The role of dietary fatty acids for early human adipose tissue growth\textsuperscript{1–4}

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ABSTRACT
Childhood obesity is increasing worldwide, and all previous attempts to stop this epidemic have shown little success. There is now growing evidence that the risk of childhood obesity is strongly influenced by perinatal determinants, including prepregnancy body mass index (BMI), gestational weight gain, and—at least in animal studies—dietary factors during pregnancy and lactation. This review addresses the issue of whether modulation of fat intake and its composition in this early-life period has a potential for primary prevention of childhood obesity. Of particular interest is the question of whether supplementation with \textit{n–3} long-chain PUFAs (LC-PUFAs) may exert an antiobesity effect. Retrospective analysis of human randomized controlled trials with fish-oil intervention during pregnancy and lactation gave inconsistent results concerning BMI and obesity development in offspring. A recent prospective human intervention study aimed at reducing the \textit{n–6:n–3} LC-PUFA ratio did not show an effect on adipose tissue growth in offspring up to the age of 1 y. Therefore, there is currently little evidence to support the hypothesis that dietary intervention to modify fat composition during pregnancy and lactation would be a promising strategy to prevent childhood obesity in humans, but more research is clearly needed to address the question if and how the risk of developing obesity can be modified by dietary intervention early in life. \textit{Am J Clin Nutr} 2013;98(suppl):549S–55S.

INTRODUCTION
Childhood overweight and obesity represent a rapidly growing problem in many health care systems worldwide and are considered to represent a public health crisis (1). A recent review showed an unchanged rate of increase in the prevalence of childhood obesity in developing as well as in developed countries (2). To date, there is little evidence that current attempts to control this threat by community- or family-based prevention programs or other efforts are effective and generalizable. The pandemic was recently attributed to global factors such as policy and economic systems, food supply, and marketing as well as to individual factors such as the genetic makeup and an inappropriate coping behavior (2).

Early development of obesity may cause a wide spectrum of serious complications from childhood to adulthood and increases the risk of premature chronic diseases and death (1). Obesity-associated disturbances with regard to vascular and metabolic functions can be already seen at young ages and may progress toward significant complications (3, 4).

Thus, there is an urgent need to develop and evaluate new approaches to address this challenge. One of the new concepts is to consider pregnancy and lactation as a critical time window for early preventive actions. There are some lines of evidence that indicate that pregnancy and the early postpartum period represent life phases in which the individual’s susceptibility for later adiposity development is determined or “programmed.” In this context, it is essential to understand the early steps of adipose tissue growth and to evaluate opportunities for early intervention.

DEVELOPMENTAL ORIGIN OF HUMAN ADIPOSE TISSUE
There are some older studies that suggest that early life stages can be seen as critical periods for fat cell development and adipose tissue growth in humans. A high proliferation and differentiation capacity of cells isolated from early fat depots has been reported, which may contribute to defining the space for later adipose tissue expansion. This acquisition of fat cells early in life appears to be an irreversible process (5). The early fixation of fat cell numbers was recently confirmed by a study that used an isotope technique (6). This study further described an annual turnover rate of fat cells of \textit{\textasciitilde}10\% at all ages and ranges of BMI by analyzing integrated \textsuperscript{14}C from nuclear bomb tests performed between 1955 and 1963 in genomic DNA.

To date, the development of white adipose tissue in human fetal life has been poorly studied. However, an anatomic study suggested that the first traces of adipose tissue are detectable between the 14th and 16th week of gestation, and thereafter fat lobules slowly develop with predominating multilocular fat cells. At the beginning of the third trimester, adipocytes are found in the characteristic fat depot areas but are still rather small (7). After
birth, the number of fat lobules remains constant, whereas the size of lobules is continuously growing. Available data have not shown any evidence for site- and sex-related differences in early adipose tissue distribution in males and females, and the sexual dimorphism of adipose tissue distribution seems to develop during puberty and later in life under the control of sex steroids, growth hormone, and other mediators (5). Immediately after birth, body fat accounts for ~14% of total body mass as assessed by skinfold thickness measurement and increases up to 20% at the age of 1 y (8). This increase in fat mass is mainly a result of an enlargement of existing fat cell size (5, 9, 10).

Adipose tissue growth and cellularity vary between different age and sex groups. Sensitive developmental periods occur directly after birth and between 9 and 13 y of age (11). This hypothesis was confirmed in a study that measured the highest thymidine kinase activity as an index of cellular proliferation in adipose tissue in infants during the first year of life and as a second peak in the preadolescent stage (12). In a large cohort study, 2-y-old children showed a small but continuous increase in both fat cell size and number during early childhood over a period of 4 y (13). Interestingly, later in vitro studies also have shown that stromal adipocyte precursor cells from human adipose tissue exhibit the highest proliferation and differentiation capacity during the first year of life and at prepuberty, which also supports the concept of sensitive periods of adipose tissue growth early in life (14).

PERINATAL INFLUENCES ON OBESITY RISK IN OFFSPRING

Multiple perinatal factors, which are in large part modifiable, have been shown to be associated with subsequent obesity development in offspring. Some studies suggest a direct relation between maternal prepregnancy BMI and a newborn’s body fat mass (15, 16) and obesity risk in the offspring later in life (17, 18). There is also strong evidence for a direct and positive association between gestational weight gain (GWG) and the offspring’s BMI from infancy through adulthood (19–26). As recently reviewed, GWG is also a predictor of postpartum weight retention of the mother, thereby contributing to the risk of overweight and obesity in women of childbearing age (27).

Lifestyle intervention studies that comprise physical activity and dietary counseling were reported to represent effective tools for reducing excessive GWG (28–30) and for improving some maternal and neonatal outcomes (30), but evidence for further benefits on infant and maternal health is limited. Most of the intervention studies were rather small, and there is clearly a need for large-scale intervention studies to examine whether other complications are reduced and whether a benefit concerning weight development in the offspring is achieved.

Another early determinant of an increased risk of overweight and obesity assessed by BMI in the offspring up to adult age is maternal smoking during pregnancy as shown by recent meta-analyses (31, 32).

Among postnatal factors that affect BMI development in the offspring, breastfeeding is probably the most important. Three meta-analyses of observational studies reported significant protective effects of breastfeeding on the risk of overweight or obesity in later life (33–35), whereas another analysis suggested confounding sociocultural factors and publication bias to be responsible for the apparent beneficial effects (36). Likewise, the results from a large cluster-randomized study of breastfeeding promotion did not provide any evidence for a beneficial effect of an intervention to endorse breastfeeding related to measures of adiposity in children at 6.5 y of age, although the degree and duration of breastfeeding could be significantly increased (37). Breast milk contains a variety of bioactive components, including immunomodulatory factors, adipokines, and long-chain PUFAs (LC-PUFAs) (38), which are absent or have not always been included in infant formula, and the health benefits of breastfeeding might in part be attributable to these factors. However, whether fatty acids present in breast milk mediate potential associations of breastfeeding with later obesity risk has not been extensively studied up to now (39). Thus, despite many advances, our understanding of the multiple perinatal factors and mechanisms that contribute to early childhood obesity is still rather limited.

TRACKING OF BODY FAT MASS AND OBESITY FROM EARLY LIFE TO ADULTHOOD

The rapidly increasing rates of childhood and adulthood obesity urgently demand more insight into the persistence of a high BMI over the life span. Hereby, the greatest challenge is to use sensitive and robust methods for measuring body composition as precisely as possible over the life course, particularly during childhood and adolescence.

The Bogalusa Heart Study found evidence for tracking of obesity from childhood into midadulthood. Childhood BMIs and skinfold thicknesses were associated with adult BMIs and obesity, although the BMIs of the youngest (aged 2–5 y) children were only moderately associated with adult obesity (40). The Avon Longitudinal Study of Parents and Children assessed anthropometric and bioimpedance data of children aged 7 and 11 y. The most important outcome was that children of obese parents had higher fat mass at age 7 y and showed a greater gain in fat mass up to 11 y, which supports the hypothesis of a genetic propensity but also the influence of a shared familial environment (41). A recent prospective cohort study from France showed that large infant size and rapid early infant growth were associated with overweight at ages 7–9 y, further supporting the concept that early infancy constitutes a critical period for the onset of obesity later in life (42).

Therefore, intervention strategies that target early infant overweight and obesity development may have potential to limit the health burden associated with obesity and its metabolic consequences later in life.

FATTY ACIDS AND ADIPOSE TISSUE GROWTH

Many animal studies suggested a role of dietary fat intake during pregnancy and lactation for the development of obesity in offspring. In most of these studies, overnutrition of pregnant rodents by using high-fat diets resulted in increased body weight and fat mass gain in the offspring (43). In addition, there is some evidence that not only the amount of ingested fat but also the qualitative composition of fatty acids in the diet during pregnancy...
and lactation may play an independent role in determining the risk in the offspring to become overweight or obese (44).

n−6:n−3 FATTY ACID RATIO AND CHILDHOOD OBESITY: EPIDEMIOLOGIC DATA

More indirect evidence for a role of fatty acid composition for human adipose tissue development can be deduced from epidemiologic data, which show that the n−6:n−3 fatty acid ratio in the diet of populations in industrialized countries has changed considerably toward an increasing dominance of n−6 fatty acids over recent decades. These changes are also reflected in the fatty acid pattern of breast milk in lactating women, in particular with regard to the linoleic-α-linolenic acid ratio, which has been continuously increasing in many Western countries over the past decades. These observations coupled with increasing rates of childhood overweight and obesity over the same time period give further support to a potential relation between dietary fatty acid composition and infant adipose tissue growth (44).

n−6:n−3 FATTY ACID RATIO AND ADIPOSE TISSUE DEVELOPMENT: IN VITRO AND ANIMAL STUDIES

In vitro studies provided first evidence that the balance of n−6 compared with n−3 fatty acids may play an important role in the critical phases of adipose tissue development. In particular, it was shown that the n−6 fatty acid arachidonic acid (AA) inhibits cell proliferation and promotes differentiation to adipocytes in the preadipocyte stage mediated through action of its metabolite prostacyclin (44, 45), whereas the n−3 LC-PUFAs DHA and EPA seem to counteract this process (44–47). Furthermore, n−3 fatty acids were also shown to act on mature adipocytes in the process of lipid storage and accumulation (47).

The underlying mechanisms include effects on the regulation of transcription factors that represent key molecules for both adipocyte differentiation (eg, peroxisome proliferator-activated receptor γ and CCAAT/enhancer binding protein) and lipogenesis (eg, sterol regulatory element-binding protein 1c), which are mediated either by the fatty acids per se or by their active metabolites such as prostaglandins (46, 47).

Furthermore, there is good agreement from animal studies for antiobesity effects of n−3 LC-PUFA supplementation as evidenced by decreased cellularity of adipose tissue (48) and reduced lipid synthesis (49), which suggests a role of n−3 fatty acid in reducing both hyperplasia as well as hypertrophy of growing fat depots. However, these studies were usually performed in young or adult animals.

More recently, attention was shifted toward the potential programming effect of modifying the fatty acid composition in the maternal diet during the gestation/suckling period on offspring obesity. A recent systematic review of animal studies to investigate the effects of increased n−3 LC-PUFA supply during pregnancy and lactation on offspring body composition concluded that there is insufficient evidence to date to definitively evaluate the role of prenatal and early postnatal maternal n−3 LC-PUFA supplementation on offspring fat mass development (50). In particular, there was considerable disparity between the available studies in terms of the type of intervention (n−3 LC-PUFAs or α-linolenic acid), the time window the intervention was applied, the mode and time point of adiposity assessment, the nature of the control group, and the study quality.

The fact that most studies did not restrict the intervention to the prenatal and early postnatal period, but continued the exposure after weaning, makes it difficult to disentangle potential effects of increased n−3 LC-PUFA supply on developing fat depots in utero (eg, reduced proliferation and differentiation of adipocytes) compared with effects that occur after birth (eg, suppression of fat storage). However, a more recent study in rats in which the n−3 LC-PUFA exposure was limited to the perinatal period and the pups were weaned to a standard diet showed an increased percentage of body fat, particularly through accumulation of subcutaneous fat depots, in the offspring of mothers fed the n−3 LC-PUFA–enriched diet, without affecting the expression of major genes regulating key steps in adipogenesis and lipogenesis (51). In contrast, another study established a model of postnatal programming through increased n−3 LC-PUFA supply in early postnatal life and reported reduced fat accumulation in adult animals after being fed a Western style diet, together with improved lipid and glucose homeostasis and fewer hypertrophic adipocytes, which suggests that early postnatal nutrition may have a programming effect on body composition and metabolism (52).

These conflicting results may raise the possibility that nutritional influences during different phases of perinatal development (prenatal compared with early postnatal period) might confer different susceptibility toward increased fat storage and highlight the need for further studies addressing this issue. Moreover, effects might also differ depending on the macronutrient composition and overall quality of the diet, and it appears conceivable that n−3 LC-PUFAs might exert preventive actions, especially under exposure to a high-fat Western diet. Note that critical periods (eg, prenatal compared with early postnatal) might differ between rodents and humans, and thus findings from rodent studies cannot be generalized to humans.

FISH-OIL/n−3 LC-PUFA SUPPLEMENTATION AND ADIPOSE TISSUE DEVELOPMENT IN OFFSPRING: HUMAN TRIALS

The biological plausibility, that LC-PUFAs have the potential to impact on adipogenesis together with the increasing awareness of the perinatal phase as a sensitive period in which nutritional influences could have a long-lasting impact on future health, led to the hypothesis that altering the fatty acid composition in maternal nutrition during pregnancy and lactation could also have a significant programming effect on body composition in the offspring in humans (44).

The first randomized controlled trials (RCTs) involving supplementation with n−3 LC-PUFAs during pregnancy were focused largely on pregnancy outcome and infant neurodevelopment, whereas the effect of increased n−3 LC-PUFA exposure on infant body composition has only recently received attention. To date, this upcoming research question has almost exclusively been addressed in post hoc analyses of studies that were originally designed to assess other infant outcomes. As recently reviewed by Muhlhausler et al (53), 4 retrospective analyses of 3 RCTs addressed the potential role of prenatal n−3 LC-PUFA supplementation on body composition in infancy and childhood and came to rather inconclusive results. Since publication of this
systematic review, 2 analyses of another RCT, the Nutraceuticals for a Healthier Life trial, comparing the effects of maternal fish-oil supplementation, folate, or both, reported results on BMI as a secondary outcome but again observed no differences in children’s BMI at ages 4 and 6.5 y between the groups (54, 55) (Figure 1).

When aggregating the findings, the high variability of these studies with regard to the timing and duration of the intervention (pregnancy and/or lactation), the dosage of n-3 LC-PUFA supplementation, compliance, and other methodologic aspects has to be considered (53). Of note, most studies relied on rather indirect growth variables such as BMI or BMI z scores, and in only one study were skinfold thickness measurements performed to determine percentage body fat and thus to discriminate between fat and lean body mass (56, 57). Consequently, these inconsistent results do not allow a definitive conclusion on the role of supplementation of pregnant women/lactating mothers with n-3 LC-PUFAs during the perinatal period in determining offspring adiposity development.

Other studies in adults also do not provide clear evidence of reduced fat deposition in humans by supplementation with n-3 LC-PUFAs. Some epidemiologic studies suggested an inverse relation between markers of n-3 LC-PUFA status and variables of obesity. In addition, small human intervention trials indicated potential benefits of n-3 LC-PUFA administration, particularly in combination with energy-restricted diets or physical exercise (61).

These limited data highlight the need for prospective RCTs that involve longitudinal assessments of infant body composition. The recently published INFAT (“The Impact of Nutritional Fatty Acids during Pregnancy and Lactation on Early Adipose Tissue Development”) study is the first RCT designed to investigate the effect of reducing the dietary n-6:n-3 fatty acid ratio in the maternal diet during pregnancy and lactation on infant adipose tissue development as the primary endpoint (8). In total, 208 women before the 15th week of gestation were enrolled and randomly assigned either to an intervention (1200 mg n-3 LC-PUFAs as a supplement per day and concomitant reduction in dietary AA intake) or a control group (general information on a healthy diet) from the 15th week of pregnancy until 4 mo postpartum. It is important to note that the intervention started early in pregnancy close to the first appearance of adipocytes in the human fetus (7) and lasted until 4 mo of lactation. The primary outcome was infant fat mass estimated by skinfold-thickness measurements at 4 body sites at ages 3–5 d, 6 wk, 4 mo, and 12 mo postpartum. Secondary endpoints included sonographic assessment of abdominal subcutaneous and periretinal fat, fat distribution, and child growth.

The study found no evidence that supplementation with n-3 fatty acids combined with instructions to reduce dietary AA

FIGURE 1. Overview of available human data from randomized controlled trials on the effect of maternal n-3 LC-PUFA supplementation during pregnancy, lactation, or both on offspring body composition. (Figure courtesy of Daniela Much.) AA, arachidonic acid; LCPUFA, long-chain PUFA; PI, Ponderal index; pp, preperitoneal; sc, subcutaneous; SF, skinfold thicknesses; US, ultrasonography; WC, waist circumference; ø, no difference between groups.
intake during pregnancy and lactation relevantly affects fat mass in the offspring during the first year of life and thus clearly argues against the underlying hypothesis that the perinatal n-6: n-3 fatty acid ratio is a critical determinant of adipose tissue growth in early infancy (8). However, this study and the trials mentioned previously only provide data on body composition over a limited period in infancy or childhood, respectively, and do not allow a final conclusion on long-term adiposity development. Nevertheless, given the well-documented persistence of infant/ childhood obesity into adulthood (62), and the widely accepted view of early life representing a critical period of adipose tissue development in which the number of adipocytes is likely to be set (6), these data might serve as a suitable indicator for subsequent adiposity development at later stages.

The only study to date with a follow-up period up to adolescence/early adulthood was recently published and found no difference in BMI, waist circumference, and selected biochemical variables between offspring of mothers supplemented with fish oil and the control group at the age of 19 y, which clearly argues against a long-term effect of fish-oil supplementation during pregnancy on offspring adiposity in adolescence (60).

**LC-PUFA STATUS IN RELATION TO INFANT GROWTH AND ADIPOSE TISSUE DEVELOPMENT**

Little is currently known about the role of circulating LC-PUFAs in maternal or umbilical cord plasma for adipose tissue growth in the newborns. Several observational studies examined the relation of maternal and fetal biomarkers of LC-PUFA status with fetal growth; however, the results were inconclusive, and these studies merely reported on birth weight but did not provide data on proxy measures of adiposity in the neonates (63–66).

Maternal plasma phospholipid n-3 LC-PUFA concentrations in early pregnancy were shown to be positively related with birth weight (63, 64), whereas a negative association was found for concentrations of AA in late pregnancy (64). In contrast, negative associations for both cord blood DHA and AA proportions with birth-weight-for-gestational-age SD scores have been reported in another study, which argues against previously suggested growth-promoting effects of AA or DHA, respectively (65, 66). With regard to longer-term associations, the Project Viva Study, a US pregnancy cohort, investigated the relation of maternal and cord blood n-3 LC-PUFA concentrations with infant BMI percentiles and skinfold thicknesses at the age of 3 y (67). In a subgroup of 302 children, an inverse association of DHA and EPA concentrations in umbilical cord plasma with obesity risk and fat mass assessed as the sum of subscapular and triceps skinfold thickness was found. In contrast, maternal midpregnancy DHA + EPA concentrations were not associated with childhood adiposity. On the other hand, a higher ratio of cord blood n-6:n-3 LC-PUFAs was associated with a greater fat mass and odds of obesity in the children, which indicates that enhanced maternal-fetal n-3 LC-PUFA status is associated with a lower risk of childhood obesity. Note that women who reported prenatal fish-oil supplementation were excluded from the analysis, and thus the exposure to n-3 LC-PUFAs covers only the intake from natural dietary sources (eg, ocean fish) and is not comparable to the RCTs that used isolated high-dose n-3 LC-PUFA supplements.

**CONCLUSIONS**

On the basis of current evidence from RCTs during pregnancy and lactation, the fatty acid ratio in early nutrition does not seem to be a critical determinant of early infant adiposity development. However, results from long-term follow-up investigations of ongoing studies are awaited and will need to be considered before drawing definite conclusions on the long-term consequences of increased perinatal n-3 LC-PUFA intake or reduced n-6:n-3 LC-PUFA ratio in influencing infant body composition and other health outcomes. Likewise, clinical studies are needed to examine the potential role of other environmentally modifiable determinants of early obesity risk, such as GWG and prepregnancy BMI or the role of lifestyle factors such as maternal diet or physical activity, to be able to develop and establish comprehensive lifestyle intervention strategies for pregnancy and lactation to achieve primary prevention of obesity. Such data may contribute to finally answer the question of if and to what extent fetal programming of obesity is relevant in humans.

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