The Crucial Role of Dietary n-6 Polyunsaturated Fatty Acids in Excessive Adipose Tissue Development: Relationship to Childhood Obesity

Florence Massiera\textsuperscript{a}, Philippe Guesnet\textsuperscript{b}, Gérard Ailhaud\textsuperscript{a}

\textsuperscript{a}ISDBC, Centre de Biochimie UMR 6543 CNRS, Faculté des Sciences, Nice, et, and \textsuperscript{b}Laboratoire de Nutrition et Sécurité Alimentaire, INRA, Jouy-en-Josas, France

Introduction

Childhood obesity can be considered a non-infectious epidemic. According to the International Obesity Task Force’s childhood obesity working group ‘the epidemic of European Union childhood obesity appears to be accelerating out of control. Things are worse than our gloomiest predictions’. Consistent with this statement, cardiovascular risk factors are now becoming ‘routinely reported’ among children in many populations. Among the social trends favoring childhood obesity, increased energy intake and decreased energy expenditure have been substantiated. However the importance of qualitative changes (i.e. the fatty acid composition of fats) has been largely disregarded despite a dramatic alteration in the balance of essential polyunsaturated fatty acids (PUFAs). Since the 1960s, indiscriminate recommendations have been made to substitute vegetable oils, high in n-6 PUFAs and low in n-3 PUFAs, for saturated fats. Moreover significant changes in animal feed and in the food chain have been introduced. For example, the n-6/n-3 ratios in food commonly consumed in the American diet range from 17 to 41, largely above official recommendations. Among the consequences, these changes have led to a 4-fold increase in the supply of dietary arachidonic acid (ARA; 20:4n-6) in the last 50 years \cite{1}. Importantly, these changes are translated into changes in the lipid composition of cell tissues and secretions in humans. Equally important, the requirement for linoleic acid (LA; 18:2n-6) for growth and development has been significantly overestimated \cite{2} whereas the recommendation to reduce n-6 PUFAs even as the n-3 PUFAs are increased has
not been followed [3]. Thus, in addition to a positive energy balance, qualitative changes in the fatty acid composition of fats may help to gain insight into the increasing prevalence of overweight and obesity in children and adults despite the slight decrease in energy and fat intake in the last decades [4], an observation which appears at odds with cross-sectional and longitudinal studies showing an association between a high fat intake and a subsequent fat mass enhancement [5]. The evidence from animal and human studies discussed herein favors the possibility that changes in the balance of essential PUFAs are altering the early stages of adipose tissue development, i.e. during fetal life and infancy which are life periods showing the highest adaptability and vulnerability to external factors.

White Adipose Tissue in Early Life and Developmental Issues

How important is the development of white adipose tissue in early life? In humans, it is known that this tissue develops as early as the second trimester of pregnancy and more extensively during the last trimester and after birth. It is also well known that the cellularity of human subcutaneous adipose tissue from obese patients depends on the age at obesity onset, particularly the adipocyte number more than the adipocyte size [6]. It should be pointed out that cellularity measurements are taking place a posteriori, i.e. after excessive proliferation of precursor cells able to divide in vitro and in vivo, in contrast to non-dividing adipocytes. Clearly, proliferation of precursor cells remains an undetectable ‘weightless’ phenomenon as these cells exhibit a 30- to 50-fold smaller volume than adipocytes. Postnatally, white adipose tissue develops extensively in various depots. Available data show that clones of precursor cells may vary in their capacity to proliferate and differentiate into adipocytes in vitro but, although this ability differs between fat depots in rodents, it is highest in humans during the first year of life [6]. Thus, early age is a highly sensitive period during which the adipose tissue expands dramatically. Of note, the formation of mature adipocytes from precursor cells may take as long as a few weeks in rodents, i.e. up to a couple of years when extrapolated to humans. Of note also, the size and self-renewal of the adipose precursor pools in various depots as a function of age or in response to different diets is presently unknown. These issues are critical as subpopulations have recently been characterized in the stromal-vascular fraction of human adipose tissue where they likely represent the true potential of white adipose tissue development.

Adipogenesis and Fatty Acids as Adipogenic Hormones

The differentiation of clonal and non-clonal precursor cells into adipocytes is a sequential process in which glucocorticoids, insulin and insulin-like
growth factor-1 have been identified as the major adipogenic hormones. However, both in rodents and humans, long-chain fatty acids act at the precursor stage and enhance the formation of adipocytes [7]. Fatty acids as well as eicosanoids arising from ARA metabolism through the action of cyclooxygenases and lipoxygenases, i.e. prostaglandins and leukotrienes, behave as activators/ligands of two members of the peroxisome proliferator-activator receptor (PPAR) family, i.e. PPARγ and PPARγ, which are sequentially expressed during adipocyte differentiation (fig. 1) [7]. In vitro, among dietary fatty acids promoting adipogenesis, ARA (arising in vivo from the metabolism of LA or directly from dietary sources) plays a unique role as a precursor of prostacyclin [8]. Once released from precursor cells, prostacyclin binds externally to its cognate receptor IP, activates the protein kinase A pathway via cAMP production and promotes adipogenesis of clonal precursor cells (fig. 1) [9, 10]. A tight correlation is observed between both short-term cAMP production and long-term adipogenesis induced by ARA (r = 0.963) [11]. Both the protein kinase A and extracellular signal-regulated kinase pathways are involved in these early events [12]. Importantly, only ARA triggers cAMP production, whereas eicosapentaenoic acid (EPA) > docosahexaenoic acid (DHA); arising in vivo from the metabolism of α-linolenic acid (ALA; 18:3n-3) or directly from dietary sources, inhibits the production of cAMP stimulated by ARA. Interestingly, in the presence of a specific PPARγ agonist, the ARA-mediated

Fig. 1. Main transcription factors, nuclear receptors and activators/ligands of adipogenesis. Arachidonic acid gives rise in preadipocytes to prostacyclin which acts through its cognate cell surface receptor IP in upregulating C/EBP-β and δ, which in turn, upregulate PPARγ and promote adipogenesis. Arachidonic acid also gives rise to other metabolites which, as well as other dietary long-chain fatty acids, play the role of activators/ligands of PPARβ/δ and PPARγ, and also subsequently promote adipogenesis.
pathway is more potent than a specific PPARβ/δ agonist in promoting adipogenesis through the upregulation and activity of PPARγ2, the master gene of terminal differentiation [13]. Of note, dihomo-γ-linolenic acid (20:3n-6) also promotes adipogenesis (unpublished). In contrast to these n-6 PUFAs, saturated, monounsaturated and n-3 PUFAs (EPA and DHA) are no more adipogenic than a specific β/δ agonist, emphasizing the unique adipogenic role of prostacyclin which has been extended to adipose precursor cells isolated from human adipose tissue. Last but not least, the prostacyclin effect takes place only at the precursor stage as both the ligand production and the expression of functional IP cease in mature adipocytes [10].

**Fatty Acid Composition of Fats and Adipose Tissue during Development**

Based upon in vitro data, in vivo experiments have been carried out to investigate whether a LA-enriched diet modulates fat mass. Under isocaloric conditions, comparative experiments have been performed with wild-type (WT) mice and mice invalidated for the cell surface prostacyclin receptor (ip−/− mice). During pregnancy and the suckling period both WT and ip−/− mice were fed high-fat diets enriched with either 15% corn oil (LA predominant; LA diet) or 10% corn-oil and 5% perilla oil (LA and ALA predominant; LA/ALA diet). These studies have shown that (1) pups from WT mothers fed the LA diet are 40% heavier 1 week after weaning than those from mothers fed either the LA/ALA or standard diet. Thus inclusion of ALA in the LA-enriched diet counteracts the LA-induced enhancement of fat mass. (2) This enhancement is abolished in ip−/− mice, demonstrating the critical role of the prostacyclin receptor in this phenomenon, and (3) this enhancing effect of the LA diet on body weight and fat mass is confined to the gestation/lactation period; importantly, the weight difference between mice fed the LA or the LA/ALA diet is maintained in adult animals. In other words, PUFAs of the n-6 and n-3 series are not equipotent in promoting adipogenesis in vitro and adipose tissue development in vivo [13]. Feeding rats isocaloric diets with varying n-6/n-3 ratios for the last 10 days of gestation and throughout lactation has led to major changes in the milk fatty acid composition. A shift from 0.4 to 8.9 of this ratio increases by 52% the inguinal fat pad weight 1 week after birth [14]. Clearly, varying the proportions of these essential fatty acids in vivo may alter the proportion of ARA and its metabolites versus EPA and DHA (fig. 2). Evidence that ALA modulates ARA and prostacyclin production from LA has been obtained. In adult humans, a reduction in LA supply results in a decrease in prostaglandins measured in urine. Moreover, ALA intake severely decreases prostaglandin synthesis in human platelets but not the conversion of LA to ARA [15]. In normal term infants receiving 16%
LA and from 0.4 to 3.2% ALA (LA/ALA ratios from 44 to 5), lower ARA and higher DHA levels are observed in plasma phospholipids and, interestingly, are associated with lower body weight [11]. In mice increasing total fat (5–20% fat) and decreasing n-6/n-3 ratios (from >100 to 0.1) lead to a dramatic decrease in prostacyclin production in peritoneal macrophages which share many properties with preadipocytes [16].

A direct and important role played by ARA on prostaglandin production must be emphasized as ARA, present in tissue lipids and mainly derived from dietary sources [1], increases the amount of prostaglandins recovered in human urine [17] and in pig lung [18], whereas DHA [18] but not EPA [19] decreases it. As the fatty acid composition of adipose tissue lipids is a fair reflection of ingested fats [20] and as preadipocytes synthesize prostaglandins including prostacyclin [8], it is assumed that the modulation of prostaglandin synthesis by varying the amount and the balance between essential PUFAs may exhibit a pattern in adipose tissue similar to that observed in other tissues.

**Fig. 2.** Metabolic pathways of the essential polyunsaturated fatty acids of the n-6 and n-3 series. Linoleic acid (LA) and α-linolenic acid (ALA) are both substrates of Δ6-desaturase. Thus absolute amounts of LA and ALA and the LA/ALA ratio modulate the fluxes of n-6 and n-3 PUFA metabolites.
Considering the adipogenic role of the LA-enriched diet and the counteracting effect of ALA in rodents, the key question to be addressed in humans is whether the balance of PUFAs has changed over decades during the gestation/lactation period so that it could favor excessive adipose tissue development and subsequent metabolic disturbances. Comparative US data from the National Health and Nutrition Survey II (1976–1980) and the NAHNES III (1988–1994) indicate that the adiposity indices for 6- to 11-month-old infants of all races has increased 1.9- and 1.7-fold for boys and girls, respectively [21], i.e. at ages where a positive energy balance or the quality of carbohydrates [22] cannot be advocated. In contrast, these data suggest that qualitative nutrient changes have occurred during gestation and/or during breast milk/formula milk consumption. In humans, recent data show that low intrauterine availability of γ-linolenic acid (18:3n-6) is related to low birth weight and presumably to low fat mass, consistent with the important role played by LA metabolites in adipose tissue development. Interestingly, low birth weight is associated with increased body fatness and insulin resistance at 7 years of age [23]. The rate of weight regain to normalize the body weight of these newborns appears important and suggests that adipose tissue development then occurring rapidly is potentially harmful [24].

As shown earlier, the content of LA in the mature breast milk of US women has steadily increased from 6–7 to 15–16% of total fatty acids between the early 1950s and the mid-1990s, whereas the percentage of ALA has remained essentially unchanged (~1%) [25], consistent with the PUFA content of adipose tissue triglycerides in US women [20]. The tight correlation between the fatty acid composition of ingested fats and that of breast milk has indeed been demonstrated in female baboons [26]. A great variation in PUFA content has been reported in the breast milk of European women, but it should be noted that both the LA content and the LA/ALA ratio in the mature breast milk of US women are higher than those of European women. Furthermore, the ratio of ARA to DHA is 2-fold higher in the breast milk of US women because of its lower DHA content [25]. These differences are of interest if one considers the differences observed between school-age American and European children with regard to the prevalence of overweight (approximately 32 versus 19%) and obesity (approximately 7.5 versus 4%). Of note, comparisons of energy intake and prevalence of overweight and obesity in the late 1980s between US and French children of 1–2 years of age show that protein, carbohydrate and lipid intake are very similar but that the percentage of PUFAs are 1.5-fold higher in American than in French infants, consistent with a role of n-6 PUFAs in promoting excessive adipose tissue development (Rolland-Cachera MF, personal communication).
Although breastfeeding may help reduce the prevalence of overweight and obesity in childhood, it is not the most frequent way of feeding newborns in industrial countries, and the enhanced fatness has been attributed to the higher energy intake of formula-fed infants [25]. However, and unfortunately, the fatty acid composition of infant formula, enriched or not with n-3 PUFAs, indicates that the percentage of LA has remained very high (~16–18%), mimicking that of mature breast milk of US women. Last but not least, the food industry in the USA has recently advertised the use of preterm infant formulas supplemented with DHA and ARA for term infants, despite the fact that no clear-cut benefits for brain and eye development have been demonstrated with this fatty acid supplementation in normal term infants [27, 28], and despite the fact that ARA acts as a potent adipogenic nutrient in vitro [13]. Moreover, 5-day-old piglets supplemented for 2 weeks with ARA exhibit a 27% increase in body weight without a change in body length [5, 29].

**Conclusion**

During the last decades, many changes have taken place with respect to the nutritional environment of human beings. The steady increase in the body mass index of children has been correlated with an early adiposity rebound which has been associated with high protein but not fat intake at age 2 years [30]. It has been suggested that eating more carbohydrates with a higher glycemic index has a major impact on the prevalence of childhood obesity in 2- to 8-year-old children [22]. We propose that unnoticed changes in the fatty acid composition of dietary fats during pregnancy, and that of mature breast and formula milk, are responsible at least in part of the dramatic increase in the prevalence of childhood overweight and obesity. In the worst-case scenario, since adipocytes once formed exhibit little or no turnover in the body [6], the continuous intake of n-6 PUFA-enriched food could only lead to further overweight and obesity in adolescents and adults.

**References**

Discussion

Dr. Hanson: Thank you very much for that. It agrees in every detail with what I just talked about, not only as to the late outcome in the rats on high n-6/n-3 ratios having increased weight and insulin levels and blood pressure and all that, but also the failure to become tolerant. I think this is a very significant contribution you made and I believe we really have to listen to these findings and do something about them.

Dr. Steenhout: Thank you very much also for your nice talk. In your mouse models, you showed a reduction in weight with the addition of n-3 to the n-6 diet, but you didn’t show us the results with only n-3. Did you do that or not?

Dr. Massiera: No, we didn’t do only n-3. That is right.

Dr. Jensen: In our study, as you pointed out, infants received a very low α-linolenic acid formula in which the ratio of linoleic acid to α-linolenic acid was very very high, higher than anyone would do today. Those infants had higher rates of weight gain during the first 4 months than infants fed a formula containing 3.2% of total fatty acids as α-linolenic acid. When we did a follow-up study we obviously were not going to use a formula that contains as little as 0.4% of total fatty acid as α-linolenic acid. We used 3 formulas, two of which contained about 16% of total fatty acids as linoleic acid and either 1 or 2% of total fatty acids as α-linolenic acid and then another formula contained 8%, half of the amount of linoleic acid, and in that study we included the usual anthropometric measures outcomes, weight, length, head circumference, skin folds, etc., and measures of both total and resting energy expenditure, and found no differences between those groups. So it suggests that the range over which this operates may be a little more limited. But that does have relevance, because if you look at the diet that the low α-linolenic acid group infants were consuming in the first study, that is exactly what most toddlers, children, adolescents and adults Americans consume in the US, but it does appear that perhaps it is what would be considered, from an evolutionary stand point, the extremes of high relative linoleic acid intakes, i.e. exactly what we are consuming. I would be interested in your comments.

Dr. Massiera: I think the ratio is very important and the key because a previous study has been following total PUFAs in general, so we can’t say that there are no effects. There is a lot of controversy regarding the human data, but we definitely must reconsider this n-6/n-3 ratio in a more balanced manner.

Dr. Laron: At the beginning when you listed the adipogenic hormones you listed insulin and IGF-1 in the same line. Do you think they have the same kind of mechanism?

Dr. Massiera: We don’t know precisely whether they both act in the same way, but at high levels they interact with each other’s receptors.

Dr. Laron: How do you think that IGF-1 acts as an adipogenic agent? The question is do growth hormone and IGF-1 act on adipose tissue by the same mechanism or do they act by differential mechanisms?

Dr. Massiera: I am not a specialist in the IGF-1-signaling pathway or even effect. I think it has been demonstrated that the effect is not the same but quite a redundant pathway.

Dr. Laron: I can tell you that IGF-1 has an adipogenic effect in men [1] but I don’t understand the mechanism, and wonder whether it acts through insulin receptors.

Dr. Massiera: In cell culture, IGF-1 has an adipogenic effect but it is not very clear whether this effect is mediated through IGF or the insulin receptor.

Dr. Lucas: This is just a sort of spontaneous thought. DHA alone has been shown to suppress growth in at least 3 studies, and actually there is some evidence for growth suppression even with arachidonic acid (AA) plus DHA. But I am just speculating if
what we are seeing as a potential negative could be a plus as far as future metabolic syndrome risk is concerned.

Dr. Massiera: You mean supplementation by DHA?

Dr. Lucas: I mean in formulas that had DHA alone, in the early formulas that were just supplemented with fish oil, which had quite high EPA as well as DHA, there was great suppression. It was always argued to be due to unbalanced addition of DHA without AA, and in fact there have been a couple of studies with DHA and AA as a suppressor, but whenever this has occurred it has been seen as a negative. In view of the data it occurred to me that slow early growth is good probably at every stage in terms of metabolic syndrome risk and whether this could be a potential explanation.

Dr. Massiera: I think adipose tissue plays a central role in the metabolic syndrome in general, so if the decrease in adipose tissue development can be done in some way by DHA supplementation, it can be good for the metabolic syndrome as a consequence [2].

Dr. Steenhout: My first comment concerns the results of a paper by Clandinin et al. [3] studying formulas for premature babies with different mixtures of LCPUFAs. The group receiving algal-DHA 0.3% and Fungal-ARA 0.6% is reported to have a better growth and faster catch-up. But when you look at the results in detail you realize that this principally concerns the weight growth and not the height or head circumference. Even though it may not be the best indicator for obesity development, the BMI for this group is higher and this is probably not the best thing to do in terms of obesity prevention. My second comment concerns the feeding recommendations based on the wish to have infants following growth curve references that have been continuously adapted. I have the feeling that during that last 50 years we have indirectly induced a secular trend of increasing weight and consequently the risk of later obesity. Should we not come back to growth curves based on breastfeeding reference groups as recently suggested in a British Medical Journal editorial by Wright [4]? Would you like to comment?

Dr. Massiera: No.

Dr. Hursting: I have a comment and a question regarding the IGF question. We have seen in ASA BAF1 mice, the whole CBP family of transcription factors as well as in CEP-β knockout mice. We actually see very low fat white adipose tissue but high IGF-1 and high insulin levels, and in fact they are diabetic as adults. So there is something going on there but I don’t fully understand it. My question relates to the PPARγ, a sort of central path, and I am wondering if you manipulate that pathway with agents or other dietary factors to interact with that, would that have the same effect as dropping the AA intake do you think?

Dr. Massiera: In fact it has been demonstrated that rosiglitazone treatment in mice in vivo increases adipose tissue development and that blocking PPARγ completely counteracts adipose differentiation. The best proof is the knockout mouse.

Dr. Jensen: I would just mention that at our institution some animal studies were also done in which animals were given diets with rather extreme n-6 versus n-3 fatty acids in different directions and with gene arrays and so forth. Those genes that encode for enzymes would promote fatty acid synthesis and deposition seem to be upregulated, and in some cases enzymes responsible for fatty acid oxidation tend to be upregulated with high n-3 fatty acid intakes. However, these were rats, and when we actually did animal calorimetry, there were no differences in body composition or energy expenditure but there was almost a difference in the risk quotient. But these gene expression differences didn’t seem to translate into physiologic effects so we felt disappointed.

Dr. Massiera: We have an explanation for that. I don’t know if the nutritional manipulation should be during the lactation period or the pregnancy period, because
if you compare those data to classical nutritional manipulation at weaning, giving high-fat diets to these rats, there is no significant effect even in our case with the mice.

*Dr. Jensen:* These were relatively young rats but the window of impact may be different from species to species.

*Dr. Massiera:* Yes, but we are giving the diets to the mothers during pregnancy and not only after birth. I think that is what makes the difference.

*Dr. Jensen:* We did not do prenatal intervention.

**References**


