Adiposity and Comorbidities: Favorable Impact of Caloric Restriction

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Abstract
The focus here is on research involving long-term calorie restriction (CR) to prevent or delay the incidence of the metabolic syndrome with age. The current societal environment is marked by overabundant accessibility of food coupled with a strong trend to reduced physical activity, both leading to the development of a constellation of disorders including central obesity, insulin resistance, dyslipidemia and hypertension (metabolic syndrome). Prolonged CR has been shown to extend median and maximal lifespan in a variety of lower species (yeast, worms, fish, rats, and mice). Mechanisms of this lifespan extension by CR are not fully elucidated, but possibly involve alterations in energy metabolism, oxidative damage, insulin sensitivity, and functional changes in neuroendocrine systems. Ongoing studies of CR in humans now makes it possible to identify changes in ‘biomarkers of aging’ to unravel some of the mechanisms of its anti-aging phenomenon. Analyses from controlled human trials involving long-term CR will allow investigators to link observed alterations from body composition down to changes in molecular pathways and gene expression, with their possible effects on the metabolic syndrome and aging.

Introduction
Anti-aging research by modern scientists continues to echo the quest of the Spanish explorer Ponce de Leon, who searched for the ‘Fountain of Youth’ on the shores of Florida in the early 1500s. Humans are no longer satisfied with simply living longer; they want increased quality of life and prolonged health during their senior years. Basic and clinical research is therefore conducted to understand the physiological and molecular mechanisms of aging with the intent to postpone and possibly alleviate many of the illnesses associated with the aging process.
Ironically, as researchers aim to unravel the mysteries of delaying the biological aging process, the current societal environment is marked by over-abundant accessibility of food coupled with a strong trend to reduced physical activity. As obesity rates have risen to over 30% among Americans [1], so have the prevalence of obesity-related chronic diseases such as diabetes mellitus, heart disease and stroke. This alarming increase in obesity is further coupled with a lower age at onset for the emergence of obesity-related comorbidities. It is now understood that obesity may cause up to 300,000 deaths per year in the USA [2]. Alarmingly, it now seems that babies born at the beginning of the 21st century will have shorter life expectancies than their parents [3].

Before the development of frank obesity, cardiovascular disease (CVD) and diabetes, individuals develop a constellation of disorders including central obesity, insulin resistance, dyslipidemia and hypertension, often termed the metabolic syndrome. Individuals with three or more of these key disorders have a 2–3 times greater risk of dying or being struck by heart attack or stroke and a 3–5 times greater risk of developing diabetes mellitus [4, 5]. It is estimated that worldwide 1 in 3 or 4 adults has the metabolic syndrome [6]. The first line of treatment is to adopt a healthy lifestyle [5]. However, the large individual variability in response to diet and exercise represents a huge challenge in clinical practice. A better understanding of the genetic and environmental influences in the physiopathology of the metabolic syndrome could ultimately deliver a customized treatment to those individuals who do not respond to intensive lifestyle changes and some medications.

**Etiology of Obesity**

Weight gain results from a sustained imbalance between energy intake and energy expenditure favoring positive energy balance. However, this simple statement belies the complex, multifactorial nature of obesity and the numerous biological and behavioral factors that can affect both sides of the energy balance equation. Figure 1a shows the major paths involved in obesity grouped according to behavioral, metabolic, and biological influences. These pathways and factors have been reviewed elsewhere [7, 8]. Figure 1b summarizes the major factors and the central integrators controlling energy balance.

Longitudinal studies of the same individuals over time have indicated that relative to body size, low metabolic rate, high respiratory quotient, insulin sensitivity, low sympathetic nervous system activity and low plasma leptin concentrations predict weight gain over time [9]. Upon gaining weight, the original ‘abnormal’ metabolic state becomes ‘normalized’. Such ‘normalization’ with weight gain explains why cross-sectional studies have not led to the identification of metabolic risk factors for obesity. Weight gain thus causes an increase in metabolic rate, a decrease in respiratory quotient, a decrease in insulin sensitivity, an increase in sympathetic nervous activity and an increase
Fig. 1. Etiology of obesity and control of bodyweight. Several factors are known to predispose an individual to obesity (a), these include: behavioral factors (activity level, nutrition, smoking status, socioeconomic status); metabolic factors (physiological, metabolic, endocrine factors), and biological factors (genetic, racial, gender, age, pregnancy status). Bodyweight is regulated by an intricate balance between energy intake and energy expenditure (b). The figure depicts a negative feedback model for the regulation of bodyweight. Peripheral signals from energy stores (adipose tissue, muscle, and liver) as well as hormonal and gastrointestinal signals provide information to the central controllers in the brain, indicating the state of the external and internal environment as they relate to food, metabolic rates, and activity behavior. The central controllers in turn integrate all the information and transduce messages into efferent signals governing the behavioral search for the acquisition of food as well as modulating its subsequent deposition into energy storage compartments such as adipose tissue, liver, and muscle by modulating energy expenditure.
in plasma leptin concentrations, all of which serve to counteract further weight gain. Therefore it is not surprising that weight loss plateaus after a few months of therapy.

**Aging and Obesity**

Aging is associated with increased risk of metabolic disorders including overweight, obesity, insulin resistance, type 2 diabetes, atherosclerosis and cancer. Cross-sectional and longitudinal studies suggest that over-consumption of energy-dense foods and lack of physical activity are the leading causes of weight gain, obesity and the related health issues [10]. Recently researchers have learned that while increased adipose tissue per se is a health concern, the storage and distribution of fat within the body also has important implications for health. In particular, adipose tissues stored centrally in the visceral compartment of the abdomen and in non-adipose tissues, such as liver, heart, pancreas and skeletal muscle, are considered to be metabolic abnormalities that precede the development of impaired glucose tolerance, hyperlipidemia and insulin resistance. As individuals age, body weight even if maintained, is composed of increased fat mass, decreased fat-free mass [11] and increased ectopic fat stores in the abdominal visceral compartment [12], the liver and the skeletal muscle [13] associated with an increased incidence and prevalence of glucose intolerance and diabetes in older persons [14, 15]. It therefore seems that the link between aging and chronic disease may be inevitable in our current obesogenic environment. Interventions therefore that can attenuate the age-associated changes in body composition could delay (even prevent) the onset of metabolic disturbances of aging and result in extended lifespan.

**Caloric Restriction and Lifespan**

Since the 1930s calorie restriction (CR) has been shown by McCay et al. [16] to retard the aging process, extending the median and maximal lifespan in various models [17]. While the exact mechanisms through which CR is able to extend the lifespan have yet to be fully elucidated, CR reduces metabolic rate and oxidative damage, improves markers of age-related diseases including diabetes such as insulin resistance, and has been shown to alter neuroendocrine activities in animals [18] (fig. 2). Results from studies on rhesus monkeys suggest that prolonged CR can also oppose many age-associated pathophysiological changes including learning and behavior changes, body temperature, plasma insulin concentrations and resting energy expenditure. Since many changes associated with prolonged CR are important to the health and survival of humans, and excessive caloric intake is associated
with morbidity and the development of chronic diseases, it has become an important research objective to assess the feasibility, safety and efficiency of prolonged CR in well-controlled human trials.

**CR May Alter the ‘Rate of Living’ and ‘Oxidative Stress’**

The aging process may be influenced by energy restriction through a reduction in the metabolic ‘rate of living’ [19], leading ultimately to reduced oxidative damage (fig. 2). An ongoing controversy among investigators appears to be whether chronic CR leads to ‘metabolic adaptation’, a reduction in the metabolic rate which is out of proportion to the diminished metabolic mass of the organism [18]. Results from rats and monkeys suggest that most of the collected data should be reevaluated using appropriate methods of normalizing the metabolic rate for changes in metabolic size [20]. For example, Blanc et al. [21] recently calculated a 13% reduction in resting energy expenditure.
after adjusting for fat-free mass in an 11-year-long study of energy-restricted monkeys. Recently, however, Selman et al. [22], using doubly labeled water to measure total energy expenditure, reported that calorie-restricted rats expended 30–50% more energy than expected.

The ‘free radical theory of aging’ or ‘oxidative stress’ hypotheses are well-supported theories of aging. It is widely accepted that the metabolic rate of an organism is a major factor in the rate of aging, and is inversely related to its lifespan [23]. Additionally, since 1–3% of consumed oxygen is associated with the production of reactive oxygen species (ROS), namely superoxide (O$_2^•$), hydrogen peroxide (H$_2$O$_2$), and the hydroxyl ion (OH$^•$) [24], the production of these highly reactive molecules from normal aerobic metabolism is also in direct proportion to an organism’s metabolic rate. Many investigators have shown that modulation of the oxidative stress of an organism through prolonged CR is able to retard the aging process in various species, including mammals [25, 26]. As a result of increased oxygen consumption, aerobic exercise is associated with increased production of ROS in muscle tissues [27]. However, exercise training boosts up the antioxidant capacity of skeletal muscle probably resulting in decreased overall oxidative stress [28].

CR, CVD, Insulin Resistance, Type 2 Diabetes Mellitus

Elevated levels of oxidized LDL, excessive ROS generation, hypertension and diabetes are all potential causes for the development of endothelial dysfunction, a precipitating event in the progression of atherosclerosis. These factors are believed to initiate an inflammatory response in the injured endothelial tissue. Long-term CR is associated with sustained reductions in factors related to endothelial dysfunction in humans, such as decreased blood pressure [29], reduced levels of total plasma cholesterol and triglycerides [30], and reduced markers of inflammation such as C-reactive protein, interleukin-6 and plasminogen activator inhibitor type-1 [31–33]. A recent long-term CR study in humans supports the feasibility of using CR as a protective effect against atherosclerosis by showing a 40% reduction in carotid artery intima-media thickness in CR participants relative to a control group [34].

Strong evidence shows that long-term energy restriction in lean and obese subjects improves insulin sensitivity, a mechanism by which CR may act to extend lifespan [30, 35]. Additionally, prolonged CR reduces fasting glucose and insulin concentrations, two factors believed to contribute to the aging process due to protein glycation [36] and mitogenic action [37], respectively. This compelling evidence suggests that weight loss due to CR may be the most effective means of improving insulin sensitivity, thereby decreasing the risk of the development of diabetes mellitus.
What Is Known from Humans?

Probably the most intriguing epidemiological evidence supporting the role of CR in lifespan extension in humans comes from the Okinawans [38]. Compared to most industrialized countries, Okinawa, Japan, has 4–5 times the average number of centenarians with an estimated 50 in every 100,000 people [39]. What is interesting about this population is that a low caloric intake was reported in schoolchildren on the island more than 40 years ago and later studies confirmed a 20% CR in adults residing on Okinawa compared to mainland Japan [40]. However the diets were typically rich in green leafy vegetables, soy and some fish providing adequate amounts of nutrients, essential vitamins and minerals [41].

To our knowledge there is only one other study that tested the effects of CR without malnutrition in non-obese humans [42]. This was a study of alternate day feeding in 60 male participants (1,500 kcal/day; 35% CR vs. controls) for 3 years, whereas the other 60 were ad libitum. While the initial report was brief, post-hoc analyses conducted several years later [43] indicated that the death rate tended to be lower in the CR group and hospital admissions were reduced in these individuals by approximately 50%.

The unexpected low availability of food during the 2-year Biosphere 2 experiment provided a unique opportunity to observe the effects of CR in non-obese humans. Eight individuals were completely isolated within this 3.15-acre ‘mini-world’ (ecological laboratory), where 100% of the air and water was recycled and all food was grown inside [44]. Due to unforeseen problems with agriculture early on, food supply was much lower than expected going rapidly from a projected ~2,500 to ~750 kcal/day during the first 6 months. The resulting 17 ± 5% weight loss was associated with many physiological, hematological, biochemical and metabolic alterations [44, 45] consistent with calorie-restricted primates including reductions in insulin, core temperature and metabolic rate. Furthermore CVD risk factors were improved, such as reductions in systolic/diastolic blood pressure and a 30% lowering of cholesterol [30].

Randomized Controlled Trials of Prolonged CR in Humans

As for randomized controlled trials, results from a 2-year study of CR in humans is only a few years away since the recruitment of volunteers began in March 2007. The National Institute on Aging (NIA) is sponsoring a trial called CALERIE (Comprehensive Assessment of the Long-term Effect of Reducing Intake of Energy) which for the first time is scientifically testing (randomized trial) the effects of 25% CR in ~150 non-obese (BMI 22–28) healthy men and women aged 25–45 years compared to 75 matched volunteers. Three clinical sites are involved in the trial: Washington University in St. Louis, Mo.; Tufts University in Boston, Mass., and Pennington Biomedical Research Center in Baton Rouge, La.
The Phase 1 CALERIE study conducted at Pennington involved 46 men and women randomized to 1 of 4 treatment groups for 6 months. For the CR group, the level of restriction imposed was a 25% reduction from the daily energy requirement for weight maintenance [46]. The other groups were: (1) CR plus exercise group where the calorie deficit was also set at 25% from weight maintenance with half (12.5%) achieved by CR and half (12.5%) by increased energy expenditure via structured aerobic exercise; (2) a low calorie diet group where participants consumed 890 kcal/day to achieve a 15% weight loss and thereafter followed a weight maintenance diet, and (3) a healthy diet control group that followed a weight-maintaining diet based on the American Heart Association Diet, Step 1.

Six months of CR induced favorable outcomes in terms of physiological, hormonal and biochemical parameters. The 12 participants assigned to this treatment group completed the study and reported no development of eating disorder symptoms [47] or reductions in quality of life indices [48]. After 6 months of 25% CR, the group lost 10.4 ± 0.9% of their body mass attributed to both a loss in fat mass (CR –24 ± 3%) and fat-free mass (CR –4 ± 1%). Central adiposity was reduced by 27% in both visceral (women –24 ± 4%, men –32 ± 6%) and subcutaneous fat depots (women –25 ± 2%, men –28 ± 7%). Interestingly the distribution of whole body fat, specifically within the abdomen was not altered by CR [49]. Abdominal fat cell size was reduced by ~20% and the deposition of lipid in the liver was lowered by ~37% but no change was noted in the lipid content within skeletal muscle [50]. Importantly the reduction in weight, visceral fat and abdominal fat cell size was associated with a 40% improvement in insulin sensitivity and reduced acute insulin response to glucose [50].

We also observed favorable changes in the lipoprotein profile. Triacylglycerol was reduced by 21%, HDL cholesterol increased by 9%, and factor VIIc reduced by 10%. No changes were observed in fibrinogen, homocysteine or endothelial function. Based on combined changes in lipid and blood pressure values, the estimated 10-year CVD risk declined by 29% in the CR group but as expected remained unchanged in the control group. Based on combined favorable changes in lipid and blood pressure levels, CR favorably reduces the risk of CVD [51].

With regard to longevity, 2 of 3 biomarkers of longevity [52] were improved in the CR group after 6 months [46]. Specifically, we observed significant reductions in both fasting insulin and core body temperature. Interestingly, in parallel with the decrease in core temperature, we observed a metabolic adaptation – lowering of metabolic rate larger than expected on the basis of weight loss – associated with reduced DNA damage probably due to lower production of ROS [46]. These findings of course echo results previously reported in nonhuman primates and rodents on CR and long-lived men in the Baltimore Longitudinal Study of Aging [52]. Importantly, CR was associated with an increase in the muscle expression of genes involved in mitochondrial
biogenesis and mitochondrial fusion including PGC1-α, mitochondrial transcription factor A, endothelial nitric oxide, SIRT1 and PSARL [53]. In parallel, mitochondrial content increased by 35 ± 5% in the CR group with no change in the control group (2 ± 2%). However, the activity of key mitochondrial enzyme of the TCA cycle (citrate synthase), β-oxidation (β-HAD) and electron transport chain (COX II) were all unchanged. This suggests that 6 months of CR in non-obese humans was sufficient to improve biomarkers of aging and supports the theory that energy expenditure is reduced beyond expectation. Whether the observed metabolic adaptation translates into long-term overall reduced oxidative damage remains to be determined. The increased mitochondrial content in parallel with a decrease in DNA damage is, however, an important indication that CR improves mitochondrial function in skeletal muscle, a factor which may decrease cellular senescence.

Taken together the preliminary findings from the Phase I of CALERIE indicate promising benefits of CR for 6–12 months on body composition whereby total fat mass, visceral fat and ectopic fat stores are reduced. These body composition changes are associated with improvements in plasma lipids and reductions in cardiovascular and type 2 diabetes risk. Due to the pluripotent nature of energy restriction, the exact mechanisms by which CR extends lifespan are still being investigated, and will likely remain a challenge. However, controlled human trials such as the multicenter CALERIE study, are transforming this challenging investigation into a modern scientific reality.

Could CR Increase Longevity in Humans?

The wealth of CR literature in rodents, however, allows us to address some important questions relating to the practicality and feasibility of CR in humans. Relevant and practical questions are: (1) how much CR do we need to improve age-related health and possibly longevity, and (2) how long do we need to sustain CR in order to obtain these benefits? Analysis of 24 published studies of CR in mice or rats indicated a strong negative relationship between survival and energy intake [54] with more CR (up to 55%) associated with longer maximal lifespan.

The rodent data indicate that CR has greater benefits when more extreme and sustained over a longer period of time. Using the prediction equations derived from the rodent data above, we and others estimated that a 5-year life extension could be induced by 20% CR starting at age 25 and sustained for another 52 years, i.e. the life expectancy of a male in the US. However, if a 30% CR was initiated at age 55 for the next 22 years, the gain would only be 2 months.

Certainly there are individuals who self-impose CR with the CRON (Calorie Restriction with Optimal Nutrition) diet for health and longevity. A group of 18 CRONIES (only 3 women) have recently been studied after 3–15 years
of CR [34]. Dietary analysis indicated an energy intake ~50% less than age-matched controls. In terms of body composition, the mean BMI of the males was 19.6 ± 1.9 with an extremely low percent body fat of ~7%. Atherosclerosis risk factors including total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides fell within the 50th percentile of values for people in their age group. This report provides further evidence that longer term CR is highly effective in lowering the risk of developing coronary heart disease and other age-related comorbidities [34]. It remains to be seen if the CRONIES live longer than their age- and sex-matched counterparts.

**Conclusion**

The enormous public health burden resulting from obesity and its related morbidities compel researchers to challenge some of the existing classical clinical interventions. Despite recent pharmacological advances, development of insulin sensitizers, antihypertensive and hypolipidemic drugs the clinician has limited options to offer to the obese patient in terms of safe and efficacious alternative therapies. Intensive changes in lifestyle seem to benefit very few individuals suffering from obesity and its comorbidities. Only intensive public health messages, population-wide lifestyle interventions and major remodeling of our obesogenic environment may start to reverse the increasing incidence of common disorders such as obesity, CVD and diabetes.

Facing an ‘obesogenic’ environment, it seems very unlikely that a public health message be launched towards reducing the amount of ingested calories. Understanding the mechanisms leading to retarded senescence at the molecular and physiological levels is therefore important for the successful development of CR mimetics. Such natural compounds or botanical extracts would mimic the effect of CR without depriving people of their usual energy intake. Biotechnology and pharmaceutical companies are eager to search for small molecules mimicking the effect of CR and representing the ‘Fountain of Youth’.

**References**

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Discussion

**Dr. M. Chatterjee:** You have talked much about caloric restriction. What role do bioflavanoids play, especially the very colorful ones, prunes, colored cabbages and berries?

**Dr. Ravussin:** Nutrition companies have a real interest in what is called caloric restriction mimetics. In other words if you know the mechanisms of how calorie restriction expands lifespan, you can mimic this by giving blueberries as a natural way. It is true that the redder and bluer the fruits the more resveratrol and flavonoids they have, and the better for life extension. We don't know enough about what the mechanisms are. In studies on resveratrol in rats, for example, it was the equivalent of about 16 liters of wine a day. If you extrapolate that to humans it probably would not be very good for health. But yes, nutrition companies do have an interest, and I am sure Nestlé is in the business of calorie restriction mimetics.

**Dr. Prentice:** I was very interested in both your survival plots for the rats and your final statement when you had recalculated the advantage of 30% caloric restriction on your own potential lifespan. It did seem from those initial survival curves that actually the differential mortality was happening very early and that they were probably parallel after about 700 days or so in the rats, and that would presumably tie in with the calculation when you said you could only get the 2 months advantage. So the question in terms of this conference is: would you care to speculate much further back in earlier life as to what we could achieve by modulating feeding patterns? I am rather confused by some of Barker's data at the moment which sometimes gives evidence that a high weight at 1 year is beneficial and other times it is the reverse.

**Dr. Ravussin:** That is a very good point. As I said, the earlier the better. You are absolutely right, in all the survival curves there is a gap very early, and then it does not increase very much anymore. One thing we have to be absolutely clear about is that people, who call themselves CRONies (Caloric Restriction Optimal Nutrition) and practice caloric restriction, are very well educated, they know nutrition, have no deficiency in any micronutrients, and know exactly what they are eating. I think there is a lot to be learned from these people, some of whom started very young, but of course what is the control group and what is the effect? There is no reason to doubt that caloric restriction with optimal nutrition can really expand lifespan by decreasing what is called secondary aging, which is basically chronic diseases of aging, obesity, diabetes, cardiovascular disease, and also by decreasing primary aging, which is at the cellular level, why do we have senescence in the cells. I think it would be very interesting to learn more about the mechanism of primary aging.

**Dr. Ajayi:** Due to the appreciation evolving regarding the understanding of nutritional science, I think we need to begin looking at the normal standards that have been set for BMI and caloric intake. It seems obvious from these results that we needn't eat as much as we do, so caloric intake could be less, and if we select our food very well, we will still benefit. So perhaps we need now to review the BMI standards and caloric intake set for every age group. Particularly after puberty, we need to look strictly at these and then begin to make some fresh recommendations for the populations.

**Dr. Ravussin:** Should there be a potential reappraisal of these BMI curves versus mortality? Looking at these curves, it is always a J curve, and there are people with a low BMI of around 18 like the CRONies. There is a lot of confusion about this J curve with regard to smoking; should smoking be deleted and so on. Again I would not speculate on the mortality curve, which of course is very likely to be different in different populations. In Pima Indians, for example, there is no way that the best BMI for them is 22.5 or 23, because it is really a higher BMI which is good for Pima
Indians, and in India it is also true that this BMI and the mortality curve may be displaced.

Dr. Klassen: I have a question related to the exercise part. In your very early slides you showed that running already had a small effect, but it seems that, with additional exercise, caloric restriction does not make an impact. I was wondering if you could elaborate on the extent of physical activity? Would for example a triathlon runner or marathon runner experience an inverse effect, i.e. would you expect them to produce more ROS and thus can there be a limit to the benefit of exercise?

Dr. Ravussin: There is a lot of controversy about oxidative stress and exercise. At the mitochondrial level about 1–2% of the oxygen escapes ATP production and therefore produces ROS. But on the other hand we know that exercisers have better defense; the antioxidants are upregulated. Of course we know that epidemiologically exercisers are more likely to live longer, at least the average lifespan. But once again in all the studies, the paradox is that the maximum lifespan is not extended by exercise. Exercisers have a lot of lipid in their muscle but they are not insulin-resistant. There are a few paradoxes with exercise.

Dr. Haschke: What would happen if you apply the same caloric restriction (15%) to people with a BMI of >30 without metabolic syndrome. What would be the effect of exercise? Probably these studies have been done.

Dr. Ravussin: There are a lot of weight loss studies in obese people, and we know the results of these studies. People can drop their weight, there is no problem with that, but the maintenance of that weight 5 years after stopping the active intervention is less than 5%. I don't know about those without metabolic syndrome. It has been claimed that it doesn't matter how fat you are, as long as you are metabolically fit. I have a hard time with the dissection of those epidemiological data because there are very few people who are fat and fit. I think the benefit would be much less in people who do not have the metabolic syndrome than in people who have the metabolic syndrome.

Dr. Haschke: In your phase 2 study, are you looking at test parameters that are indicative for cognitive function?

Dr. Ravussin: I teamed up with a psychologist and in phase 1 we made a lot of cognitive measurements and there was no detrimental effect. But this was too short. Now over 2 years, the concerns are bone health, immune function, and also cognitive function. What has been done, and this has not been published but I am aware of the data, is a comparison with basically calorie-restricted CRONies versus marathon runners who have the same BMI, 19–20, or very close. One group, the marathon runners, had a high energy flux, and the other group had very little, and no difference was found in cognitive function. That is all that I can say.

Dr. Popkin: The issue of resveratrol raises the question of how we can extrapolate from animal studies to human studies and this is very complex. We do not find effects in humans, so one has to be very careful on all these anti-aging issues because all the work has been done on animals, and when we pull it forward it gets very complex in humans. Although I love red wine I would not want to oversell it. I do not think we can replicate your research in infants. Can you see a way that we can go after Dr. Prentice’s question, because you are talking about a well-balanced diet plus caloric restriction and I don't think under any human circumstances we will ever be able to find that. All the Barker and fetal origins studies do not replicate those two combinations.

Dr. Ravussin: The first point is well taken, except that there are no randomized clinical trials with resveratrol, they are all epidemiology. The secondly, and I kind of escaped Dr. Prentice’s question, at the fetal age once again all the nutrients and proteins are needed for the development of this fetus. I think first of all, these are not experiments which are going to be done. I don't know the answer.
Dr. Haschke: There are data in infants showing that feeding influences the metabolic outcome from the first day of life. It has clearly been shown that the differences between breastfed infants and infants fed formulas with a high protein content are that they have much lower insulin secretion. We have much less data on growth hormone and IGF1; so there is something from early life onwards. I am not saying that this can be extrapolated clinically. It is not caloric restriction, but it is protein restriction to a certain extent. Growth of the breastfed infant is very much modulated by the protein content. We consider this healthy growth, but growth can be accelerated in an infant if more protein is given.

Dr. Giovannini: About caloric restriction, do we mean a low carbohydrate intake or a low fat intake, or does a low protein intake play a role? A European project is assessing the correlation between obesity and protein intake in the first year of life.

Dr. Ravussin: That is a good question. These people were interested in longevity and tested all that in rodents, and it is mostly the calories which count. Of course if protein intake is too low then there is a problem, but it doesn’t make any difference if it is carbohydrate or fat. The alternate-day fast in rodents is very interesting. When a rat is starved and given food ad libitum every other day for a long time, there is no difference in weight but there are all the advantages of caloric restriction. There is a lot of interest in that now. There are some mechanisms that we don’t understand: cells are needed, a stressor is needed, and is that an alternative stress or just eating less calories? We don’t know.

Dr. Arora: As prolonged caloric restriction may not be possible, what is the impact of intermittent caloric restriction? Are there some studies on that?

Dr. Ravussin: There are no studies in humans. I am embarking on a study of people with impaired glucose intolerance. Every other day they are put on 25% of the energy requirement, and then we look at insulin sensitivity. All that with the hope that we could have an intervention which is basically easier than caloric restriction, because it is tough to start counting all your calories and this kind of thing.

Dr. Ganapathy: Since the discussion is about red wine I thought why not touch upon probiotics. There was a beautiful article in Nature [1] in which two sets of mice, obese and thin, were compared. It was found that there definitely is a difference in the gut flora constitution, more saccharolytic and less proteolytic in the thin rats, and it was the other way round in the obese rats. The second thing that I want to talk about is the accelerator hypothesis of caloric restriction in diabetes, that type 1 and type 2 belong to the same spectrum but different ends, that if as a child you are exposed to a high caloric diet and you develop insulin resistance very early in life, it upregulates your pancreas and makes it more prone to an immune response. There are a lot of studies being done on that by Wilkin [2]. The last thing that I want to talk about is about sleep. A good night’s sleep just makes you save about 120 cal.

Dr. Ravussin: Let me start with the last one. First of all we did not measure the duration of sleep in our subjects. This was when the subjects were in a chamber, in an artificial environment. In phase 2 we have a questionnaire about sleep. Now your second point is about malnutrition and b-cell growth or immune resistance? We simply do not have the answers.

Dr. Ganapathy: No, the upregulation of the islet cells secondary to increased calorie exposure and hyperinsulinism. This is a new hypothesis that has come from Barker, now it is the accelerator hypothesis. We were talking about longevity and aging, but I am more interested in the probiotic part.

Dr. Ravussin: I am aware of some data in South Africa on malnutrition and basically decreased b-cell mass, but I am not familiar with the data that you are talking about.
Dr. Whitelaw: I was interested in your significant change in the DNA damage. Do you want to talk more about comet assay and what you think it means? Also was that done on blood or what tissues?

Dr. Ravussin: It was done on mononucleated blood cells. We looked at this because a reviewer asked whether it is the same if we take CD8 versus CD4, etc., and we had exactly the same answer on whole blood DNA versus sorting the T cells. There are two assays, one which is basically an electrical field and the DNA migrates. The more damage there is the more DNA is left behind and it makes a comet. For a positive control the DNA is then also treated with hydrogen peroxide, and then mostly the comet remains but there is no nucleus anymore.

Dr. Whitelaw: So chromatin might be involved in contributing to those kinds of assays, I am not sure if anybody has look at that. Telomeres come to mind if you have got damaged DNA and aging effects. So I wonder if your telomeres are getting shorter or longer?

Dr. Ravussin: That is a very interesting question because one of the theories of aging is also shortening of the telomeres and therefore more potential mutations at these genes towards the end of the chromosome. First of all if you take epidemiological data and with age you have a reduction in telomere length. I am now doing a study comparing some less mitotic tissue, like skeletal muscle versus blood cell or sperm, and it seems that the length of the telomere at birth is very variable between people. If mitotic tissue is normal, the birth telomere length is normal, and then after a turnover of skin cells or mononucleotide blood cells, and attrition can be seen, the difference being the attrition. If you have the misfortune of being born with short telomeres and have a very rapid rate of attrition, it will be the worst scenario. But this is one of these 25–100 theories of aging, and I don't know if anyone has ever put everything together.

References