrition, traveling, a change in colon flora, that I don't think would be accounted for to the extent that it is important.

**Dr. El Barbary:** Yes, but I just wanted to know whether lactase activity as enzyme decreases with age in the same person.

**Dr. Savaiano:** I don't believe the evidence is there to support that reduction.

**Dr. Sankaranarayanan:** I presume your focus is more on lactose intolerance in the adult.

**Dr. Savaiano:** Yes, correct, all the studies are with adults.

**Dr. Sankaranarayanan:** Could you answer some questions on pediatric lactose intolerance?

**Dr. Savaiano:** I can at least review the literature for you and give you an indication of what is there.

**Dr. Okai Brako:** What is the extent of nonpersistence in children younger than 3 years old?
Dr. Savaiano: The scientific literature would suggest that it's extremely low, that almost all mammals or humans between the birth and 3 years have high levels of lactase. There are few examples of congenital abnormalities where there is no lactase at all, but very few examples. I would ask the question about milk protein and milk protein allergy because oftentimes those issues can be confused. If one takes milk proteins early in development and the gut is not closed up and you get immune response and allergic response, and as you will know that's a whole different clinical scenario; but I think oftentimes those scenarios get confused. There isn't very much evidence that would support low lactase levels prior to the age of 3 years, and I know clinically you may have experiences that are different, but I am not suggesting those are not real experiences, the literature doesn't have much.

Dr. Sarwar Ferdaus: As pediatricians, we often deal with diarrheal diseases. After rotaviral diarrhea, infants or small children do not digest milk. In those cases, we don't see too much flatulence or gas production, but what we see is that lactic acid comes out in the stool. There are perianal disorders apparent with lactic acid, and in those cases we do a test that reduces lactic acid in the stool, we give the children some other products and their condition improves. So, can we replace the hydrogen test with reducing the substances in the stool in adults?

Dr. Savaiano: I don't know the answer to that question. On a theoretical basis, there are perhaps a lot of factors that will reduce the acidity or increase the acidity, lower the pH of the colon. I showed you that hydrogen is adaptable, so in that sense it's not a good test either. The future I think is the genetic testing. There is a literature that compares those tests, and in general what we find is the hydrogen test is the most sensitive and it's the least likely to have false positives.

Dr. Johansson: With hydrolyzed lactose you have a release of glucose and galactose, and one should consider if one is turning a noncariogenic milk product into a cariogenic milk product, which might be an issue to be studied.

Dr. Savaiano: I agree, these are control studies attempting to distinguish this hypothesis. But when you do split lactose and make lactose-hydrolyzed products, you do have a different carbohydrate composition, and that needs to be considered, and in the US these products are sold widely.

Dr. Haque: You said that the incidence of lactose intolerance is very rare in the pediatric population. But in our place, we come across many children with watery diarrhea, and when we restrict lactose and give them lactose-free milk they respond quite dramatically. How do you explain this?

Dr. Savaiano: The lactase enzyme is very sensitive to all influences that come through the intestinal lumen. It's one of the only two enzymes that sit outside the cell attached with a carbohydrate moiety into the membrane, so anything that comes by that influences it, ethanol, alcohol, infection. You could even hypothesize that certain food components might influence whether that enzyme gets knocked off or not. If you are eating fiber components, anything that could influence that enzyme is going to cause a secondary intolerance, and so I would argue that what you are likely seeing is a secondary intolerance which is very common. Don't get me wrong, it's very common and important, but once you resolve the issue of what is causing the secondary intolerance, whether it's infection, malnutrition or whatever, you can get the individual healthy. You should go back to a situation where they are tolerant, at least that's what the data would suggest.

Dr. Garg: I have one comment and one question. The comment is about lactase deficiency or activity in the <3 years age group. Let's remember that premature babies have less than normal lactase activity, and you said that even at 34 weeks there is only 30% of lactase activity compared to term newborns, and that is the logic behind formulating preterm infant formula with low lactose and glucose polymers. Still, whether
this is clinically significant is not certain. We must remember that preterm newborns tolerate mother’s milk very well, and mother’s milk contains lactose as the only carbohydrate source.

My question is about children 2–3 years old. Apart from lactose, they also consume other sugars, and some of them take large amounts of fruits, juices and candies, and these can lead to the symptoms of flatulence and abdominal discomfort. So when a child of this age group consumes both lactose and a lot of other sugars, how do you see which one is causing the symptoms?

**Dr. Savaiano:** I don’t know. I am not a clinician. Theoretically, simple sugars at even fairly high concentrations should be cleared through the small bowel very rapidly. They should not reach the large bowel.

**Dr. Gibson:** Just a question about the general applicability of your observation that you can induce tolerance to lactose by giving increasing doses of small doses, which sort of parallels what we do to overcome bee sting intolerance and inducing small amounts of bee venom. I was just wondering, in relation to celiac disease and the intolerance to gluten, do you think this could be a method? What’s the literature on this?

**Dr. Savaiano:** Let me answer different questions. Many gastroenterologists in the western world deal with irritable bowel syndrome (IBS). There is something wrong and they don’t know what it is. It’s the wonderful label that says we don’t know what’s wrong with you, you have recurring diarrhea. Could it be that in the western world we are eating such a refined diet with so few fibers, so few complex carbohydrates that we have created intestines that essentially starve the microbiota, starve these compounds that they evolved on? I don’t know. Celiac disease is probably a different mechanism. That mechanism in terms of immune function and immune response might not fit this model. IBS though might fit this model, I really don’t know. Actually we are starting to take a look at some of those questions with IBS.

**Dr. Neves:** It’s a very practical question. We used to tell our mothers and teenagers not to drink milk during meals because of iron absorption and now you are telling us to allow this. What should we do?

**Dr. Savaiano:** So, is it more important to have adequate iron status or have tolerance to lactose, that’s the answer to the question. It depends on the individuals. If their iron status is fine and they feel that they are lactose intolerant, then my advice is probably sound. If they will tolerate lactose and they are iron deficient, it’s probably a good advice. It’s probably situational.
Milk and the Risk and Progression of Cancer

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Abstract
Observational evidence suggests that nutritional factors contribute to a substantial proportion of cancer cases, and milk contains numerous bioactive substances that could affect risk and progression of cancer. Cancer results from multiple genetic and epigenetic events over time, so demonstrating a specific effect of nutrients or other bioactive food components in human cancer is challenging. Epidemiological evidence consistently suggests that milk intake is protective against colorectal cancer. Calcium supplements have been shown to reduce risk for recurrence of adenomatous polyps. Calcium supplementation has not been observed to reduce risk for colon cancer, although long latency and baseline calcium intake affect interpretation of these results. High calcium intake from both food and supplements is associated with increased risk for advanced or fatal prostate cancer. Results from epidemiological studies examining the relationship between intake of dairy foods and breast or ovarian cancer risk are not consistent. Animal studies have suggested that galactose may be toxic to ovarian cells, but results from epidemiological studies that have examined ovarian cancer risk and milk and/or lactose intakes are mixed. Dietary guidelines for cancer prevention encourage meeting recommended levels of calcium intake primarily through food choices rather than supplements, and choosing low-fat or nonfat dairy foods.

Introduction
Cancer is one of the leading causes of death worldwide, accounting for 7.6 million (13%) of all deaths according to World Health Organization statistics [1]. Notably, patterns of cancer, like dietary patterns, are highly variable across regions and countries with different levels of economic development [2]. Over the past 30 years, improvements have been observed in 5-year survival rates for all cancers combined and for several specific cancers in developed
countries, attributed primarily to improved initial treatments and to increased screening that results in diagnosis at an earlier stage [3]. An increasing population of cancer survivors, i.e. individuals with a history of cancer who are thus at risk for recurrence or new cancers, has promoted increased interest in whether dietary factors may influence this risk and long-term survival [3, 4].

Accumulated data on diet and cancer over the past several decades suggest that approximately 30–40% of cancer cases are potentially preventable via food choices and the modification of nutritional factors [5]. Observed associations between dietary patterns and cancer mortality and morbidity have led to hypotheses about cause and effect relationships, which have often been examined more specifically in laboratory studies of biological activities of dietary constituents, case-control and cohort studies within populations, and clinical trials. Food provides nutrients and numerous other bioactive compounds, many of which have been linked specifically to cellular and molecular events and activities that have been identified in the development and progression of cancer [6]. Milk contains numerous bioactive substances that could potentially affect risk and progression of cancer (see table 1). The relationship between cancer risk and progression and the intakes of milk and/or these bioactive constituents has been the focus of epidemiological and clinical investigations.

### Key Issues in Diet-Cancer Research

Examining the evidence linking milk intake to cancer risk and progression requires an appreciation of the multistage process of carcinogenesis. Cancer

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results from multiple genetic and epigenetic events involving protooncogenes, tumor suppressor genes and antimetastasis genes throughout progression [7]. Clinical cancer is not determined by a single molecular event that disrupts normal cellular function or regulation of growth, but instead results from a series of disruptions across the cancer continuum. This continuum extends from the earliest cellular changes, to a preneoplastic lesion, to a malignant tumor, and finally, to metastasis. Genetic or inherited factors play a role in determining susceptibility to molecular and genetic changes in the process of carcinogenesis, although notably, nutritional factors appear to influence risk even in the presence of highly penetrant, dominant gene mutations [8].

Demonstrating a specific molecular effect of nutrients or other bioactive food components in human cancer, in which a series of genetic and epigenetic changes has occurred over years or decades, is logistically challenging. Also, disentangling the effects of various foods, specific dietary constituents, and related lifestyle factors and characteristics (e.g. physical activity, obesity) that influence risk and progression of cancer has proven to be very challenging. The clustering of health-related behaviors, overall dietary pattern, and food choices is commonly observed [9], so attributing causality or protection to a specific dietary factor, such as milk intake, is limited by the possibility of confounding, especially in observational studies.

In general, interpretation of results from observational studies that rely on self-reported dietary data is particularly constrained by the problem of crude and imprecise methods that are used in the collection of these data, as well as a food content database that is often of limited quality. The use of suitable dietary biomarkers, rather than reliance solely on self-reported dietary intake data, is recognized increasingly as being of value for more accurately characterizing usual patterns of intake and true exposure [10].

Randomized clinical trials, especially those involving isolated dietary constituents as supplements, would seem to be a preferable approach to testing the specific effect of dietary factors on cancer risk and progression, but interpretation of the results of such studies in cancer prevention has been difficult. These studies have been relatively short-term in nature, especially in consideration of the long process and several genetic and molecular steps in the development of clinical cancer. As a specific example, there is a long latency associated with the development of colon cancer, so administering nutrients or other bioactive compounds and examining cancer outcomes in middle-aged and older individuals over a span of 5–7 years in a clinical trial does not address the possibility that a lifetime of high or low intake, or differential intakes at another point in the colon cancer continuum, might affect risk for cancer. Alternatively, many of these clinical trials have involved individuals with precursor lesions [11], such as clinical trials of the effect of calcium on risk for recurrence of colorectal adenomatous polyps. The rationale for using recurrence of colorectal adenomas as the primary end point is that adenomatous polyps are considered precursors of most cancers of the large
bowl, although most polyps do not progress to lesions. Thus, this approach does not truly address the possible effect on colon cancer outcomes.

Within these recognized constraints on interpretation and conclusions, available data and current evidence suggest a possible role for milk intake, or certain bioactive constituents of milk (e.g. calcium, vitamin D), in the etiology of cancer. Separating the effects of the intake of milk per se and bioactive components may not be possible or even necessary for translation into public health recommendations. For example, milk and other dairy foods are a rich source of dietary calcium. In countries in which milk and other dairy products are consumed, these foods are the major source of calcium, so evidence linking calcium intake to cancer is highly relevant to dietary recommendations regarding milk intake. Also, milk is an important source of dietary vitamin D in the US, Canada and other countries in which milk is fortified with this compound. Thus, milk intake is a potentially important determinant of vitamin D status depending on the national policies of vitamin D fortification and other factors that determine vitamin D status, such as sunlight exposure and skin pigmentation [12, 13].

Colorectal Cancer

Cancer of the colon is the fourth most commonly diagnosed cancer worldwide, and incidence rates have been increasing steadily, especially in developed countries [2]. In the US, colorectal cancer accounts for 10 and 11% of the incident cancer cases in men and women, respectively, and 10% of cancer deaths in both gender subgroups [3]. The World Health Organization statistics for colorectal cancer incidence worldwide indicate that incidence in the less developed countries is increasing dramatically [1, 14], with associated high mortality rates. Results from ecological and migrant studies have long suggested that diet is an important environmental factor that influences the risk and progression of colon cancer. Colon and rectal cancers have a well-established and defined continuum of cellular changes and associated lesions that appear to occur in the stepwise process of developing an invasive tumor.

Numerous observational epidemiological studies have examined the relationship between milk intake and risk for colorectal cancer, as summarized in several recent comprehensive reviews [2, 15, 16]. The vast majority of these observational studies, which include both cohort and case-control studies, have identified a protective effect of milk. For example, Cho et al. [17] conducted a large pooled analysis of data from ten cohorts (n = 534,536) from five countries, in whom 4,992 individuals were diagnosed with colorectal cancer at follow-up. Individuals who consumed more than a glass of milk (≥250 g/day) had a 15% reduced risk of developing colorectal cancer (relative risk, RR, 0.85; 95% confidence interval, CI, 0.78–0.94), compared to those who consumed <70 g/day. For each 500 g/day increase in milk intake, the risk of
colorectal cancer was reduced by 12% (RR 0.88, 95% CI 0.82–0.95). Few studies have examined whether the effects are similar across milk products containing higher versus lower levels of fat, which is relevant because of the potential adverse effect of dairy fat intake on cardiovascular disease risk, and possibly, risk for cancer. Data on the relationship between intakes of other dairy foods and colorectal cancer incidence are less consistent. In the large meta-analysis described above [17], relationships between colorectal cancer risk and intakes of other dairy products (measured in five of the ten cohorts) were inverse but not significant. Meta-analysis of studies that examined the relationship between intake of cheese, a dairy food which is typically high in fat, and colorectal cancer risk suggests an increased risk that was not significant [2, 16].

Similarly, calcium intake has been found to be inversely associated with reduced risk for colorectal cancer in the majority of observational studies. Cho et al. [17] found dietary calcium intake to be associated with a significantly reduced risk of colorectal cancer (RR 0.86, 95% CI 0.78–0.95 for the highest versus lowest intake group). In another meta-analysis of ten cohort studies that was conducted for the second World Cancer Research Fund (WCRF) report [2], the RR was 0.98 (95% CI 0.95–1.00) per 200 mg calcium/day. Both dietary calcium and total calcium intake (including supplements) have generally been observed to be protective in a dose-response manner up to a threshold of about 1,200–1,400 mg/day.

The effect of calcium supplementation on recurrence of adenomatous polyps and colon cancer incidence has been tested in a few clinical trials. In a randomized controlled trial of calcium (1,400–1,500 mg/day), calcium plus vitamin D or placebo conducted in postmenopausal women, total cancer incidence at all common cancer sites was significantly lower among the women taking calcium plus vitamin D, but the number of cases was so small that inferences about the effectiveness are unclear [18]. Among secondary outcomes in the Women's Health Initiative (WHI) randomized controlled trial of calcium (1,000 mg/day) plus vitamin D or placebo, colon cancer incidence did not vary between the intervention and placebo groups over 7 years of follow-up [19]. Notably, these WHI participants reported a mean total calcium intake of 1,151 mg/day at baseline, and personal use of a calcium supplement was reported by 54% at baseline and 69% at follow-up clinic visits, with a similar usage pattern in treatment and control groups. In both of the two clinical trials that examined the effect of calcium supplementation on risk for recurrence of adenomatous polyps (reviewed in Weingarten et al. [20]), beneficial effects were observed. At doses of 1,200 and 2,000 mg/day for 3–4 years, a reduction in the risk for recurrent adenoma was observed (odds ratio, 0.74, 95% CI 0.58–0.95) when the results were combined. The beneficial effect of supplemental calcium was observed to be most important in individuals who had baseline levels of serum 25-hydroxyvitamin D that was above the median in one of these trials [14], supporting the synergistic effect of these compounds.
Rock

In numerous epidemiological studies, good vitamin D status (reflected in adequate levels of serum 25-hydroxyvitamin D) has been consistently linked with lower risk for colorectal cancer, as previously reviewed [14, 16].

Several mechanisms of action have been proposed to explain a protective effect of milk intake on colorectal cancer. Calcium influences cell growth and apoptosis and may also sequester tumor-promoting secondary bile acids in the intestine. Vitamin D has a demonstrated role in cell growth regulation via gene transcription, which affects differentiation and apoptosis, including the epithelium of the colon. Additional bioactive components of milk that may specifically affect colorectal cancer progression include magnesium, butyric acid produced by microflora, and conjugated linoleic acid, all of which have been observed to inhibit cell proliferation and tumor cell growth in laboratory studies [14–16].

Prostate Cancer

Cancer of the prostate is the most commonly diagnosed invasive cancer among men in most developed countries [2]. Worldwide, it is the second most common cancer in men, accounting for 12% of incidence cancer cases in men. In the US, it accounts for 33% of new cases [3], and approximately 9% of cancer deaths among men in the US are attributable to prostate cancer. Evidence from migrant populations supports the likelihood that environmental factors, such as diet, are among the important etiologic factors determining risk for prostate cancer.

As recently reviewed [2, 21], numerous observational epidemiological studies, including several meta-analyses, have examined whether milk, dairy foods and/or calcium are associated with risk for prostate cancer. In contrast to the evidence linking higher milk intake to reduced risk for colorectal cancer, the relationship between intake of milk and/or dairy foods and prostate cancer risk is suggestive of an adverse, rather than a protective, effect. The adverse effect is modest and considered suggestive rather than conclusive, as summarized in the WCRF second report [2]. For example, one recent meta-analysis of eleven cohort studies reported a summary RR of 1.11 (95% CI 1.03–1.19) per serving for total dairy foods, 1.06 (95% CI 0.91–1.23) for milk, and 1.11 (95% CI 0.99–1.25) for cheese, similar to results from other large cohorts and pooled studies [reviewed in 15, 21]. The most recent meta-analysis on eighteen cohort studies also found a slightly increased risk [22], although three of the five large cohort studies subsequently published found no strong evidence for the positive association between dairy food intake and prostate cancer risk. Some of these observational studies attempted to examine the effect of higher- versus lower-fat milk in the analysis, but a differential effect is not apparent based on data in the studies reported to date.
In comparison with the large number of epidemiological studies of diet and risk for incident prostate cancer, a limited number of investigations of the relationship between pre- and postdiagnostic diet and the risk of prostate cancer progression have been conducted. In a large US cohort, Chan et al. [23] identified 392 progression outcomes in 1,202 men diagnosed with incident localized/regional prostate cancer and found that milk intake was associated with a small elevation in risk (adjusted hazard ratio, 1.12 for one serving/day increase, p < 0.05).

A more consistent and stronger positive association between calcium intake, whether from dietary sources or supplements, and prostate cancer risk has been observed in these cohort studies and also case-control studies. For example, Giovannucci et al. [24] examined data from a large cohort (n = 51,529) and found calcium intake to be particularly associated with increased risk for fatal or advanced prostate cancer (RR 2.08, 95% CI 1.05–4.10), although the effect was not evident until the level of intake exceeded 1,000 mg/day. Notably, secondary analysis of data from one of the calcium supplement trials aimed at reducing adenomatous polyp recurrence revealed a null or even inverse association between calcium supplementation and incident prostate cancer risk [25].

One proposed mechanism by which intakes of milk, dairy products and/or calcium have been suggested to affect prostate cancer is by downregulating the synthesis of 1,25-dihydroxyvitamin D, the bioactive vitamin D metabolite involved in regulating cellular differentiation and proliferation of prostate epithelia. However, a relationship between 1,25-dihydroxyvitamin D (or even 25-hydroxyvitamin D) in the circulation and prostate cancer risk has not been demonstrated, so this hypothesis is not well supported. Another hypothesis relates to circulating insulin-like growth factor (IGF)-I, which stimulates prostate cell growth and has been shown to be moderately increased by milk intake. However, circulating IGF-I concentration has not been consistently linked with increased prostate cancer risk, so this hypothesis also is not well supported by the evidence.

**Breast and Ovarian Cancer**

Carcinomas of the breast and ovary are hormone-related cancers that have biological similarities. Breast cancer is the most common invasive cancer among women worldwide, accounting for approximately 23% of all cancers in women [1, 2]. Rates are highest in more developed countries; for example, it accounts for 26% of new invasive cancer cases and 15% of cancer deaths in women in the US [3], and rates are increasing rapidly in middle- and low-income countries. Ovarian cancer is much less common than breast cancer but is more likely to have a worse prognosis. Rates are generally higher in more developed and high-income countries, with observed increases in incidence.
in countries undergoing economic transition. Ovarian cancer accounts for 3% of incident cancers in women in the US but 6% of cancer deaths [3].

Estrogens are thought to play an important role in breast and ovarian cancers. Normal cell proliferation and differentiation in these tissues is highly responsive to estrogens and the other gonadal hormones, as well as cellular factors and mitogens that affect growth regulation and apoptosis and thus influence carcinogenesis in all cell types. In addition to the ovarian steroids, other growth factors and mitogens influenced by nutritional factors and dietary patterns also appear to play an important role in the initiation and promotion of breast cancer.

A small proportion of breast cancers can be linked to a specific inherited susceptibility, and inherited variations in genes relevant to biochemical or metabolic pathways involved in mammary cell biology also are likely to increase risk for breast cancer. The incidence of breast cancer varies widely by geographical location and with migration, suggesting that environmental factors, such as diet, contribute substantially to risk. Dietary factors are believed to influence the risk and progression of breast and ovarian cancer, either through effects on hormonal status or via direct tumor-promoting or anticarcinogenic effects.

Over the past several decades, numerous epidemiological studies have examined relationships between dietary intake of milk and dairy foods and the risk and progression of breast cancer. As previously reviewed [2, 15], results from these epidemiological studies have not consistently demonstrated an association between intake of dairy foods (high- or low-fat) and breast cancer risk. Instead, the overwhelming evidence suggests that adiposity, low levels of physical activity and weight gain during adulthood appear to be the most important diet-related determinants of risk, which is reflected in current recommendations for the prevention of breast cancer [2, 26].

Three randomized clinical trials have tested whether a change in diet composition (a reduction in fat intake, and in one study, increased vegetable, fruit and fiber intake) can affect primary breast cancer risk or recurrence and/or survival in women diagnosed with early stage breast cancer. In the WHI Dietary Modification Trial, 48,835 women were assigned to a low-fat dietary pattern or control group and followed for approximately 8 years, and no overall effect on incident cancer was observed [27]. Milk and dairy foods were among the foods targeted for reducing fat content, because these foods contributed 10% of total dietary fat at baseline. Although a change in total milk or dairy food intake was not encouraged, a change in the type of milk or dairy product was among the goals, and a reduction of −2.5 g fat/day from cheese at year one (and −2.4 g fat/day from cheese at year two) was observed in response to the intervention [28].

Two trials have tested whether diet modification following the diagnosis of early stage breast cancer affects cancer outcomes [29]. The Women’s Intervention Nutrition Study (WINS) tested a low-fat diet (≤15% of energy...
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intake) in 2,437 postmenopausal women with early stage breast cancer. With a median follow-up of 5 years, the WINS intervention resulted in a difference in dietary fat intake (33.3 vs. 51.3 g fat/day in the intervention versus control groups at year one), which was associated with modest weight loss (averaging 2.7 kg) and a 24% reduction in new breast cancer events in the intervention group, although a stronger protective effect (42% reduction) was observed in the subgroup of women with estrogen receptor-negative tumors. The Women's Healthy Eating and Living (WHEL) Study tested the effect of a diet very high in vegetables, fruit, and fiber and low in fat (20% of energy intake) on prognosis in 3,088 pre- and postmenopausal breast cancer survivors who were followed for an average of 7.3 years. At baseline, the WHEL Study participants reported an average intake of 7.3 servings/day of vegetables and fruit, which averaged 9.2 in the intervention group and 6.2 in the control group at 6 years. Recurrence-free survival did not differ across the two study arms. In that study, serum estrogen at baseline was found to be independently associated with poor prognosis, and a protective effect of the diet was observed in the subgroup of women who did not report hot flashes at enrollment. These findings suggest that reproductive hormone status may determine whether a high-vegetable, fruit and fiber diet may improve prognosis. Another finding from the WHEL Study was that total exposure to carotenoids over the course of the study, which was largely determined by the level at enrollment, was associated with breast cancer-free survival regardless of study group assignment. In additional subsequent analysis, milk consumption (a variable that combined milk, cream, and yogurt) at year one was observed to be slightly higher in the intervention versus control group (a difference of 0.1 serving/day) but unrelated to breast cancer-free survival in a Cox proportional hazards model controlled for stage, grade, ethnicity, and age.

The relationship between vitamin D status and breast cancer is an area of current interest, because several epidemiological studies have linked lower serum 25-hydroxyvitamin D levels with higher risk for breast cancer [30]. Notably, results from the WHI supplement study revealed no effect of calcium or calcium plus vitamin D supplements on breast cancer risk [31].

Although fewer epidemiological studies have been reported for ovarian cancer than for breast cancer, the available evidence for a relationship with intake of milk or dairy foods and cancer risk is not consistent [reviewed in 2, 15]. A possible role for intake of lactose (or more specifically, galactose, a unique monosaccharide constituent of lactose) in the development of ovarian cancer has been the focus of some research. The suggestion was based on animal studies showing that a diet very high in galactose is toxic to oocytes and evidence that the genetic disorder of galactosemia causes premature ovarian failure in women. A few case-control and cohort studies have investigated this relationship, and the results are mixed. In one follow-up study of the influence of dietary factors on survival in a group of 609 women who had been diagnosed with ovarian cancer, lactose intake was found to be modestly
Rock

associated with mortality (hazard ratio 1.32, 95% CI 0.99–1.75 for the highest versus lowest tertile), although an association between intake of total dairy products and survival was not evident [32].

Conclusions

Currently, the weight of the evidence suggests that intake of milk and/or constituents such as calcium and vitamin D may influence the risk and progression of large bowel (colon and rectum) and prostate cancer, while the evidence is mixed or lacking for other cancers. The WCRF report concluded that there is a probable link between milk intake and colorectal cancer in decreasing risk, and a probable link between diets high in calcium and prostate cancer in increasing risk [2]. In the WCRF personal recommendations, milk or dairy food intake is not specifically addressed, although meeting nutritional needs through foods is encouraged and dietary supplements are not recommended for cancer prevention. The current American Cancer Society guidelines for cancer prevention advise individuals to consume recommended levels of calcium intake, primarily through food choices (rather than supplements), and that people who obtain much of their calcium intake from dairy products should select low-fat or nonfat choices [26]. These strategies are consistent with general public health guidelines to promote good health and reduce risk for chronic disease.

References

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Discussion

Dr. Gibson: I was wondering whether milk is a biomarker for some other dietary habits or lifestyle or whatever in the same way that smoking often is. I mean smoking isn't healthy, I understand that, but it often coincides with low socioeconomic status.
So, to what extent do you feel that the milk situation is actually representing something else?

Dr. Rock: That’s a good question, and that’s why I am talking about different quality of studies that are done; it’s because we try to identify those components. I examined the data in the Women’s Healthy Eating and Living study to see if milk or dairy products were associated with a risk of recurrence in those women. The relationship was not linear, with grade and stage being the primary predictors. Ethnicity confounds the relationship because the Asian-American women in that study had much lower risk for recurrence, and they represent a group that consumes lower amounts of milk. Another interesting point is that the more we learn about bioactive ingredients in food, the more we need to do adjusting, for example soy and genistein have been shown to actually reduce the effect of that CYP24 that degrades the vitamin D active compounds.

Dr. Melnik: I would like to hear your opinion concerning high progesterone levels in the fraction of milk lipids due to the technique of milk production by permanently pregnant cows. According to my calculations, heavy meals including the intake of butter, cream and cheese could result in a daily ingestion of about 50 µg of progesterone. So, this is in the range of an oral contraceptive. This may have an effect on fertility. Furthermore, progesterone is an important hormone involved in the pathogenesis of breast cancer [1].

Dr. Rock: There are two things that make me worry less than you. We know for example the soy estrogens have such low affinity with binding to estrogen receptors so that they really only get important in older women. There is little competition against the natural endogenous hormones. It is important to look for consistency across different types of studies: if they were that bad, we would see an adverse effect of milk and dairy food intake on breast cancer incidence and progression, and that just hasn’t emerged. So I am not concerned about that for that reason. People are getting carried away by vitamin D right now, especially in the countries where milk is the major source of vitamin D. I think we should just wait and see because that’s a vitamin that clearly at high amounts is associated with adverse effects. In the ATBC study, with 29,000 Norwegian men, higher vitamin D in the blood was actually associated with a much higher risk for pancreatic cancer, which is a problem in smokers in particular; that’s a cancer that is driven by smoking.

Dr. Martin: You nicely explain the problems with epidemiology. In fact, one of our doctors in the UK who writes for The Times once wrote that the best thing that we could do for public health is to close all departments of epidemiology. So I wondered whether we should focus on randomized controlled trials instead. Specifically, whether we should focus on primary or secondary prevention trials, particularly since you showed that dietary modification in primary intervention trials is almost impossible, but secondary prevention trials can modify diet.

Dr. Rock: That’s a very good comment. I think epidemiology is a tool like biostatistics. It’s a source of hypothesis, and it’s people eating food, and usually we can follow them long enough to see cancer outcomes. The problem with clinical trials when you have a cancer outcome is that the expense is just phenomenal and that long latency with cancer makes it unlikely that funding for a clinical trial for 50 years to see who develops colon cancer would be achieved. Another really good point you brought up is to collect as much information as we can. In clinical trials, people can put away aliquots for future studies. As an example, I will be presenting an abstract in Experimental Biology this year showing that we were able to examine the effect of weight loss on plasma hydroxyvitamin D levels, because as Dr. Prentice mentioned, it’s usually lower in obese individuals, and we don’t know why. The question is when people lose weight, does it go up? So now in my weight loss studies completely unrelated to cancer, I can
get blood samples, and 20 years from now, if we have aliquots put away and there are emerging hypotheses of factors and mechanisms that can be explored, that's where we will get the most out of clinical trials.

Dr. Anderson: When you talked about epidemiology giving rise to hypothesis and mechanisms, I missed the one on calcium in prostate cancer. Do you have one there?

Dr. Rock: The evidence for a mechanism is weak. The milk connection is not so much driven by milk but that calcium is a marker of milk intake. The major mechanism proposed is that calcium downregulates the synthesis of 1,25-hydroxyvitamin D, but a relationship between this metabolite and prostate cancer risk has not been observed. With regard to colon cancer, I think there is a more biologically feasible mechanistic explanation. I think the discussions also need to note that milk is consumed in developed countries, and that's where they are getting vitamin D, and maybe overconsumption promotes the effect on the insulin-like growth factor.

Dr. Prentice: It is also linked to the high 1-25-dihydroxy vitamin D associated with a low calcium intake, and that's regarded as protective, but as a mechanism it is fairly lean, as you say. I was interested that amongst the plausible mechanisms for the colorectal cancer association you were not including the formation of fecal soaps by calcium. Has the hypothesis that calcium reduces fat absorption now been knocked on the head? I would be interested in hearing where the evidence has gone to on that now.

Dr. Rock: There have been at least a few short-term studies in which people were fed calcium and/or dietary fiber, and/or β-carotene, and samples were examined to identify effects in the lumen. Short-term effects on secondary bile acids were observed, but in the long-term there was no effect on colorectal adenoma recurrence. And that's also true with proliferation, in those reviews from clinical studies it would be wonderful if we had some good markers of proliferation in tissue, but they are too highly variable, and in a lot of those short-term studies, changing these dietary factors did not change the proliferation differentiation or epitosis of colonic epithelial cells.

Dr. Mouane: Several studies show that breastfeeding protects against cancers, particularly ovarian cancer and breast cancer. What do you think?

Dr. Rock: With breast cancer, there have been some studies suggesting that breastfeeding may be helpful, but it's not consistent and it presumably would be because of the effects on estrogen. When a woman breastfeeds, she is not going back into the usual ovulatory cycle that has spikes of estrogen. So there is theoretically an argument. I think the data are there because there are so many studies examining the link between breastfeeding and osteoporosis. Once you have those cohorts identified, it shouldn't be that difficult to find out their breast cancer risk, but the bone scientists aren't always in the same room as the cancer scientists unlike this meeting, where we are. So, maybe we need more meetings like this that focus on foods rather than on simple biological systems.

Reference

Milk A1 and A2 Peptides and Diabetes

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Abstract

Food-derived peptides, specifically those derived from milk, may adversely affect health by increasing the risk of insulin-dependent diabetes. This position is based on the relationship of type 1 diabetes (T1D) and the consumption of variants A1 and B β-casein from cow's milk. It appears that β-casomorphin-7 (BCM-7) from β-casein may function as an immunosuppressant and impair tolerance to dietary antigens in the gut immune system, which, in turn, may contribute to the onset of T1D. There are thirteen genetic variants of β-casein in dairy cattle. Among those variants are A1, A2, and B, which are also found in human milk. The amino acid sequences of β-casomorphins among these bovine variants and those found in human milk are similar, often differing only by a single amino acid. In vitro studies indicate BCM-7 can be produced from A1 and B during typical digestive processes; however, BCM-7 is not a product of A2 digestion. Evidence from several epidemiological studies and animal models does not support the association of milk proteins, even proteins in breast milk, and the development of T1D. Ecological data, primarily based on A1/A2 variations among livestock breeds, do not demonstrate causation, even among countries where there is considerable dairy consumption.

Introduction

Bovine milk is a rich source of energy and many essential nutrients, and is often consumed from infancy throughout life. The 2005 United States Dietary Guidelines for American recommends the daily consumption of 2–3 cups of low-fat or fat-free milk or complementary amounts of dairy products, such as yogurt and cheese. Evidence-based health benefits of dairy products have been supported by numerous health organizations and regulatory agencies.
The diabetogenicity of bovine milk protein was suggested based on the observational relationship of its consumption and the apparent incidence of type 1 diabetes (T1D). Subsequent studies suggested the casomorphins derived from specific variants of β-casein may have unique structural properties that, in part, explain their potential bioactivity. One peptide, β-casomorphin-7 (BCM-7) is a peptide derived from the A1 variant of β-casein which is primarily found in high frequencies among Holstein or Holstein-Friesian, Hereford, Ayrshire and Braham cows. Originating in Europe, approximately 95% of today’s dairy cows in the US are Holstein or grade Holstein.

There are thirteen genetic variants of β-casein in dairy cattle. Among those variants are A1, A2, and B, which are also found in human milk. The amino acid sequences of β-casomorphins among these bovine variants and those found in human milk are similar, often differing only by a single amino acid. Studies indicate BCM-7 can be produced from A1 and B during in vitro digestive processes; however, BCM-7 is not a product of A2 digestion. The evidence of peptide absorption from a normal, healthy human gastrointestinal tract is scant. However, antibodies to this peptide have been detected in individuals with celiac disease and those diagnosed with autism and schizophrenia in which there are several immune and gastrointestinal disorders.

Evidence from epidemiological studies and animal models is inconsistent with respect to the association of milk proteins, even proteins in breast milk, and the development of T1D. Ecological data, primarily based on A1/A2 variations among livestock breeds, do not demonstrate causation, even among countries where there is considerable dairy consumption. Regardless, the implication of variant A1 consumption in the development of T1D remains controversial. This apparent relationship has prompted some changes in livestock composition and subsequent exposure to the A1 and A2 variants. The public health impact on these changes remains to be evaluated.

**Overview Dietary Peptides**

Dietary proteins and the potential health aspects of their respective peptides are of considerable interest among food scientists, health professionals, and pharmaceutical companies. These peptides may be available through several avenues, including hydrolysis during normal digestive processes, proteolytic activity of typical microflora, and fermentation actions facilitated by enzymes from plants and microorganisms.

Depending on their site of action, it may not be necessary for bioactive peptides to cross the mucosal barrier. Some peptides cross the intestinal epithelium via transcellular and paracellular pathways, peptide transport systems as well as through translocation across Peyer’s patches. Regardless, many of
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these peptides mediate physiological responses at various molecular targets, such as the cardiovascular system, nervous system, digestive system, and the immune system [1] (see fig. 1).

Inhibitory peptides of angiotensin-converting enzyme, a key enzyme in blood pressure regulation, have been identified following hydrolysis of milk, soy and fish proteins. One of the casein-derived peptides, Val-Pro-Pro, may modulate monocyte adhesion and downregulate epithelial inflammation by attenuating the c-Jun NH2-terminal kinase pathway. This peptide may reduce the risk of cardiovascular disease. Pepsin digestion of lactoferrin, a bovine milk protein, can produce lactoferricin, an antimicrobial peptide that, as in

**Fig. 1.** Food-derived bioactive peptides. From Korhonen and Pihlanto [1], with permission.
vitro studies indicate, may also have immunostimulatory and antiviral properties. The biology of human milk and colostrum demonstrates they are rich in proteins and peptides, including growth factors, hormones, antiviral and antibacterial factors, and even some potential allergens.

Opioid peptides, those which include amino acid sequences that function as opioid receptor ligands that behave as agonists, may be formed from most dietary protein sources, such as milk, grains, legumes, vegetables, meat and poultry. For example, enzymatic digests of milk proteins, such as α-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin, and lactotransferrin yield an array of exorphins, casoxins, casomorphins, lactorphins, and lactoferroxins [2]. Some of these peptides have morphine-like activity by binding opioid μ-receptors. These receptors are found in many tissues and compartments, such as the central and peripheral nervous system, the endocrine system, and the immune system. The physiological significance of these peptides derived from β-casein is the center of considerable controversy. Some studies suggest this peptide may reduce normal gastrointestinal peristalsis and may ‘trigger’ susceptible individuals to diabetes.

All proteins are subject to variations in structure and amino acid sequence. Bovine milk contains at least four caseins: αs1-casein, αs2-casein, β-casein and κ-casein. Similarly, whey protein is composed of an array of acid-soluble proteins, including α-lactalbumin, β-lactoglobulin, immunoglobulins, glycomacropeptides, bovine serum albumin and many minor proteins. These kinds of bioactive proteins and peptides are also found in breast milk.

There are thirteen variants of bovine β-casein. Alleles for A1, A2 and B have received the greatest attention for their health implications. The frequency distribution of these alleles is quite broad among cattle breeds. The most common variants among Western cattle are A1, A2, and B. Jersey (~30%), Normande (~45%) and Hereford (~25%) breeds have a relatively high frequency of the B variant. A2 is the dominant allele in Guernsey (>96%) cattle. Cattle breeds such as Angus (~95%), Ayrshire (~60%), Hereford (~75%), Holstein (~60%), and Shorthorn (~49%) primarily present the A1 allele. Nearly all milk-producing breeds contain a blend of the A1, A2 and B alleles [3].

There are several genetic polymorphisms associated with the opioid peptides derived from β-casein. For example, A1 and A2 variants differ by His to Pro amino acid substitution at position 67, respectively. A1 and B variants differ at position 122, where Ser replaces Arg, respectively. In general, the β-casomorphins from these β-caseins are stable to enzymatic degradation. In vitro digestion with pepsin, pancreatic elastase and leucine aminopeptidase of A1 and B yielded BCM-7. A2 is resistant to hydrolysis by these proteases. Thus, depending on the procedures and proteolytic enzymes used to prepare dairy products, such as milks, yogurts, and cheeses, the BCM content may vary and possibly become negligible.
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Overview Diabetes

In the US, the crude incidence of diagnosed diabetes increased 136% from 3.3 to 7.8 per 1,000 population between 1980 and 2007 based on CDC statistics. According to WHO, the global burden of diabetes is expected to reach 230 million by 2030, twice the estimated prevalence in 2000. The estimated health care expenditures, depending on the mode of treatment of this chronic disease, exceeded USD 132 billion in the USA in 2002, representing approximately 10% of the health care financial burden. This percentage increases more than twofold among many developing countries. The highest prevalence of diabetes is found in India, China, USA, Indonesia, Japan, Pakistan, Russia, Brazil, Italy and Bangladesh.

T1D, formerly known as insulin-dependent diabetes mellitus, is the second most common childhood malady after allergy. This disease reflects interactions of a constellation of complex factors that ultimately contribute to the destruction of insulin-producing β-cells and the presentation of insulitis and β-cell autoantibodies. Genetic predisposition, environmental factors, and autoimmune mechanisms are involved in the pathogenesis of this immune-mediated disease that eventually leads to insulin deficiency.

Possible dysfunction of the gastrointestinal immune system and stimulation of pancreatic antigens have been suggested to predispose individuals to T1D. Even disruptions in intestinal microflora and a compromised mucosal barrier have been implicated as contributors of decreased tolerances to dietary antigens and consequential autoimmunity that trigger T1D pathogenesis. For example, wheat-derived peptides may initiate intestinal inflammation among T1D individuals by penetrating atypical cell junctions, activating T cells, and interacting with antigen-presenting cells. The ensuing cascade production of inflammatory cytokines, such as γ-interferon and tumor necrosis factor further compromises the gut immune function [4, 5]. While there is evidence that these cereal peptides can initiate or modulate intestinal inflammation among susceptible individuals, some would suggest bovine insulin can trigger an immunologic response in an immature gastrointestinal tract, contribute to an aberrant inflammatory response, and lead to an increased risk of T1D.

Human leukocyte antigen (HLA) haplotypes have been implicated in several autoimmune diseases, including susceptibility to T1D. The frequencies of various DRB1 and DQB1 alleles may confer a predisposition of developing T1D. A 9-year longitudinal study among 254 HLA-DQB1-positive children who received cow’s milk during infancy or early childhood demonstrated that this protein source was not associated with a clinical progression of T1D. However, when corrected for several confounding factors, such as genetic susceptibility and age at cow’s milk introduction, the relative risk statistics indicated high consumption (>3 glasses/day) of cow’s milk can be diabetogenic among those genetically predisposed to T1D [6].
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About 25 years ago, the diabetogenicity of cow’s milk hypothesis was advanced based on studies with the diabetes-prone BB (bio-breeding) rat model. This study indicated the 35 and 52% incidence of diabetes among animals fed milk or wheat protein rations, respectively, was reduced to 15% when elemental amino acids replaced the protein sources [7]. Other studies with nonobese diabetic (NOD) mice suggested early introduction of diabetogenic food components even as late as puberty may trigger diabetes onset. Interestingly, the introduction of protein hydrolysates or chloroform-methanol extracted rations virtually precluded diabetes development [8]. A more recent pilot study among HLA-DQB1 allele-positive infants indicated that the early introduction of a casein hydrolysate may modulate β-cell autoimmunity.

Two noteworthy studies among NOD and BB mice yielded inconsistent results. The international study indicated a milk-free, plant-based (primarily wheat) ration was significantly more diabetogenic than rations containing 10% milk hydrolysates. These data also indicated the animals developed diabetes, regardless of the β-casein variant [9]. On the other hand, an earlier study among A1 and A2 variant-fed NOD mice presented a higher incidence of diabetes in the A1 group. Again, diabetes was evident in each study group [10].

Observational studies among Polynesian children in Somoa (formerly associated with New Zealand) in the South Pacific and those in Auckland, Australia, indicated a low incidence of T1D among children who were breast-fed or consumed little cow’s milk. Similar assessments among the Maasai in Kenya and northern Tanzania whose diets consist primarily of cow’s milk and maize meal indicated a low incidence of T1D. Interesting follow-up studies with NOD mice indicated those receiving the A2 variant of casein did not develop diabetes.

Several studies among T1D patients and with those diagnosed with celiac disease detected antibodies to bovine β-casein, particularly A1 casein. Celiac disease, previously known as celiac sprue, is an autoimmune disease prompted by undigested gluten, primarily gliadin peptides, from cereal grains, such as wheat, rye and barley. Many of these gluten-intolerant individuals are HLA DQ2-positive and often present symptoms associated with chronic gastrointestinal inflammation during early childhood.

The consumption of cow’s milk with A1 or A2 variants has been compared with the incidence of T1D. In the absence of cheese consumption, data from 16 countries indicate a significant correlation between total milk consumption and the incidence of T1D among males under the age of 15 years [11] (fig. 2). It is important to note that the correlation, $r^2 = 0.72$, occurs in the absence of cheese. This correlation diminishes to $r^2 = 0.23$ when the consumption of the B variant is included. While these data suggest a strong relationship, it is important to note that there are limited data indicating BCM-7, per se, is...
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Actually produced during in vivo digestion in humans. The in vivo release of this peptide from A1 β-casein is implied by the detection of opioid peptides in blood and urine of autistic and schizophrenic subjects. However, the clinical presentations of these conditions include gastrointestinal dysfunction and altered dietary protein and peptide digestion and absorption.

Conclusion

T1D is a chronic autoimmune disorder among genetically predisposed individuals that contributes to the progressive dysfunction of the pancreas, and the inability of the β-cells to secrete insulin. Most of those diagnosed with T1D are children typically before achieving puberty. Among the many hypotheses advanced to describe dietary factors that lead to T1D development is the early exposure to cow’s milk. Animal models, observational studies, and clinical trials to assess the diabetogenic role of β-casein peptides, specifically from A1 and A2 variants, have yielded inconsistent results. Exposure to these variants depends on the livestock breed, the amount of bovine milk consumed and the duration of breastfeeding. Individual genetics and environmental variables may be important T1D predisposing factors. There appear to be dietary components, other than milk, that may trigger or contribute to the onset of T1D. The composite of current evidence from these kinds of studies and factors is insufficient to develop a national health policy, invoke changes in livestock characteristics, and alter early dietary patterns with respect to the consumption of bovine milk [12].

Fig. 2. Relationship between the daily consumption of β-casein A1 and the incidence of T1D [11], with permission from Elsevier.
References


Discussion

Dr. Rock: I would like to ask where we can go forward with this information. I am wondering if among the bodies who have prepared recommendations and summary statements, most of them have concluded as you did, that the evidence is not very supportive?

Dr. Clemens: The US has not taken a position on that. Some countries and some regions have actually changed their livestock. I know there is a big push, and I know there is a number of organizations that actually screen their livestock and slowly remove the A1 variant from the breeds, and certainly the dominant part here is A2. So maybe we don’t have long-term data to say that in fact was going to give us the answer. I think there may well be other factors. We have not taken a position on this even though the dairy people are trying to respond to it. I think we just have to keep it in proper perspective. We examined this when I was with a major company of the world over 30 years ago, and we need to examine receptor sites, we need to examine how those receptor sites change over the span of life. We know that different receptors respond differently upon birth vs. later on in life. We certainly know that’s the case with vitamin D receptor, for example, and we certainly have seen that with various hormones. We know that some hormone receptor sites are much more sensitive at birth. We also have configurations of components in the diet that may not have the same binding affinities at birth as later on in life. So, there are a lot of variables here. I think it’s a bit premature to say the A2 or A1 variant in cow’s milk is the causative
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agent. I think it's an interesting concept to examine. I think that at the end of the day we are consuming food, and in a lot of places of the world people are grateful to have some milk to consume.

**Dr. Anderson:** Dr. Frasier Scott produced several publications on the BB rat, in which he argued that casein was a cause of type 1 diabetes. But I believe that Dr. Scott has changed his opinion as this effect of casein can happen with a number of proteins, when the intestinal barrier is weakened and proteins or peptides affect the β-cell and peptides amino acids.

**Dr. Clemens:** You remember well, and that's why we are seeing more and more research on the gut barrier function and the gut immune system, because the gut is a great immune organ. I think that's going to be a major focus of future research in this area.

**Dr. Thorsdottir:** I just want to comment on Dr. Rock's question about cow's milk intake in infancy and the incidence of type 1 diabetes. There are studies from Finland where they are doing long-term interventions and giving the infants who have a genetic risk for type 1 diabetes different kinds of formulas. They actually reported a higher concentration of antibodies in those children who received intact milk proteins. However, if the children had received something else to eat, other proteins, the risk may have been the same, they would have shown other antibodies. So, this is really a difficult task, but if you are interested in milk in general and type 1 diabetes, I would recommend looking into this Finnish intervention.

**Dr. Hernell:** I was wondering about the association between cow's milk protein and type 1 diabetes in children because if you look at Finland and Sweden, we have a very high incidence that has been increasing for many years, and I don't think that you can see a correlation between the milk intake and the increase in diabetes. Now, at least in Sweden, it seems that the increase has leveled off. Again, I don't think there is a clear association. Also, I find it difficult to believe that a change in the composition of the breed resulting in different casein patterns should be the culprit. It must be difficult to differentiate an effect of milk intake from a general effect on increased growth, which is also associated with increased diabetes risk.

**Dr. Clemens:** I appreciate that remark. Indeed, this is what I also questioned. Can we change that, and is it going to make a significant difference? I think Dr. Thorsdottir is right in that if it's not milk, it will be something else in our diet that may precipitate these kinds of conditions. I think we have to continue looking at foods without drilling down too far and actually exacerbating and making a public health problem out of it.

**Dr. Thorsdottir:** I think the problem with the discussion about β-casein A1, β-casomorphins, type 1 diabetes and even the diseases of the central nervous system was that it was too early on the news and too early on the market, even though it was mainly in New Zealand and in Australia. People were too eager to make a lot of money before they had any evidence, and from my point of view this also disturbed the research.

**Dr. Netrebenko:** As you know, infants born with severe intrauterine growth retardation have a lack of β-cells and nephrons. Do you know whether the lack of β-cells in these infants could result in diabetes?

**Dr. Clemens:** That's a very interesting question. I am not a diabetologist, I am an endocrinologist, so I can't answer that question. I would be glad to pursue it for you and get back to you on that.

**Dr. Hernell:** I think with type 1 diabetes there is an association with high birthweight and rapid growth early in life, but I do not remember seeing data on an association with low birthweight...
Milk Fat and Health Consequences

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Abstract
Dairy foods are widely recommended as part of a healthy diet mainly because of the ready availability of calcium but also because they are a good source of protein, minerals and fat soluble vitamins. On the other hand, dairy foods have been viewed with suspicion by many because dairy fats contain saturated fatty acids and cholesterol. It has been thought, particularly by consumers, that dairy fats may increase the risk of coronary heart disease because of the contribution they make to total saturated fat intake. However, dairy fats contain other lipid bioactives (e.g. omega-3 fatty acids, gangliosides, conjugated linoleic acid) that may counteract the effect of saturates in a well balanced diet. Surprisingly, there have been few studies that have addressed this issue.

Dairy Fat Composition
Dairy fats are a complex mixture of lipids, but the bulk (~98%) is contained in the triglyceride form. The fatty acids are made up primarily of saturates and monounsaturates with polyunsaturates present in minor amounts (<3% total fatty acids).

Saturated Fats and Cholesterol
The saturated fats have received the most attention because they contribute to the total saturated fat intake of many people living in Western societies, and this in turn has been associated with elevated risk of cardiovascular disease (CVD) through increased plasma cholesterol and LDL. However, unlike the fats from most animal sources, dairy saturated fats are composed of fatty acids ranging in carbon length from 4 to 20. Because not all saturated fatty
acids are hypercholesterolemic in nature, there is debate about the negative
effect of dairy fats on human health. Only lauric (C12:0, 3% total fatty acids),
myristic (C14:0, 10%) and palmitic acids (C16:0, 26%) are hypercholester-
olemic [1]. Stearic acid (C18:0) is poorly absorbed by the gut and is not con-
sidered potent at raising cholesterol levels in humans [2]. Furthermore, it has
recently been proposed that stearate-rich fats are suitable replacements for
trans fatty acids (TFAs) and possibly other hypercholesterolemic fats [3]. The
short- and medium-chain saturates including butyrate (C4:0, 6%), caproate,
(C6:0, 3%), caprylate (C8:0, 3%) and capric (C10:0, 3%) are not thought to
be hypercholesterolemic. In fact, caprylic and caproic acids are the major
constituents of medium chain triglycerides (MCT oil) that are widely used
in clinical nutrition as highly available sources of energy since they directly
enter the portal system and bypass the liver [4]. The role of butyric acid in
foods is less well known. It is interesting to note that the protective effect of
dietary fiber against bowel cancer is thought to be mediated by butyric acid
produced by commensal bacteria. Butyrate induces transcriptional changes
in human colonic mucosa, nucleotide binding and oligomerization domain
2-dependent mucosal immune responses, and is reported to stimulate rumen
development in calves [5–7]. There are only limited studies that address the
role of dietary butyrate in humans.

Cholesterol and some plant sterols are present in milk fats, and again, the
effect of dietary cholesterol is controversial. While it is scientifically accepted
that dietary cholesterol contributes little to plasma cholesterol levels, foods
containing cholesterol are often grouped as unhealthy. However, cholesterol
is present in human breast milk, and because there is emerging evidence to
suggest that dietary cholesterol could play a positive role in the growth and
development of infants, some manufacturers are including cholesterol in their
infant formulas. There is no doubt that animals (e.g. rats, pigs) fed cholesterol
can be shown to accumulate cholesterol as measured by increases in cere-
brum weight and cholesterol level, but there is little in the way of functional
data to show that this can result in a practical difference in brain function [8].
There are no data relating to the brains of breast milk-fed infants compared to
the brains of formula-fed infants in the way that was used to establish a need
for DHA in infants [9, 10]. It is important to note that human infants thrive on
cholesterol-free diets. Smith-Lemli-Opitz syndrome, marked with extremely
low serum cholesterol, has served as a human model for the evaluation of
dietary cholesterol [11] in the same way that Zellweger syndrome enabled the
definition of DHA requirement for neural function.

Effect of Dairy Fats in the Diet

Of major interest is the role of dairy foods in the whole diet. Gibson
et al. [12] recently conducted a systematic review that had the specific
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aim to assess the effect of dairy foods on coronary heart disease using all available prospective cohort studies. They found 12 studies that were eligible for inclusion assessing in total over 215,000 subjects. Most studies had close to or greater than 80% follow-up rate, made adjustment for three or more confounders in the statistical analysis and used standard criteria to determine CHD/IHD end points. About half the studies used a validated food frequency questionnaire (FFQ), administered the FFQ more than once, or had a follow-up duration of 20 years or more. Less than half the studies involved subjects with characteristics representative of the general population. Eight of twelve studies reported no association between dairy intake and CHD/IHD. Four of twelve studies suggested some association between some aspect of dairy intake and CHD/IHD. It is important to note that very few studies were designed specifically to address the issue of dairy consumption and heart disease. In most of the studies, the information for dairy intake was collected as part of FFQ, and these tools do not robustly assess dietary fatty acids. In general, attempting to ascribe effects of a single food to multifactorial events such as CVD is extremely difficult. However, in general the studies show that high intakes of saturated fats from all foods are associated with increased risk of CHD, and generally vegetarian diets were protective.

Cohort studies are beset by confounders or effect modifiers including social factors and changes to diet and other risk factors over time. For example, eight of the studies were set up prior to the introduction of low fat dairy products. The Nurses Health Study examined the effect of low fat vs. high fat dairy products in the diet [13]. The study indicated that the ratio of high- to low-fat dairy was positively associated with increased CHD and that the effect could be mainly ascribed to C16:0 and C18:0 in the diet. However, dairy only accounts for 15% total saturates in the diet and 10% of the C16:0 and C18:0 from all foods. Therefore, there appears to be a lack of clarity about the contribution dairy foods make to the risk factors for CVD in our diets. In conclusion, this assessment of twelve prospective cohort studies indicated that there is no consistent evidence in support of the concept that dairy intake is consistently associated with higher CHD/IHD risk.

Most studies support the concept that high saturated fat intake is associated with higher risks of CHD/IHD, but corrections for effect modifiers is not always complete. Thus, it is almost impossible to tease out the relative effects of single food constituents, such as dairy fats. These conclusions have been broadly confirmed by a recent report from Australia. When subjects with the lowest intake of full-fat dairy were compared with participants with the highest intake (median intake 339 g/day), the latter had reduced death due to CVD (HR: 0.31; 95% CI: 0.12–0.79; p for trend = 0.04) after adjustment for calcium intake and other confounders [14]. In contrast, a recent meta-analysis of cohort studies included all studies regardless of whether dairy was mentioned [15]. Surprisingly, this review could not find
significant evidence for concluding that dietary saturated fat is associated with an increased risk of CHD or CVD. This result challenges the strong belief that many consumers and health care professional have that CHD/CVD is mediated through high intakes of saturated fat that result in elevated plasma cholesterol levels.

To put this in context, there is still uncertainty over the actual role of diets with low polyunsaturated fat to saturated fat (P/S) ratios. In a systematic review and meta-analysis of randomized controlled trials, Skeaff and Miller [16] determined that the relative risk of fatal CHD was not reduced by either low-fat diets or high P/S diets. However, when they restricted the meta-analysis to intervention trials of P/S diets in which mean serum cholesterol concentration was significantly lower in the treatment group, the risk of fatal CHD was significantly reduced by the P/S diets. Similarly, high P/S diets reduced the risk of CHD events. Whether the benefit of high P/S diets is independent of cholesterol lowering deserves attention.

**Trans Fatty Acids**

Dairy fats also contain TFAs that are also thought to be hypercholesterolemic. However, there is debate as to the relative effect of the ‘natural’ TFAs in milk fats at the typical consumption levels that arise from rumen microbiota and so-called industrial TFAs that arise from industrial transesterification of polyunsaturated fats. Chardigny et al. [17] reported on the results of the TRANSFACT study in which the consumption of TFAs from industrially produced sources was compared with TFAs from natural sources among 40 normolipidemic subjects (19 men and 21 women). In this 3-week study, the subjects were fed 11–12 g/day of these TFAs, and the natural or ruminant TFAs led to increased HDL and LDL cholesterol in women but not in men. Importantly, this intake level represented about 10 times that normally consumed (~5 vs. 0.5%en). The mechanism for this is not clear, but the data point to our limited understanding of one aspect of dairy fats. Finally, conjugated linoleic acid (CLA) is also found in bovine milk. While the efficacy of the use of CLA in the diet of animals is well documented, the effect of this fatty acid on human health remains debatable.

**Omega-3 Long-Chain Polyunsaturated Fatty Acids**

The importance of dietary omega-3 fats in reducing the risk of CHD/CVD has attracted worldwide attention. Metcalf et al. [18] recently demonstrated the close relationship between dietary omega-3 intake, adequate intake status as measured by the level of omega-3 fatty acids in RBC and the level of omega-3 fatty acids in cardiac tissue. There have been few attempts to
examine the effects of dairy diets on omega-3 long-chain (LC)-PUFA status. This is surprising for two reasons. Firstly, although dairy fat is low in PUFAs, the balance of the essential fatty acids linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) at 2:1 is considered desirable as it allows the endogenous conversion of these PUFAs to LC-PUFAs. In addition, dairy fats contain low levels of two important omega-3 LC-PUFAs, namely eicosapentaenoic acid and docosapentaenoic acid. Each is a precursor of the very important DHA (docosahexaenoic acid) that has proven health benefits when provided in an adequate amount in the diets of infants. There are clues that a low intake of both precursor fatty acids may result in better omega-3 LC-PUFA status. Clark et al. [19] demonstrated that if the linoleic acid:α-linolenic acid ratio of infant formulas was lowered from the levels of 10:1 to around 3–4:1, the DHA status of infants improved. Equally, pertinent are the findings of others who have shown that infants fed formulas based on dairy fats [20] or indeed evaporated milk [21] have LC-PUFA status midway between those fed formulas enriched with vegetable oils and those fed breast milk. Finally, in their interesting monastery study, Lasserre et al. [22] reported that the level of omega-3 LC-PUFAs in nuns consuming a dairy fat-based diet was superior to the same group when they consumed a sunflower oil-based diet. Confirmation of these findings comes from animal studies. When rats were fed butter, as a model of a saturated fat diet, their omega-3 LC-PUFA status was better than those fed plant oils [23]. Clearly, the influence of dairy fats in a well-balanced diet needs to be investigated in regard to omega-3 LC-PUFA status and the metabolites they produce.

**Bioactive Phospholipids**

Some of the LC-PUFAs are located in the phospholipid and glycophospholipid fractions of cell membranes. These components contribute about 1% of the total fats, and contain some interesting bioactive compounds that have putative health benefits. Those compounds include ceramides, cerebrosides, and gangliosides. Gangliosides are particularly interesting. They are naturally present in milk and have been shown to increase ganglioside levels in tissues, including gut and brain (human and animal data). Dietary gangliosides have also been reported to enhance learning ability in animals [24]. Park et al. [25] reported that animals fed gangliosides have increased gangliosides in their retinas and alterations in membrane phospholipids. Interestingly, their work points to an interaction with dietary LC-PUFAs since they also showed an increase in retinal gangliosides in animals fed LC-PUFAs [26]. There are no good human intervention studies with gangliosides, although it is established that breastfed infants have higher levels of brain gangliosides than formula-fed infants.
Gibson

Conclusions

The paucity of data that relate to the biochemical, physiological and health effects of dairy fats in the diet is surprising considering the ubiquitous nature of messages from learned organizations to include 3–4 serves of dairy as part of a healthy, well-balanced diet. Unraveling the relative health benefits of all the lipid constituents of bovine milk will provide a challenge for researchers for many years to come.

References

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Discussion

Dr. Hernell: You pointed out that there is a difference between unsaturates. We often talk about saturated fat and lump saturated fatty acids together, and likewise we talk about dairy fat as a lump. But there are clearly differences between the various saturated fatty acids. Is it just too simple to talk about saturated fats or dairy fats? Shouldn’t we be more cautious and differentiate between the various saturated fatty acids?

Dr. Gibson: I think that’s the point I was trying to make. Dairy fats are very peculiar in the sense that their saturated moieties have molecular weights that are quite small and ones that are quite large. That is in contrast, for example, in meat in which the smallest would be around about C14 and they range through to C20. In dairy fats, the fatty acids are as small as C4, which we know have beneficial effects if they go through the gut. All of the literatures lump everything together in terms of saturated fats, and thus misunderstanding has occurred. I don’t believe that dairy fats have been looked at in a fair and a logical manner, particularly given their range of nutrients. Particularly in infant nutrition I think that’s where we have missed out enormously. I was one of the proponents many years ago of saying we have got to get the dairy fats out of infant formulas and replace them with vegetable oils, and I think I was dead wrong about that. I think we need to do the trials and look again at what the relative benefits of dairy fats are. I haven’t had time to talk about cholesterol, but people are now starting to put cholesterol back into infant formulas as you know, and that’s because we think there is now a role for dietary cholesterol in infant nutrition but cholesterol in the diet is something we have been avoiding. So, I think that in past years we have grouped all saturated fats together. Now we know that it’s not fair. For example, stearic acid doesn’t seem to be absorbed very well, so it can’t do much harm, and there are other fatty acids that are not hypercholesterolemic. We are even starting to question the whole cholesterol hypothesis, that is, that saturated fats increase your cholesterol level.
**Dr. Hernell:** You mentioned infant formulas, and that we have been very careful to get rid of all milk fat when formulas are manufactured. Some companies now use milk fat in formulas. How much dairy fat, in your opinion, should formulas contain? Because there is still the problem with trans fatty acids in bovine milk fat.

**Dr. Gibson:** A popular combination used to be to have about 80% of the total fat from dairy, and then about 20% vegetable oil was added. What we've tended to do is add an awful lot of linoleic acid in the form of corn oil or soy oil or whatever and ignore the fact that linoleic acid is such a strong inhibitor of two processes: the synthesis of omega-3 long-chain polyunsaturates on the one hand and the incorporation of long-chain polyunsaturates in the cells, so that if I eat a piece of fish, for example, I can get more benefit out of it if I eat it cooked in saturated fat than I would have if cooked in polyunsaturated fat because linoleic acid can compete for the incorporation. So then, what vegetable oil would we use if we added it to the 80% dairy fat? I would be advising to be putting in the minimum you can to comply with the regulations about linoleic acid but not so high as to swamp the whole system and prevent any endogenous synthesis.

**Dr. Saviano:** Would you comment on the ability of the feed stuffs that animals are fed to modify the lipid composition of the milk?

**Dr. Gibson:** Are you talking about changing the diet of the cow, for example? There is a lot of talk about. You can change the composition of milk slightly, naturally by different diets, for example grass vs. corn and so forth, but the changes are small due to the fact that almost everything that the cow eats goes into one of four different stomachs and gets churned up, and most of the fats that we see that are coming to milk fat are not originated from the grass that they eat but in fact from the biota which is in the gut of the ruminant. So, we are looking at ruminant type fats, that's why so many of them have got small molecular weight. We can't change them very much without actually doing something tricky. You can give protective fats, you can wrap them up in activated proteins and get them through to another part of the ruminant to allow that there is some absorption of long-chain polyunsaturates, for example, to enrich them. There is a lot of controversy about whether that's the sort of thing we want to be doing to the animal and whether the milk is actually safe, but you can do that.

**Dr. Mølgaard:** What was your final conclusion on the industrial trans fat? I think I missed it.

**Dr. Gibson:** No, you didn't miss it, I was carefully avoiding it. Absolutely, I have read the review, I have read the paper and I have read the follow-up reviews, and I am totally confused about that one way or the other. Fortunately Dr. Clemens is going to elucidate on that.

**Dr. Melnik:** You did not mention in your talk the branched-chain fatty acids. There is some concern in the field of prostate cancer. Correlations between phytanic acid intake and prostate cancer have been reported [1]. Dairy products are a rich source of branched-chain fatty acids, especially phytanic acid. Can you comment on that?

**Dr. Gibson:** I would like to have a bit more expertise before I commented it definitively. My understanding is the branched chains are there absolutely. We measured them ourselves. But they are there in small amounts, and when people say there is a rich source of something, I often wonder what they actually mean by that because about 98% of all the fats are in triglyceride form of standard known fatty acids, so there can't be much more than point something of a percentage. That also brings us to the point of trans fatty acids. Many of the studies that have been performed about trans fatty acids have used the sort of levels that it's impossible to eat even if you ate a very high dairy diet, and hydrogenation of fats to make them more solid for the use of making margarines and so forth ceased a long time ago and we now use randomization
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procedures to make fats harder. So, many of the studies that have raised these issues with feeding large doses of a single fatty acid, whether it’s a trans or a phytic acid, is somewhat spurious. I think anything, if you give it in huge doses, is going to have deleterious effects on health. It’s a personal viewpoint.

Dr. Johansson: You mentioned the different reactions in relation to the trans fatty acids, and my question is if there is anything known about other fatty acids or types of fats. The reason I am asking is that we found a protective effect of dairy products in women, but not men, based on biomarkers measured in plasma and also food frequency questionnaire data.

Dr. Gibson: Thank you for the question, and I don’t want to be flippant but I will point out to you that there was a book that came out some years ago that men are from Mars and women are from Venus. I think it was supposed to be about our different characters. The number of reports that are coming out in the literature at the moment about different responses of males and females to different dietary conditions is truly amazing, and I don’t think that I can explain it. In the DINO trial which we reported in JAMA, we got a strong beneficial effect of increasing the DHA level in the breast milk or the formula up to around 1% of the total fatty acids, which is about three times the level that is normally present. The benefit was mostly in very small infants as you would expect, but it was almost exclusively in the girl babies and not the boy babies. Now, why would this be so, why would girls respond and boys not? In the trans fat study there were strange effects that benefited the women but not the men. I don’t have an answer for you, but I think we are increasingly becoming aware that the studies need to be large enough and have enough power so that you can actually determine whether there is a gender effect.

Dr. Prentice: A very quick question about the ontogeny of the desaturases. Are newborn babies able to desaturase sufficiently for this mechanism to be useful in young babies?

Dr. Gibson: Absolutely. We know that in adults these is a relatively a slow rate. In fact, the conversion is reported to be somewhere between point zero zero something and one or two percent depending on who you believe. But all those studies have been done with a background of very high levels of linoleic acid in the diet. They haven’t done what our animal data are telling us, that we have got to lower the total PUFAS out of it. If you have an appropriate ratio of omega-6 to omega-3, the total level of PUFA must also be low, otherwise you flood the desaturase system and shut down all of the activity of the enzyme. Having said that, there was a theory around for many years that preterm babies and babies in the first months of life had very low delta-6 desaturate activity and they therefore needed preformed fatty acids, but that has proven to be false. There are good conversion rates being found in preterm babies, and we have shown that in term babies.

Dr. Thorsdottir: I just want to comment on the discussion about long-chain omega-3 in the diet and the concentration in milk. There are several studies in breastfeeding women that show the consumption of omega-3 long-chain fatty acids is mirrored in breast milk. There are also studies in breast-feeding women that show the consumption of omega-3 long-chain fatty acids is mirrored in breast milk. We also know very well that in the dietary studies the concentration of omega-3 in red blood cells is strongly associated with the intake. About milk in general, you indicated that giving omega-3 long-chain fatty acids to the cows would not be a suitable way to increase its concentration in cow’s milk. Could this be because of the generally high PUFA intake? Is that possible?

Dr. Gibson: It could be, I think it has more to do with the fact that they are ruminants and that most fat or energy that goes into the ruminant is going into these four stomachs, each of which has huge biota which are converting it to other things.

Dr. Thorsdottir: The fact is that we have seen this in the Nordic cow’s milk from Iceland, where the cow’s diet contains fish, and we see higher DHA and EPA than in
the milk collected in Scandinavia and Finland. I thought that was simple, I have not
done any intervention or study on that except collected the milk and measured the
content. We thought that was because of a different diet.

Dr. Gibson: It is from the different diet, and there is some accumulation of EPA
and DHA from fish meal which is often given to some animals in the winter time. So,
you can increase the levels of EPA and DHA in the cow’s milk, but it’s considered of
low efficiency and most of it is getting chewed up by the biota.

Dr. Haschke: I would like to follow up a little bit on Dr. Prentice’s question. You said
that dairy products are beneficial because it’s possible that the endogenous omega-3
synthesis will be promoted. From which age on would you say it is beneficial? We
have discussed what happens in the premature infant and the term infant, but from
the fatty acid synthesis side, from which age on do you see a benefit of giving perhaps
dairy products with low LC-PUFA levels?

Dr. Gibson: This is a great controversy. The one thing that I can stand here with
my hand over my heart and say is that my data or the data that Dr. Makrides and I have
produced in preterm infants now convince us that long-chain polyunsaturates of the
omega-3 variety have a beneficial effect on cognitive outcomes and visual outcomes
in children, we know at least to 18 months, if they are born preterm. We started the
7-year follow-up of those infants, and we will know whether this effect disappears or
is enhanced. With term infants, as you know, we have also done a number of studies,
some of them with Nestlé, about the inclusion of long-chain polyunsaturates into
infant formula and compared them with breast milk. The studies haven’t been any-
where near the size that we think is necessary. We suspect that to see any true effects,
that is to correct for all possible confounders, the sort of sample size will have to be in
the excess of about 3,000, that is about 1,500 per group according to our calculations.
But I don’t see any data that convince me that term infants from well-nourished moth-
ers actually benefit from the inclusion of long-chain polyunsaturates of the omega-3
variety in the infant formulas.

Dr. Gibson: Dr. Makrides, do you have anything to add?

Dr. Makrides: The only thing that I will add comes back to clarifying Dr. Prentice
and Dr. Haschke’s questions relating to when the infant is able to synthesize and
accumulate DHA. The Clark study from 1992 started with infants from birth, and the
changes that we saw in plasma and red cell EPA and DHA were seen in the first 6–10
weeks of life [2]. By simply modifying the balance of vegetable oils in the formula, the
concentrations of EPA and DHA in infant blood were increased, although they didn’t
exactly match the concentrations of the breastfed infant. So, there was evidence of
synthesis and accumulation of EPA and DHA from a very early age.

Dr. Okolo: I want to ask if the composition of dairy products varies from location
to location. The reason why I ask this is that we analyzed the milk of mothers from
northern Nigeria and discovered that the levels of DHA and linoleic acid were on
the low side. We wanted to look at what they consume and if they consume more
products from their cows; butter oil is the common oil they use for cooking. When
we analyzed the butter oil, we discovered that it had a very low level of linoleic
acid and DHA, even though the level of linolenic acid was high. So I wonder if the
composition of dairy products varies from location to location based on what the
cows are fed.

Dr. Gibson: I think from looking at the studies around the world, it seems that
the fat composition of a milk from dairy cows is pretty independent of the variety of
cows, whether they are long horn or short horn, whether they are Friesians or Jerseys.
Concerning butter in the diet of the mothers that you are talking about, we’ve shown
a number of times now that the level of DHA in the diet is directly proportional to the
level of DHA that you can see in the breast milk. Whether there is better conversion of
the α-linolenic acid that women might be getting from other sources, from vegetables and so on, allows better conversion of their bodies to make ALA into DHA for their breast milk, I do not know.

References

Concluding Remarks

It is our privilege to summarize the workshop and to make some concluding remarks. Let us begin by thanking the speakers for their excellent contributions and all participants for actively participating in the discussions.

The first session started with Ann Prentice who addressed the effects on maternal, fetal and infant bone of milk, calcium and vitamin D intake during pregnancy and lactation. Animal milks are an important source of dietary calcium and energy, protein, vitamins, minerals, growth factors and other bioactive components, and contribute to dietary vitamin D intake, especially in countries where the milk is fortified with vitamin D. Despite the transfer from mother to infant of 200–300 mg calcium/day during the last trimester of pregnancy and during breastfeeding, physiological changes during these conditions are independent of maternal calcium intake. Neither are increases in maternal calcium intake necessary, nor are they effective in reducing maternal losses. There is no evidence of an increase in biological requirement of vitamin D during pregnancy, but many mothers and infants in the world do have hypovitaminosis D (defined as a plasma concentration of 25OHD below 25 nm, or 10 ng/ml), with increased risk of clinical vitamin D deficiency including rickets and osteomalacia. Vitamin D deficiency in the mother during pregnancy is associated with vitamin D deficiency in the newborn infant with its many sequelae. Safe sun exposure and dietary supplementation are effective and should be promoted for all pregnant and lactating mothers as a measure to reduce these risks.

Next, I spoke on the differences between bovine and human milk and the evolution of infant formulas. Not only does each species have a unique composition of its milk reflecting the specific needs of its offspring, but the composition varies also within a species, and an individual. The goal to minimize the difference in performance between breastfed and formula-fed infants will drive future development of infant formulas. Some of the many bioactive milk components are attractive ingredients in future formulas to achieve that goal. However, some of them have species-specific activities and others are truly species specific as milk components. The bile salt-stimulated lipase in human
milk, which compensates for low endogenous capacity to digest dietary fat in the newborn, is absent from bovine milk but can now be produced by recombinant techniques in quantities making it possible for supplementation of formulas. With novel ingredients, some with potent biological activities, produced with new techniques, it will be extremely important to rigorously evaluate and document safety and efficacy also beyond the first few months of life. All improvements may not be worth a higher cost – ‘functional effects’ are not necessarily the same as beneficial health effects.

Inga Thorsdottir pointed out that one of the major reasons for refraining from consumption of whole cow’s milk in infancy is the risk for iron deficiency anemia and its consequences. Other potential risks relate to the high renal solute load, of particular concern to infants and children suffering from dehydration. A population-based infant cohort study in Iceland, carried out in 1995–1997 when the tradition was to wean infants from the breast to whole cow’s milk, showed that low iron status at 12 months of age was indeed strongly associated with whole cow’s milk intake at 9 and 12 months of age, and a follow-up at 6 years revealed an increased risk of overweight among boys who had higher protein and milk intake in infancy, and iron deficiency in infancy was associated with lower developmental scores at 6 years. A second cohort study conducted between 2005 and 2007, when new national public health recommendations, including use of follow-on formula (iron fortified and with lower protein content than cow’s milk) rather than whole cow’s milk had been adopted, showed improved iron status and slightly reduced weight gain velocity during a period of the 1st year of life. Suggested associations between the consumption of whole cow’s milk and several chronic diseases were weak in these studies. The Icelandic experience illustrates the importance of evidence-based guidelines on infant feeding.

Bo Lönnerdal spoke on biological effects of novel bovine milk fractions. Besides its ideal amino acid composition, α-lactalbumin, which is the dominant human whey protein, seems to have several functions. It is digested to peptides with antimicrobial and prebiotic activities, has immune stimulatory effects as well as a promoting effect on mineral absorption. The iron-binding protein lactoferrin is an even better example of a milk protein with several biological activities, both in its native and partly digested forms. Besides its known antibacterial and antiviral effects, it increases iron absorption, regulates immune functions and has trophic effects on the intestinal mucosa. Some effects have been disputed, and the lack of evidence in some earlier studies may have been due to contamination by lipopolysaccharide of commercial bovine lactoferrin fractions. Osteopontin is a possible key molecule in the induction of Th1 responses and may stimulate the postnatal Th1/Th2 switching, but also affects bone mineralization and growth. Osteopontin binds lactoferrin and some effects of lactoferrin may in fact be facilitated by osteopontin. A novel bovine milk fraction is enriched in milk fat globule membrane, which contains a number of proteins, e.g. lactadherin, butyrophilin, xantine
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oxidase and mucin with antimicrobial effects in vitro, and lipids e.g. sphingomyelin and gangliosides which may affect signal transduction and neurodevelopment. This fraction has been low in formulas, but it is now available as a possible ingredient and its potential effects are evaluated in clinical studies.

Finally, Ingegerd Johansson mentioned that there has been a striking reduction in dental caries and periodontitis in industrialized countries, but contrasting to this the proportion with severe disease has remained at 10–15%, and now even increases in less developed countries. Breastfed infants differ distinctly from non-breastfed infants in their composition of the oral microbiota. There is evidence that breastfeeding might promote a health-associated microbiota in the dental biofilm, which is unique compared to other compartments of the digestive tract, and hinder establishment of cariogenic mutans streptococci. Focus has shifted from specific cariogenic bacteria to the composition of the eco system of this biofilm, as an important factor in caries development. Non-sweetened dairy products, in particular caseins and casein-derived peptides, which are proven non-cariogenic, or specific bioactive components derived from such products might prove to be part of future preventive strategies against caries. For instance, studies in children have shown that consumption of milk or hard cheese is associated with less caries. However, although in vitro and observational studies are promising, randomized clinical trials are needed to reveal if dairy products could indeed be a future cost-effective complementary treatment to proper oral hygiene, sugar restriction and use of fluoride for oral health.

Olle Hernell

The second session was on milk during childhood in low- and high-income countries.

First Christian Mølgaard talked about milk and linear growth. There is strong evidence that milk stimulates linear growth both from observational and intervention studies in low-income countries, but also observational studies and a few intervention studies in industrialized countries suggest an effect. The mechanism is not clear. It is quite obvious that IGF-I and perhaps insulin play an essential role, but it is not known which components in milk stimulate growth. More research, especially with long-term follow-up of intervention studies, is needed to better understand this, which was also recommended by Richard Martin. Whey and/or casein seem to have a positive effect on lean body mass, but whether it is also the case in malnourished children is not clear. The long-term consequences of the effects of milk on linear growth are most likely mixed with both positive and negative effects.

Then, Richard Martin talked about the role of milk in programming of the IGF-I axis and which implications this can have for health in adulthood. He presented evidence that milk intake is positively associated with higher levels of circulating IGF-I in children and adults and that higher circulating
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IGF-I promotes linear growth in children, but also that milk intake in early life is inversely associated with IGF-I levels in adult life. Thus, there seems to be a long-term programming of the IGF-I axis, with high milk intake early in life being associated with lower IGF-I levels as adults, which could have implications for risk of cancer and ischemic heart disease later in life.

IGF-I was also a theme in the presentation given by Bodo Melnik. As a dermatologist, he has studied the epidemiology of acne vulgaris, which is the most common skin disease in industrialized countries. He suggested that milk, especially whey, plays a central role in acne pathogenesis through stimulation of IGF-I and insulin. The epidemic of acne is most likely the visible metabolic syndrome of skin caused by an exaggerated insulinotropic diet, which also includes high intake of carbohydrates, he said. If milk intake and thereby stimulation of IGF-I and insulin is reduced, he suggested that this would reduce the incidence of obesity, diabetes mellitus, cancer, neurodegenerative disease and acne. However, the available epidemiological data on milk intake and lifestyle diseases do not support this view, as presented in several of the talks in the third session of this meeting.

Lindsay Allen talked about the effects of animal source foods in low-income countries with emphasis on milk. Many observational studies show positive associations between intake of animal source foods and better growth, cognitive and motor development and physical activity, but there are only few intervention studies available. These showed that milk had a positive effect on height and weight, especially in the younger children, and meat improved cognitive function and physical activity. Some of these effects could be caused by the high content of vitamin B₁₂ in animal source foods.

I talked about the role of cow’s milk in the treatment of moderate and severe malnutrition. The development of products to treat severe acute undernutrition, F100 and RUTFs, have reduced mortality and increased weight gain considerably, and part of the success is likely to be due to the high content of milk in these products. The beneficial effects are most likely due to the high content of bioavailable proteins and minerals and because there are no fibers and antinutrients as in plant-based foods. Furthermore, the high lactose content might add beneficial effects. Milk-based products would also be beneficial for the many millions of children with moderate undernutrition, but milk-based products are expensive. Therefore, one strategy is to add small amounts of milk powder to cereal-based fortified blended foods, but there is a need for randomized controlled trials to determine the amount of milk protein that has the optimal cost effectiveness in treating moderate undernutrition.

Kim Fleischer Michaelsen

I would like to extend my appreciation to Harvey Anderson, Robert Gibson, Cheryl Rock and Dennis Savaiano for their contribution to this wonderful session with Nestlé.
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The preponderance of evidence indicates milk proteins can affect a reduction in food intake, lower postprandial blood glucose, with a concomitant increase in blood insulin concentration. These effects suggest that the consumption of dairy products may be important in bodyweight management and reducing comorbidities of metabolic syndrome. Even those with a self-report history of lactose intolerance may benefit since the more typical response following milk intake is symptoms of maldigestion. Thus, even among mal-digesters who have a limited ability to digest lactose, dairy products can provide a spectrum of essential nutrients for growth and development. Equally important, epidemiological evidence suggests milk-derived nutrients, such as calcium, may reduce the risk of colorectal cancer, possibly increase the risk of prostate cancer, while the impact of calcium on other cancers, such as breast and ovarian, is mixed or lacking. Similarly, the association of milk proteins with the risk of type 1 diabetes is inconsistent, although dairy fats, including saturates and omega-3 fatty acids, may be cardioprotective. Emerging evidence indicates that stearic acid, a saturate, and that the naturally occurring trans fatty acids, such as conjugated linoleic acid, are nonatherogenic. Collectively, many components of milk can provide health benefits beyond normal growth and development.

Roger Clemens

It’s time to close the scientific part of this workshop. I must say, it turned out as we expected, more questions have been raised than answers have been provided, and I think it’s good that we are in a continuous discussion and that we are critical. We are challenging paradigms and are moving to the next step.

I said 10 years ago in a workshop that perhaps full fat milk is not bad for your health if you consume it long-term. Today, a lot of people would criticize me for this and would say that we promote industry products. Today, I think the opinion that was expressed by both, also by the committee, is a little bit different, so we can be happy that dairy products, which play a major role in our lives, are good for our long-term health. Still, I have been in my job for some decades now, and I must tell you that whatever you say the probability of it being wrong 10 years later is 50%, so there is always time for a new workshop in 10 years to review what we have said today and move forward.

This brings me to thank the three chairmen of this workshop; they have been working with us since June last year, and I think they have brought together a very good comprehensive program; it was not easy because we were combining fetal life with early infancy, poor countries and the long-term outcome of health effects.

I want to thank the three moderators, Nezha Mouane, Fatima Debbi and Rachida Boukari. I think they did their job in a very charming way, and even when it was not so clear who was raising the hand at the back, I think most of
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the people who wanted to speak could speak up. The discussion in my opinion always contributes significantly to the outcome and to the content of the workshop.

This workshop would not have been possible without the support of local organization and I would like to thank Sophie and Samir. Last but not least Petra, I think she did a great job, thank you.

Ferdinand Haschke
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