

Review

Temporal changes in dietary fats: Role of $n-6$ polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity

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Received 9 November 2005; received in revised form 22 December 2005; accepted 10 January 2006

Abstract

The importance of a high fat intake in the increasing prevalence of childhood and adult obesity remains controversial. Moreover, qualitative changes (i.e. the fatty acid composition of fats) have been largely disregarded. Herein is reviewed the role of polyunsaturated fatty acids (PUFAs) of the $n-6$ series in promoting adipogenesis *in vitro* and favouring adipose tissue development in rodents during the gestation/suckling period. Epidemiological data from infant studies as well as the assessment of the fatty acid composition of mature breast milk and infant formulas over the last decades in the Western industrialized world are revisited and appear consistent with animal data. Changes over decades in the intake of $n-6$ and $n-3$ PUFAs, with a striking increase in the linoleic acid/ α -linolenic ratio, are observed. In adults, using a consumption model based upon production data, similar changes in the PUFA content of ingested lipids have been found for France, and are associated with an increase of fat consumption over the last 40 years. These profound quantitative and qualitative alterations can be traced in the food chain and shown to be due to changes in human dietary habits as well as in the feeding pattern of breeding stock. If prevention of obesity is a key issue for future generations, agricultural and food industry policies should be thoroughly reevaluated.

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Keywords: Childhood obesity; Adult obesity; White adipose tissue; Development; Sensitive periods; Adipogenesis; Fatty acids; Arachidonic acid; Signaling pathways; Diet fatty acid composition; $n-6$ and $n-3$ fatty acids; Metabolic fluxes; Breast milk fatty acids; Formula milk; Animal feed; Food chain

Abbreviations: ALBP, adipocyte lipid binding protein; ARA, arachidonic acid (20:4, $n-6$); COX, cyclooxygenase; DGLA, dihomo- γ -linolenic acid (20:3, $n-6$); DHA, docosahexaenoic acid (22:6, $n-3$); eLOX-3, epidermal lipoxygenase 3; EPA, eicosapentaenoic acid (20:5, $n-3$); FA, fatty acid; FABP, fatty acid binding protein; IGF, insulin-like growth factor; IP, prostacyclin receptor; LA, linoleic acid (18:2, $n-6$); LCFA, long chain fatty acid (14–18 C); LNA, α -linolenic acid (18:3, $n-3$); LOX, lipoxygenase; PKA, protein kinase A; TNF- α , tumour necrosis factor- α ; VLCFA, very long chain fatty acid (20C or longer).

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1. Introduction

Both childhood and adult obesity can be considered a non-infectious epidemic. According to the childhood obesity working group of the International Obesity Task Force (2004) “the epidemic of European Union childhood obesity appears to be accelerating out of control. Things are worse than our gloomiest predictions”. As a result, cardiovascular risk factors are now becoming “routinely reported” among children in many populations. In adults, according to a recent report of WHO consultation on obesity, a continuous trend in overweight and obesity has also been observed in most industrialized countries. Of interest, in studies conducted in males and females (BMI > 30; age range ~20–70 years), a significant upward shift in the prevalence figures occurred in USA, Canada and UK in the 1980–1990 period whereas in France, according to the WHO Monica project published in 2000, a similar shift was observed later, in the 1985–1995 period. These observations suggest important changes in various social trends favouring energy intake and decreasing energy expenditure among which sedentary lifestyle appears as an important player. However, qualitative changes in food consumption have been largely ignored in the last 40 years despite dramatic changes in food habits and in the food chain, including the feeding pattern of breeding stock.

The role of dietary fat as a major player in adult human obesity has remained a controversial issue [1,2] because the prevalence of overweight and obesity has increased dramatically over the last decades despite no

recent major change in the amount of ingested fats. However, as we shall see, this conclusion needs to be reassessed. Moreover, the importance of qualitative changes in the fatty acid composition of fats has been largely disregarded despite a dramatic alteration over decades of the balance of essential polyunsaturated fatty acids (PUFAs). Since the sixties, 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 feeds and the food chain have been introduced. For example, the $n-6/n-3$ PUFA ratios in food commonly consumed in the American diet range from 10 to 41, considerably above official recommendations. In the last 50 years, these changes have been accompanied by a significant increase in the supply of dietary arachidonic acid (ARA; 20:4 $n-6$). Of note, the amounts of ARA in the most consumed foods are significantly higher than those previously published [3]. Equally important, the requirement for linoleic acid (LA; 18:2 $n-6$) for growth and development as a precursor of arachidonic acid and its metabolites has been significantly overestimated [4] whereas the recommendation to reduce $n-6$ PUFAs even as the $n-3$ PUFAs are increased has not been followed [5]. Thus, in addition to a positive energy balance, qualitative changes in the fatty acid composition of ingested fats, including breast milk and formula milk, may help to gain insight in the increasing prevalence of overweight and obesity in children and adults which add to the cross-sectional and longitudinal studies showing an association between high fat intake and a subsequent fat mass enhancement [6–9]. Evidence from animal and human studies discussed herein favour 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 the periods showing the highest adaptability and vulnerability to external factors but also at the adult age during which adipose precursor cells remain present and potentially able to differentiate into adipocytes.

2. White adipose tissue development in early life and responses to dietary fats

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 in various sites (buccal, neck, shoulder, gluteal, perirenal) and after birth [10]. Adipocytes represent between one third and two thirds of the total number of cells in adipose tissue. The remaining cells present in the stromal-vascular fraction are various blood cells, endothelial cells, macrophages, pericytes and adipose precursor cells of varying degrees of differentiation. Methods of determining adipocytes number and size have been used to study the postnatal development of WAT, particularly in rodents. Unfortunately, they are not sufficiently accurate to detect modest changes in cell number and, in any event, the methods only count lipid-filled cells. In other words preadipocytes, i.e. cells that have expressed early phenotypes but do not yet contain triacylglycerol droplets, are not counted. It should be pointed out that cellularity measurements are taking place a posteriori, i.e. after excessive proliferation of precursor cells which are able to divide in vitro and in vivo, in contrast to non-dividing adipocytes. Despite these pitfalls, compared to non-obese subjects, the cellularity of sub-cutaneous adipose tissue from obese patients depends on the age of obesity onset with an increase of the adipocyte number greater than the adipocyte size [11].

In humans, the proliferative capacity of adipose precursor cells from sub-cutaneous adipose tissue is highest during the first year of life and before puberty [12]. Thus, early age is a highly sensitive period during which adipose tissue expands dramatically. Most importantly, proliferation of precursor cells remains an undetectable “weightless” phenomenon as these cells exhibit a 30- to 50-fold smaller volume than adipocytes. Numerous studies using ^3H -thymidine have been performed in order to distinguish between cell proliferation and the lipid-filling process. A dramatic decrease of the labeling index of stromal-vascular cells containing adipose precursors precedes the rise of glycerol-3-phosphate dehydrogenase activity (a key enzyme for triacylglycerol synthesis). Interestingly, the shift in cell labeling from stromal-vascular cells to adipocytes may take as long as a few weeks in rodents, i.e. up to a couple of years when extrapolated to humans [13]. Post-natally, white adipose tissue develops extensively in various depots. Available data show that clones of murine precursor cells may vary in their capacity to proliferate and differentiate into adipocytes in vitro. Although this ability differs between fat depots, it decreases but is not abolished with age in all sites [14]. Last but not least, adipose precursor cells remain present in male and female octogenarians [15]. This point is critical as it emphasizes that

throughout life (i) potent nutritional and adipogenesis stimuli can be potentially active at the adult age and (ii) adipocytes can be formed in sub-cutaneous adipose tissue as it is clinically observed for severely obese adults. Of note, 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 in humans. This point is important as sub-populations 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 [16,17]. Of note also, adipocyte turnover is low, if any, and apoptosis has only been observed under drastic conditions, making questionable its quantitative importance under physiological conditions. Altogether, these observations emphasize the fact that adipocyte formation (adipogenesis) is de facto an irreversible process and that prevention of this phenomenon should represent a key issue from a health perspective.

Clearly, a key point for any given individual is whether excess of adipose tissue at early age is predictive of subsequent overweight or obesity. Longitudinal studies have been performed to answer this question in subjects from 10 months to 18 years of age. The results indicate that BMI shows the best correlation between childhood at 1 year of age to adulthood at 16 years of age, and that the relative risk of becoming fat adults is twice as high for fat as for lean babies [18]. Similarly, a recent study shows that BMI of children at 8 years of age was positively predicted by their BMI at 2 years of age [19].

Another important developmental issue is whether adipose tissue has the ability to expand at any given age in response to high-fat diets. Contrary to highly popular claims made in the past, high-fat diets are known to induce in adult rodents an increase in the weight of various adipose depots by hypertrophy often accompanied by hyperplasia [15]. With similar caloric intake, hypertrophy of perirenal and epididymal adipose tissues is lower with high-fat diets enriched in mono- and poly-unsaturated FA than with high-fat diets enriched with saturated FA [20–22]. In young male Sprague–Dawley rats fed for 4 months with various high-fat diets, perilla oil (high in α -linolenic acid) leads to hypoplasia and mainly to hypotrophy as compared to other dietary fats [23]. More recently, mother rats have been fed diets containing either coconut oil resembling milk fat (high in lauric, myristic and palmitic acid) or safflower oil (high in oleic and linoleic acids), and have been mated to provide Fa/Fa and Fa/fa lean offsprings as well as fa/fa obese offsprings. Despite similar quantitative fat intake, the data indicate qualitative differences regardless of the genotype, where long-chain mono- and poly-unsaturated FA favour hyperplasia whereas long-chain saturated FA favour hypertrophy [24]. Thus it appears that the hyperplastic versus hypertrophic response of expanding adipose tissue depends upon the age of rodents and the fatty acid composition of ingested fats (vide infra).

3. Adipogenesis and fatty acids as adipogenic hormones

Knowledge of adipogenesis has increased dramatically over the last two decades with the use of clonal and non-clonal adipose precursor cells from rodents and humans. Adipogenesis is a sequential process in which glucocorticoids, insulin and IGF-I have been identified as the major adipogenic hormones [15]. Both in rodents and humans, long-chain fatty acids act also at the precursor stage and enhance the formation of adipocytes. The first line of evidence that fatty acids (FA) are involved has been obtained after purification of the main adipogenic component of fetal bovine serum which was characterized as arachidonic acid (ARA). In vitro, ARA is very adipogenic and plays in preadipocytes the role of a precursor of prostacyclin [25,26]. In contrast, the $n-3$ isomer of ARA (only present at trace levels in nature) and two major metabolites of the α -linolenic acid (LNA) pathway [i.e. eicosapentaenoic acid (EPA, C20:5 $n-3$) and docosahexaenoic acid (DHA, C22:6 $n-3$)], which are not metabolised to prostacyclin, are less potent than ARA. The adipogenic effect of ARA is partially blocked by cyclooxygenase inhibitors and anti-prostacyclin antibodies added externally, and is mimicked by carbacyclin, a stable analogue of prostacyclin [25–28]. This strongly suggests an adipogenic role of prostacyclin through the cell surface prostacyclin receptor IP as an autocrine/paracrine mechanism (Fig. 1). Among all natural fatty acids (saturated, mono- and poly-unsaturated FA), only ARA triggers cAMP production and activates, through the IP/prostacyclin system, the protein kinase A pathway. Interestingly, EPA and to a lesser extent DHA, while being inactive as cAMP-elevating agents, inhibit the stimulatory effect of ARA on cAMP production [29,30]. Of interest, prostacyclin production ceases in adipocytes in which the cell surface prostacyclin receptor IP is no longer functional [29,31]. In other words, prostacyclin production and its triggering effect, i.e. the rise in cAMP levels represent *transient* events of

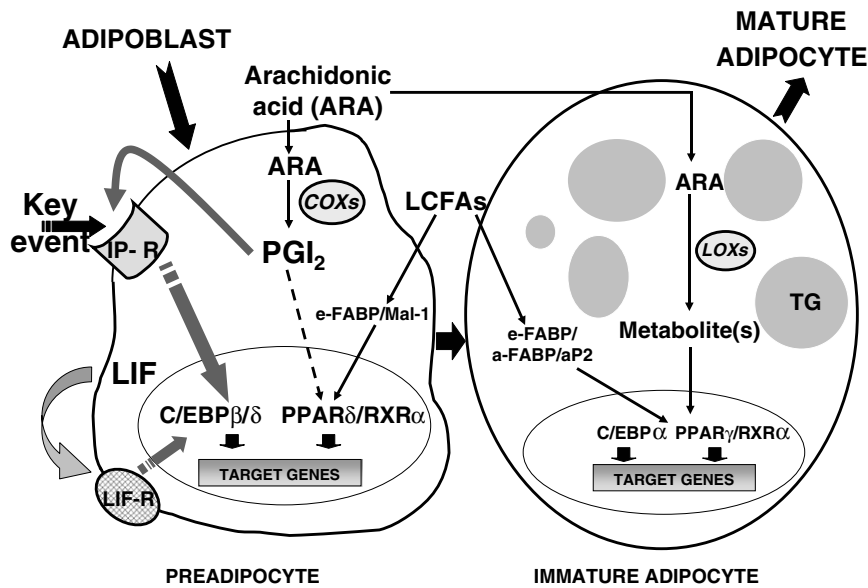


Fig. 1. Redundant pathways and long chain-fatty acids (LCFA) implicated in adipogenesis. At least two cell surface receptor/ligand systems concur to up-regulate the expression of CCAAT/enhancer binding protein β (C/EBP β) and C/EBP δ [i.e. prostacyclin receptor (IP)/prostacyclin [25–30,41], and leukemia inhibitor factor (LIF) receptor/LIF [41], and to promote adipogenesis. Dietary linoleic acid (LA) and/or arachidonic acid (ARA) favour in preadipocytes the synthesis of prostacyclin which is then released. Thus, ARA via prostacyclin triggers a key event, plays a unique role in activating the protein kinase A pathway by means of IP, and enhances the differentiation process [25–30]. Furthermore prostacyclin is assumed to bind to peroxisome proliferator-activated receptor β/δ (PPAR β/δ) [39]. Other dietary long-chain fatty acids (LCFAs) act as activators/ligands of PPAR β/δ and PPAR γ [34–37]. Upon terminal differentiation, LIF is no longer produced [41]. Production of prostacyclin and other prostaglandins ceases and is accompanied by reduced expression and loss of functional IP [29,31]. In addition to ARA metabolites synthesized through cyclooxygenases (COXs) at early step(s), involvement of ARA metabolites synthesized through lipoxygenases (LOXs) as ligands of PPAR γ are also implicated at later step(s) [33,38,40]. Epidermal (keratinocyte) fatty acid binding protein (e-FABP/Mal1) in preadipocytes and also adipocyte fatty acid binding protein (a-FABP/aP2) in adipocytes are assumed to bind and transport LCFA [36]. TG, triglycerides.

adipogenesis. Not surprisingly, any *sustained* expression of cyclooxygenases in differentiating cells by tumor necrosis factor- α (TNF- α) [32] or any *sustained* activation of the PKA pathway by cAMP-elevating agents in the presence of ARA [33] will prevent terminal differentiation to occur and therefore inhibit adipogenesis. Consequently, and as observed, selective COX inhibitors will in this case partially relieve this inhibition [29,31–33].

The second line of evidence that FA are important regulators of adipogenesis has been obtained when it was shown that all long-chain FA (LCFA) could act as transcriptional regulators of some lipid-related genes, as first reported for the gene encoding the adipocyte lipid-binding protein (ALBP), also termed adipocyte fatty acid-binding protein (a-FABP) or aP2 protein [34]. In contrast to ARA which is metabolized to prostacyclin in clonal preadipocytes, the metabolism of the other LCFAs is not required to bring this effect as α -bromopalmitate, a non-metabolized LCFA, is more potent than natural LCFAs in activating those genes [35]. When compared to control adipogenic conditions, a brief exposure of preadipocytes to LCFAs appears sufficient to trigger *in vitro* both hyperplasia and hypertrophy, in a way similar to the situation observed *in vivo* upon long-term high-fat feeding [36]. These observations on the adipogenic role of LCFA as transcriptional regulators have been extended to rat and human non-clonal preadipocytes [15].

The intracellular sensors of LCFA have been identified as nuclear receptors of the family of peroxisome proliferator-activated receptor (PPARs) [37]. Interestingly, ARA and some of its metabolites generated through cyclooxygenase and lipoxygenase activities are implicated in adipogenesis [38] and behave as activators/ligands of PPARs. Recent observations indicate that prostacyclin is a ligand of PPAR β/δ [39] and that epidermal eLOX-3 generates endogenous PPAR γ ligands in differentiating preadipocytes [40]. If it were so, ARA could therefore be involved in early and also late events of adipogenesis. From the bulk of *in vitro* data,

Fig. 1 depicts the main ARA-related events which favour adipocyte formation. First, ARA up-regulates the expression of CCAAT/enhancer binding protein β (C/EBP β) and C/EBP δ via the prostacyclin/IP receptor system and the PKA pathway [41]. C/EBP β and C/EBP δ are *trans*-acting factors which up-regulate the expression of PPAR γ [42] known to be critical for late events of adipogenesis [43]. Second, ARA may also act through prostacyclin as activator/ligand of PPAR β/δ which, in turn, up-regulates the expression of PPAR γ [44]. In vivo, invalidation (deletion or insertion within the gene) of cyclooxygenase (COX) genes has not led to a decrease in the body weight of both COX-1^{-/-} and COX-2^{-/-} mice but a decrease of epididymal fat pad weight is observed [45]. Invalidation of C/EBP β and C/EBP δ genes impairs severely but does not abolish adipose tissue formation [46], whereas that of the PPAR β/δ gene leads to a slight decrease in fat mass [44]. This suggests that prostacyclin signaling, arising from ARA metabolism, may play a more important adipogenic role through C/EBP β and C/EBP δ than through PPAR β/δ in up-regulating PPAR γ .

In order to estimate the relative importance of these two regulatory pathways, we have taken advantage in vitro of the recent availability of specific PPAR agonists. In the presence of a specific PPAR γ agonist, the ARA-mediated pathway is more potent (\sim 3-fold) than a specific PPAR β/δ agonist in promoting adipogenesis [30]. Of note, dihomo- γ -linolenic acid (20:3 *n*-6) promotes also 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 PPAR β/δ agonist, emphasizing the unique adipogenic and early role of ARA. Thus LCFA are *not* equipotent in promoting adipogenesis and ARA appears as a remarkable adipogenic “booster”. This proposal is supported by recent data showing that more than 95% of adipose precursors established from human adipose tissue [47,48] differentiate into functional adipocytes in the presence of carbaprostacyclin (unpublished).

To gain insights into the contribution of ARA and prostacyclin signalling in adipose tissue development, wild-type mother mice have been fed before mating and during the gestation/suckling period with either a high-fat diet rich in LA (LA diet) or the same isocaloric diet enriched in LA and LNA (LA/LNA diet). The ratios of *n*-6 PUFAs versus *n*-3 PUFAs were 59/1 for the LA diet and 2/1 for the LA/LNA diet, respectively. Body weight from weaning onwards, fat mass, epididymal fat pad weight and adipocyte size at 8 weeks age were found to be higher with the LA diet than with the LA/LNA diet. Remarkably, pups from mother mice fed the LA diet were 50% heavier at weaning than those fed the LA/LNA diet. This is a time when adipose tissue is being extensively formed and, furthermore, this difference in body weight persists until the pups reach adulthood. Thus inclusion of LNA in the isocaloric diet rich in LA prevents the enhancement of fat mass, which is consistent with our in vitro observations (i.e. the adipogenic effect of *n*-6 PUFAs compared to that of *n*-3 PUFAs) [30].

The importance of the gestation/suckling period is demonstrated by the fact that when wild-type mother mice are fed a standard laboratory, i.e. a high-carbohydrate low-fat diet, the body weight of pups fed after weaning only with the LA diet remains similar, in the following weeks, to that of pups fed the LA/LNA diet. In contrast to wild-type mice, prostacyclin receptor null (*ip-r*^{-/-}) mice exhibit no additional increase of body weight and fat mass in pups from mothers fed LA diet compared to mothers fed LA/LNA or standard diet. This observation demonstrates that the prostacyclin signalling generated through ARA via metabolism of LA is the key effector which is responsible of the enhanced fat mass observed in wild-type pups during the gestation/suckling period in response to LA-enriched diet [30]. Consistent with the adipogenic role played by LA and the anti-adipogenic role played by LNA, when rats are fed isocaloric diets with varying *n*-6/*n*-3 ratios for the last 10 days of gestation and throughout lactation, major changes in the milk fatty acid composition are observed. A shift from 0.4 to 8.9 of this ratio increases by 52% the inguinal fat pad weight one week after birth [49]. Moreover, in 5 week-old mice, among five different high-fat semi-purified diets given for 5 weeks, the linoleic acid group of animals has the highest and the α -linolenic acid group the lowest proportion of body fat [50].

Taken together, these observations suggest that varying the proportion of α -linolenic acid in the diet should alter the production of ARA through competitive inhibition of Δ 6 desaturase which uses both LA and LNA as substrates (Fig. 2). Conversion of [¹³C]LA and [¹³C]LNA to long-chain metabolites is low but varies directly with precursor concentrations of each series and inversely with the polyunsaturated fatty acid concentrations of the alternative series, suggesting that the dietary LA/LNA ratio downregulates LA conversion to ARA [51]. Interestingly, in normal term infants receiving 16% LA, inclusion of 0.4–3.2% LNA lowers ARA

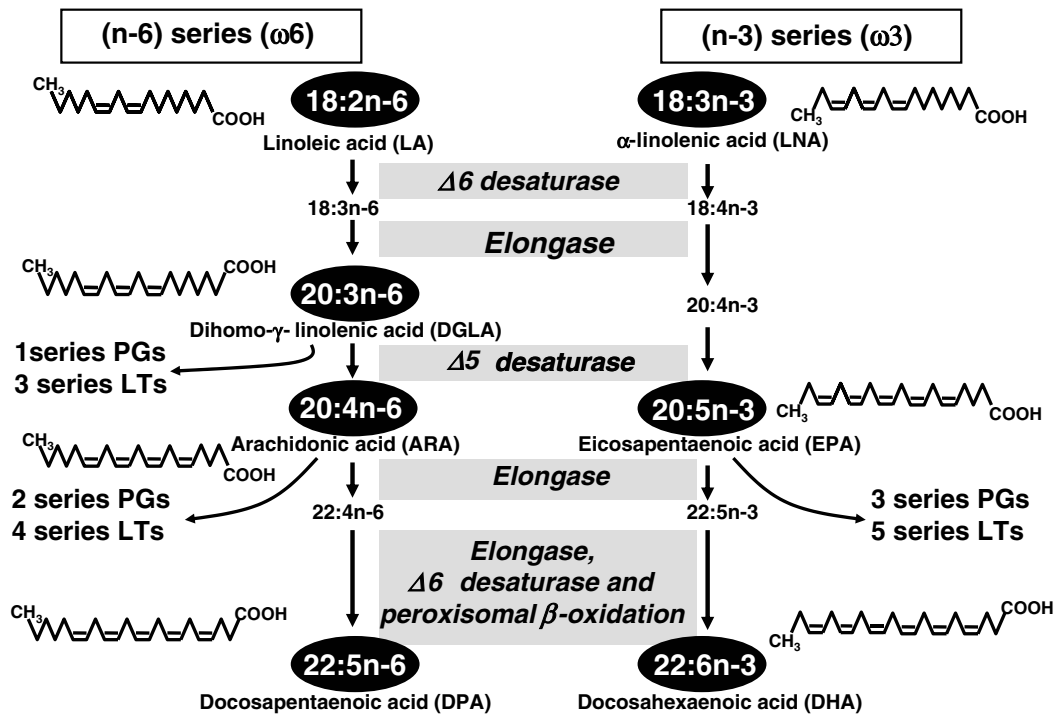


Fig. 2. Metabolic fluxes of linoleic acid and α -linolenic acid.

and increases DHA levels, and this was associated with lower body weight [52]. In adult humans, LNA supply decreases severely prostaglandin synthesis in human platelets but not the conversion of LA to ARA [53]. In mice, increasing total fat (5–20%) and decreasing $n-6/n-3$ ratios (from >100 to 0.1) leads to a dramatic decrease of prostacyclin production [54].

A direct and important role played by ARA on prostaglandin production is therefore assumed as ARA, present in tissue lipids and mainly derived from dietary sources (3), increases the amount of prostaglandins recovered in human urine [55] whereas DHA decreases prostaglandin production in neonatal pig lung [56]. Moreover dietary ARA increases this production in vivo in peritoneal cells but EPA does not reverse this effect [57]. As the fatty acid composition of adipose tissue lipids is a fair reflection of ingested fats [58] and as pre-adipocytes synthesize only a few prostaglandins that include prostacyclin [25,26], it is envisioned that the modulation of prostacyclin synthesis by varying the amount and the balance between essential PUFAs may exhibit in adipose tissue a pattern similar to that observed in other tissues and thus may favour excessive adipose tissue development.

4. Fatty acid composition of fats in early life and relationships to childhood obesity and health

4.1. General considerations

As mentioned above, the importance of a high lipid intake in childhood obesity has been seriously challenged because of a lack of evidence for the increased energy intake as fat despite a striking increase in the prevalence of overweight and obesity among youths [59]. According to most investigators, physical inactivity is considered to be a major contributor to this alarming trend. However, distinct and important weight-related events appear to take place during pregnancy and at a very early age. Regarding pregnancy, recent data show the importance of LA metabolites in development, as low intrauterine availability of γ -linolenic acid (18:3 $n-6$) is related to low birth weight and presumably to low fat mass. Interestingly, low birth weight is associated with increased body fatness and insulin resistance at 7 years of age [60]. It appears that a high rate of

weight regain to normalize body weight of low-birth weight newborns is critical and potentially harmful [61,62]. The percentage of US children between 6 and 11 months of age above the 95th percentile of the weight-for-length growth reference curve has increased in boys from 4.0% between 1976 and 1980 to 7.5% between 1988 and 1994, and in girls from 6.2% to 10.8% during the same periods of time [63]. Obviously, a doubling of the adiposity indices of babies over roughly a 13 year interval can hardly be explained by an increased energy and fat intake or by increased sedentarity but rather emphasizes qualitative issues. Considering the adipogenic role of LA-enriched diet and the counteracting effect of LNA in rodents, *one key question to be addressed in humans is whether the balance of PUFAs has changed over decades during pregnancy and/or the lactation period such as it could produce metabolic disturbances and favour excessive adipose tissue development*. Consistent with a role played by LA metabolites in this development, a positive association between adipose tissue ARA and body mass index has been recently reported in children of Cyprus and Crete [64]. Another key question is whether the fatty acid composition of ingested fats will determine that of breast milk and adipose tissue triglycerides. This appears to be the case. First, when very young infants (before the 5th day of life) are fed formula milks differing widely in their LA content (1.2% versus 39.2%), the fatty acid composition of triglycerides from sub-cutaneous fat becomes similar within 6 weeks to that of the ingested diets, i.e. 2.5% and 25.8%, respectively [65]. Second, the FA composition of breast milk from female baboons is a fair reflection of that of animal feed [58].

4.2. Lactation period and PUFA composition of mature breast milk

4.2.1. Status of linolenic and α -linolenic acid

Regarding the lactation period, the content of LA in mature breast milk of US women has increased between the early fifties and the mid-nineties whereas the percentage of LNA has remained essentially unchanged [59], consistent with the PUFA content of adipose tissue triglycerides of US women [66]. Of note, robust data are available for US women as dozens of reports on milk fatty acid composition have been periodically published after 1944 in that country [67–84]. As illustrated in Fig. 3, the LA content of the mature milk of US women steadily increased from 6% to 7% to 15% of total fatty acids between 1944 and 1990 and then plateaued at 16%. As early as 1965, the LA content passed beyond the adequate level which is now recommended by the ISSFAL for infant formulas (10% of total fatty acids) [5]. To date, the LA mean content is between the ISSFAL (International Society for the Study of Fatty Acids and Lipids) recommended value and the upper limit fixed by several committees of nutrition [85–88]. In contrast, the LNA content has remained nearly constant during the last 40 years (approximately 1.0–1.3%) (Fig. 3). Therefore, the LA/LNA ratio has increased from the value of 6.0–8.0 before 1970 [89,90] to 14–16 since 1980. Such an imbalance of the precursor ratio may favour the conversion of LA to ARA, to the detriment of the synthesis of EPA and DHA

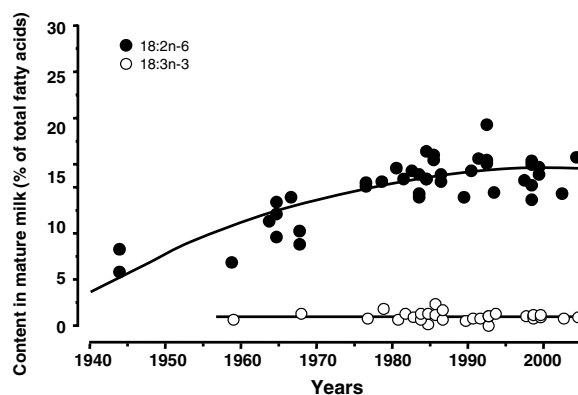


Fig. 3. Linoleic acid (LA) and α -linolenic acid (LNA) content in mature breast milk of US women from 1944 to 2005 (adapted from [67–84]). For 18:2n–6, the best regression was a 2-order polynomial fit $y = -0.0029x^2 + 11.9x - 1189$, $n = 40$, $r = 0.82$, $P < 0.001$. For 18:3n–3, no significant relationship was noted ($y = -0.00035x^2 + 1.39x - 1373$, $n = 28$, $r = 0.14$, NS).

from LNA. A great variation of the PUFA content has also been reported in the breast milk of Canadian and European women because a similar but less marked increase in the LA content has been observed between 1950 and 1990, whereas that of the other PUFAs, particularly that of LNA, has not changed substantially. Indeed, in the sixties, milks of US women contained ~10% of LA [91–96] similar to those of Canadian women (10%) [97], whereas those of British women contained 7.1% [98]. Higher percentages were reported in the eighties though still below the present ones: 9.0% in France and UK [99–102], 10.8% in Australia [103], 11.0% in Canada [104] but a markedly higher value was already found in USA, 14.0% [105–107]. Only a significant comparison between USA and UK can be made as several reports on milk fatty acid composition have also been published in UK since 1965 [98,100–102,108–113]. As illustrated in Fig. 4, the trend regarding UK is similar to that of USA but the range of variations was much less marked, since the LA content increased from 7% in 1965 [98] to 11–12% in the nineties [102,112,113]. Apparently, the LNA content has also increased in UK, leading to no significant change in the LA/LNA ratio.

The content in PUFAs found in human milk reflects the various types of dietary fats consumed by the mothers, including those consumed on a long term basis. Hence, *both the daily intakes and the amounts present in lipid-filled organs (adipose tissue, liver)* will determine the PUFA content of breast milk, explaining the difficulties of revealing a tight relationship between LA or LNA intake as assessed by food records and their content in human milk. Using a bolus of ^{13}C -labeled LA in lactating women, it was estimated that only 30% of LA found in milk originates from the diet (short term impact) and 70% from maternal body stores (long term impact) [114]. Clearly, on a long term basis, changes in LA milk content reflect changes in LA intake by the mothers and result from significant modifications of food practices in Western countries (see Sections 5.2 to 5.4). In USA, consumption and use of corn oil were favoured, progressively replacing and supplanting animal fats in salad and cooking, so that corn and other vegetable oils finally are now representing more than 70% of the PUFA dietary source [1,115–117]. The increasing use of margarines and LA rich-vegetable oils have also occurred in UK, but the increased use of rapeseed oil offers a possible explanation of the parallel rise in LNA intake [90]. The consumption of sunflower and peanut oil in replacement of that of rapeseed oil probably explains the marked increase of LA content in the milk of French mothers [118].

Unfortunately, time-trend changes in dietary intakes of LA and especially LNA in these Western populations during the last decades are difficult to estimate accurately. This is mainly due to differences among studies conducted on food consumption surveys (24-h recall, 3- and 7-days dietary records, food determination) and to food composition tables which are sometimes lacking accuracy. To illustrate this critical point, a compilation of more than 100 studies reporting individual fatty acid consumption between 1940 and 1985 shows that the total PUFA intake steadily increased in USA from 2.5% of the energy intake to 7.5% in the overall population [119]. In men, despite a decreased consumption of total fat between the 1950s and the 1980s, daily PUFA intake appears to have risen gradually and to plateau around 17 g/day. Rather confusing, in women, daily intake appears to have reached a peak in the 1970s (17 g/day) and to have decreased

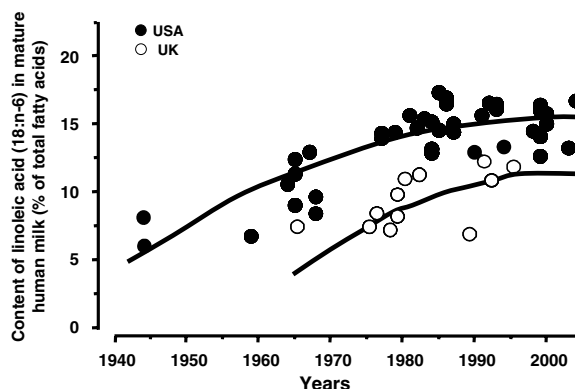


Fig. 4. Linoleic acid content in mature breast milk of women living in USA and UK from 1944 to 2005 (adapted from Refs. [67–84] and Refs. [98,100–102,108–113]. The best regressions were 2-order polynomial fits: for US milk, $y = -0.0029x^2 + 11.9x - 1189$, $n = 40$, $r = 0.82$, $P < 0.001$; for British milk, $y = -0.0057x^2 + 22.8x - 22787$, $n = 12$, $r = 0.61$, $P < 0.05$.

thereafter (10 g/day), whereas PUFA content in breast milk has continued at the same time to increase (Fig. 3). Although these reported values raise a major concern regarding the reliability of food composition tables, it appears that intake of PUFAs, including that of LA, also increased in Western European countries but that this trend has taken place *later* than in USA [120]. As an example, the daily intake of LA of UK male has increased by 50% between 1980 and 1992 from 10 g/day up to 15 g/day as well as that of LNA from 1.0 g/day up to 1.9 g/day [120]. However, comparison between US and British data (Fig. 4) raises questions because, although LA intakes were reported to be similar in the nineties, the LA content of breast milk was ~16% for US mothers and only 11% for British mothers [69–75,102,111,112].

To circumvent the obvious difficulties in interpreting data from food composition tables, trends in LA consumption can also be evaluated from seed oils consumed by humans (Fig. 5) because seed oils constitute one of the major dietary sources for this PUFA [121]. Recently, the apparent consumption of LA in Canada, USA, Australia and UK for the 1961–2000 years has been reported (Fig. 5). As observed for the LA content of human milk, similar increases in LA consumption are seen in both USA and UK with higher levels for USA whereas the trends for Australia and Canada appear also similar to those observed in the both countries. Taken together, these results would favour the use of apparent consumption of PUFAs to get a better estimate of LA intake rather than the use of food composition tables.

A more direct and reliable way to estimate LA and LNA consumption is to determine the PUFA content of adipose tissue triglycerides as the latter is known to equilibrate over time with that of ingested fats. Indeed adipose tissue is a suitable biomarker of dietary intake of fatty acids, particularly for *n*–3 and *n*–6 PUFAs, as endogenous fatty acid synthesis from glucose is low (<5%) [122,123]. As shown in Table 1, content of LA in adipose tissue of US people during the last decades had a similar trend to that previously noted in human milk [124–132]. It increased from 9% to about 16% of total fatty acids between the 1960s and the 1980s. It then plateaued around this value whereas the adipose LNA content remained under 1%. During the same period of time, a similar trend was observed in adipose tissue of European people but with a range of time-changes less marked than in USA [120,133–150]. Indeed, the LA content increased from 7.5% to only 12% thus maintaining a lower LA:LNA ratio (Europe < 15 < USA).

Table 2 shows the mean contents of LA and LNA reported throughout the last 25 years in the mature milk lipids of women living on unrestricted diets living in USA, Canada, Australia and Europe (France, Germany, Italy, Netherlands, Nordic countries, Spain, and UK). These countries were selected because sufficient data are available, regarding their evolution during the last decades. Solely the data on mature milk (i.e. at least 1 month of lactation) published since 1980 were taken into account to calculate the mean contents as the

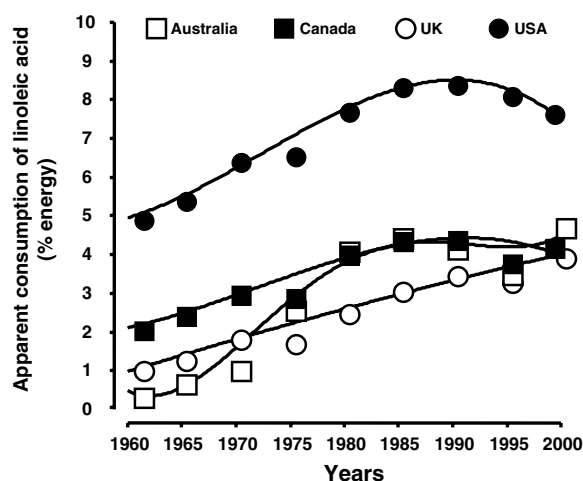


Fig. 5. Apparent consumption of linoleic acid (% of dietary energy) among Australia, Canada, UK and USA for the years 1961–2000 (adapted from Ref. [121]). The best regressions were 2-order polynomial fits: Australia $y = -0.0036x^2 + 14.2x - 14198$, $r = 0.94$, $n = 9$, $P < 0.01$; Canada $y = -0.0022x^2 + 8.6x - 8581$, $n = 9$, $r = 0.94$, $P < 0.01$; UK $y = -0.00025x^2 + 1.08x - 1146$, $r = 0.98$, $n = 9$, $P < 0.01$; USA $y = -0.0038x^2 + 15.2x - 15118$, $n = 9$, $r = 0.82$, $P < 0.01$.

Table 1

Median values (\pm SD) and ranges of essential PUFA precursors (% of total fatty acids) in adipose tissue from US and European adults during the last decades^a

	Linoleic acid (LA, 18:2n-6)	α -Linolenic acid (LNA, 18:3n-3)	LA/LNA ratio
<i>USA</i>			
• 1960–1970 (n = 10)	9.5 \pm 1.4 (7.5–11.4)	–	–
• 1980–1990 (n = 6)	15.6 \pm 2.3 (12.9–17.6)	0.92 \pm 0.50 (0.60–1.91)	17.0 \pm 5.8 (8.5–25.3)
• 1990–2005 (n = 3)	15.9 \pm 2.3 (14.4–18.5)	0.70 \pm 0.10 (0.62–0.70)	21.7 \pm 1.2 (21.0–23.2)
<i>European countries</i>			
• 1960–1970 (n = 2)	7.5	–	–
• 1980–1990 (n = 7)	11.5 \pm 2.7 (8.3–15.0)	1.68 \pm 1.16 (0.70–3.0)	6.8 \pm 5.1 (3.3–14.6)
• 1990–2005 (n = 9)	12.3 \pm 2.0 (9.2–14.4)	0.91 \pm 0.46 (0.4–1.7)	13.6 \pm 11.6 (6.4–35.8)

n refers to the number of studies.

^a Adipose tissue of both women and men were considered because no effect of sex on PUFA content was noted. Adapted from Refs. [120,124–150].

LA content varies during the maturation of milk (lower content in colostrum and transitional milk) [67–69], and as reliable values have been obtained after the eighties [59], i.e. at a time when the use of capillary GLC columns allowed clear-cut separation of PUFAs [67]. The mean values of LA and LNA content of breast milk show important differences between 1990 and 2005 (Table 2) [70–84,102–104,110–112,151–222]. Although a great variation of PUFA precursor contents is noted in the breast milk of European women, it should be stated that LA in mature breast milk of US women is higher than those of European, Canadian and Australian women.

The milk of Scandinavian and Italian mothers (respectively, 9.7% and 10.3% of LA of the total fatty acids) exhibits the lowest LA contents which actually would correspond to adequate intake levels for the suckling infant (10%) [5]. The milk of Canadian, Australian, Spanish, French and Dutch mothers shows intermediate levels (from 12.8% to 13.8%) and that of American women the highest level (15.5%, i.e. 1.5-fold higher than the recommended level). These differences result from wide differences in the range values, which are distributed between 7.5% and 10.5% in Scandinavian countries and between 12.7% and 20.2% in USA, a value close to the upper level (22%) recommended by several committees of nutrition. These average values mask individual variations which are often more substantial within the same group of women from the same country than mean differences between countries. We have followed a group of French women during the course of lactation and found that \sim 20% of them had an imbalance of LA content in their milk, reaching the upper limit (16–22%), whereas the mean LA content was around 13% of total fatty acids [192,222]. High LA values were also found in Hungary [223,224], Chile [225] and Japan [226,227], with the highest LA concentrations (18.4% and 22.7%) being found in the milk of Chinese women [228,229].

It must be emphasized that the distribution of LNA content among North American and European countries does not follow that of LA. The milk from Italian, Spanish and French mothers is relatively poor in LNA (0.58–0.62%), whereas that of Scandinavian and Canadian mothers exhibits a 2-fold higher content (1.4%). Therefore, the LA/LNA ratio varies from 7.0 (Nordic countries) to 8.9 (Canada) and 13–14 (Netherlands, UK, USA) and reaches a high value of 22 in France and Spain. These differences in breast milk PUFA content are likely to be ascribable to food practices and particularly to the consumption of margarines and edible oils which generally contribute up to 40% of the PUFA total intake [230,231]. Indeed, soybean and corn oils are the edible oils mainly consumed in the USA, while canola oil is consumed in Canada and sunflower oil in France. Differences between these countries are also found again for the 1980–1990 period, suggesting that food practices with respect to fat and PUFA intake were already present at the beginning of the eighties. During the 1980–1990 and 1990–2005 periods, the milk LNA content has increased by 1.8-fold in Canada and slightly increased in UK whereas it has decreased in Spain.

Altogether, keeping in mind methodological pitfalls, despite the fact that a truly Western diet does not exist with respect to PUFA intake and despite the variability and time-trends in the PUFA content of breast milk as a result of dietary intakes, a significant time-increase of LA content has been systematically reported in breast milk of women living in major industrialized countries of the Western world whereas the LNA content has not

Table 2

Median values (\pm SD) and ranges of essential PUFA precursors (% of total fatty acids) in mature milk from North American, Australian and European women during the last 25 years^a

	Linoleic acid (LA, 18:2n-6)	α -Linolenic acid (LNA, 18:3n-3)	LA/LNA ratio
<i>Australia</i>			
• 1980–1989	10.8	0.59	18.3
• 1990–2005 (n = 6)	12.8 \pm 2.3 (8.5–15.2)	0.87 \pm 0.11 (0.66–0.95)	14.7 \pm 1.9 (12.9–18.1)
North America			
<i>USA</i>			
• 1980–1990 (n = 12)	15.3 \pm 1.4 (12.9–17.6)	1.26 \pm 0.56 (0.40–2.40)	12.2 \pm 10.4 (7.0–44.0)
• 1990–2005 (n = 16)	15.5 \pm 1.9 (12.7–20.2)	1.08 \pm 0.27 (0.28–1.50)	14.3 \pm 11.3 (8.9–58.2)
<i>Canada</i>			
• 1980–1990 (n = 4)	11.3 \pm 1.8 (8.9–13.0)	0.78 \pm 0.28 (0.50–1.10)	14.6 \pm 6.9 (10.3–26.0)
• 1990–2005 (n = 6)	12.8 \pm 1.6 (10.5–14.2)	1.44 \pm 0.27 (1.16–1.90)	8.9 \pm 1.7 (7.5–12.2)
Europe			
• 1980–1990 (n = 14)	12.4 \pm 2.8 (6.6–16.4)	0.92 \pm 0.33 (0.40–1.40)	13.5 \pm 4.5 (9.2–22.2)
• 1990–2005 (n = 44)	12.0 \pm 2.2 (7.5–16.5)	0.88 \pm 0.38 (0.35–2.04)	16.2 \pm 7.2 (4.5–33.3)
<i>France</i>			
• 1980–1990	13.2	0.7	18.9
• 1990–2005 (n = 6)	13.8 \pm 1.5 (11.8–15.3)	0.62 \pm 0.12 (0.46–0.82)	22.1 \pm 5.3 (18.4–33.3)
<i>Germany</i>			
• 1980–1990 (n = 2)	10.4 (10.0–10.8)	0.82 (0.81–0.83)	12.7 (12.0–13.5)
• 1990–2005 (n = 7)	11.3 \pm 2.4 (8.7–16.5)	0.83 \pm 0.12 (0.57–0.90)	13.6 \pm 2.5 (12.1–18.7)
<i>Italy</i>			
• 1980–1990	–	–	–
• 1990–2005 (n = 6)	10.3 \pm 1.2 (9.6–12.7)	0.58 \pm 0.21 (0.35–0.83)	17.7 \pm 7.7 (12.1–31.2)
<i>Netherlands</i>			
• 1980–1990	–	–	–
• 1990–2005 (n = 5)	13.8 \pm 1.4 (11.8–15.2)	1.03 \pm 0.06 (0.96–1.11)	13.4 \pm 1.3 (12.3–14.9)
<i>Nordic countries</i>			
• 1980–1990 (n = 2)	12.5 (12.0–12.9)	1.4	9.2
• 1990–2005 (n = 9)	10.0 \pm 1.4 (7.5–10.5)	1.40 \pm 0.36 (0.90–2.04)	7.3 \pm 2.6 (4.5–13.1)
<i>Spain</i>			
• 1980–1990 (n = 4)	15.4 \pm 0.9 (14.5–16.4)	1.08 \pm 0.24 (0.80–1.30)	14.3 \pm 4.2 (11.6–20.0)
• 1990–2005 (n = 8)	13.6 \pm 1.8 (11.7–15.9)	0.62 \pm 0.15 (0.47–0.79)	21.8 \pm 6.3 (14.8–32.5)
<i>United Kingdom</i>			
• 1980–1990 (n = 3)	9.6 \pm 2.6 (6.6–11.2)	0.70 \pm 0.44 (0.40–1.20)	13.7 \pm 6.5 (9.3–22.2)
• 1990–2005 (n = 3)	11.7 \pm 0.7 (10.9–12.3)	0.91 \pm 0.49 (0.49–1.45)	12.8 \pm 6.9 (8.5–22.2)

n refers to the number of studies.

^a Human milks were considered mature at 1 month of lactation. Nordic countries for which several data were available were Denmark, Finland, Norway and Sweden. Adapted from Guesnet (unpublished results) and from Refs. [70–84,102–104,110–112,151–222].

changed in a parallel way, leading to a continuous increase in the LA/LNA ratio. This increase started as early as the fifties in the USA and in the sixties for the other countries. Moreover, the LA content in mature breast milk of US women seems to have plateaued as early as the 1980s but is still currently at a higher level than that of European women (Table 2).

4.2.2. Status of long-chain polyunsaturated fatty acids

The amount of long-chain PUFA in breast milk will depend both on exogenous supply and endogenous adipose stores, and also on the biosynthesis rate from their respective precursors. However, biosynthesis occurs to a low extent for the n-6 fatty acids (3% of LA ingested is found as milk ARA) [114] and probably at a rate even lower for n-3 fatty acids. Moreover, the content in milk long-chain PUFAs of the two

series is not correlated to that of their precursors LA and LNA [165,192,232], and supplementing the diet of lactating women with LNA did not increase the DHA content in their milk [83]. Therefore ARA and DHA content of breast milk are more closely related on a long-term basis to their dietary intake rather than to LA and LNA intakes. The ARA and DHA contents in the milk of Western women currently noted throughout the last 25 years are given in Table 3 [70–72,74–77,80–84,102–104,111–113,151–158,161,163–165,167,170–180,182,183,185–202,204–206,208,210,212–215,217–221,233]. Contrasting with the great variations of LA, a relative stability of ARA is observed among countries (range of values: 0.40–0.66%). On the other hand, a wide range in DHA content is found for the 1990–2005 period, probably reflecting different levels of sea food consumption which is the main dietary supply in long-chain *n*–3 fatty acids

Table 3
Median values (\pm SD) and ranges of arachidonic and docosahexaenoic acids (% of total fatty acids) in mature milk (>1 month of lactation) from North American, Australian and European women during the last 25 years^a

	Arachidonic acid (ARA, 20:4 <i>n</i> –6)	Docosahexaenoic acid (DHA, 22:6 <i>n</i> –3)	ARA/DHA ratio
Australia			
• 1980–1989	0.4	0.32	1.25
• 1990–2005 (<i>n</i> = 6)	0.40 \pm 0.03 (0.37–0.45)	0.22 \pm 0.04 (0.18–0.26)	1.84 \pm 0.24 (1.46–2.11)
North America			
<i>USA</i>			
• 1980–1990 (<i>n</i> = 10)	0.46 \pm 0.17 (0.10–0.69)	0.18 \pm 0.09 (0.06–0.30)	2.38 \pm 1.49 (1.33–6.00)
• 1990–2005 (<i>n</i> = 13)	0.50 \pm 0.11 (0.24–0.67)	0.19 \pm 0.07 (0.09–0.37)	2.61 \pm 0.96 (1.81–4.89)
<i>Canada</i>			
• 1980–1990 (<i>n</i> = 4)	0.53 \pm 0.05 (0.50–0.60)	0.37 \pm 0.05 (0.30–0.40)	1.44 \pm 0.18 (1.25–1.67)
• 1990–2005 (<i>n</i> = 6)	0.44 \pm 0.07 (0.35–0.50)	0.22 \pm 0.06 (0.14–0.30)	1.98 \pm 0.50 (1.33–2.50)
Europe			
• 1980–1990 (<i>n</i> = 11)	0.47 \pm 0.16 (0.30–0.80)	0.28 \pm 0.11 (0.10–0.40)	1.69 \pm 0.61 (1.33–3.00)
• 1990–2005 (<i>n</i> = 39)	0.47 \pm 0.20 (0.15–1.50)	0.31 \pm 0.14 (0.10–0.97)	1.95 \pm 2.20 (0.55–15.0)
<i>France</i>			
• 1980–1990	0.45	0.25	1.80
• 1990–2005 (<i>n</i> = 6)	0.42 \pm 0.10 (0.24–0.52)	0.30 \pm 0.10 (0.14–0.41)	1.38 \pm 0.26 (1.02–1.71)
<i>Germany</i>			
• 1980–1990 (<i>n</i> = 2)	0.38 (0.36–0.39)	0.19 (0.16–0.22)	1.97 (1.64–2.44)
• 1990–2005 (<i>n</i> = 7)	0.49 \pm 0.14 (0.36–0.77)	0.28 \pm 0.13 (0.16–0.55)	1.74 \pm 0.38 (1.40–2.56)
<i>Italy</i>			
• 1980–1990	–	–	–
• 1990–2005 (<i>n</i> = 6)	0.50 \pm 0.20 (0.15–0.70)	0.23 \pm 0.07 (0.16–0.30)	2.20 \pm 1.06 (0.83–4.12)
<i>Netherlands</i>			
• 1980–1990	–	–	–
• 1990–2005 (<i>n</i> = 5)	0.41 \pm 0.07 (0.34–0.50)	0.29 \pm 0.09 (0.19–0.40)	1.43 \pm 0.32 (1.09–1.79)
<i>Nordic countries</i>			
• 1980–1990 (<i>n</i> = 1)	0.4	0.3	1.33
• 1990–2005 (<i>n</i> = 6)	0.54 \pm 0.32 (0.30–1.50)	0.32 \pm 0.13 (0.10–0.49)	1.68 \pm 1.18 (1.00–15)
<i>Spain</i>			
• 1980–1990 (<i>n</i> = 3)	0.66 \pm 0.13 (0.57–0.80)	0.37 \pm 0.06 (0.30–0.40)	1.79 \pm 0.26 (1.50–2.00)
• 1990–2005 (<i>n</i> = 6)	0.47 \pm 0.15 (0.21–0.69)	0.42 \pm 0.28 (0.18–0.97)	1.12 \pm 0.57 (0.55–2.28)
<i>United Kingdom</i>			
• 1980–1990 (<i>n</i> = 2)	0.33 (0.30–0.35)	0.24 (0.10–0.37)	1.98 (0.95–3.00)
• 1990–2005 (<i>n</i> = 3)	0.42 \pm 0.07 (0.36–0.50)	0.36 \pm 0.05 (0.30–0.40)	1.18 \pm 0.09 (1.08–1.25)

n refers to the number of studies.

^a See abbreviations Table 2. Adapted from Guesnet (unpublished results) and from Refs. [70–72,74–77,80–84,102–104,111–113,151–158,161,163–165,167,170–180,182,183,185–202,204–206,208,210,212–215,217–221,233].

[230]. In Australia, USA and Canada, the mean concentration of DHA in breast milk is 0.2% (range: 0.19–0.22%). A complete food consumption survey conducted in Canadian pregnant women showed that 84% women consumed less than 150 mg/day, i.e. half the adequate level recommended during late pregnancy (300 mg DHA/day) [230]. In Canada, and possibly in Australia, the milk DHA content has decreased by 30–40% during the past 15 years. Breast milk DHA content is reaching 0.3% in France, Germany, the Netherlands, and in Nordic countries (range: 0.28–0.30) and up to 0.4% in Spain and UK (range: 0.36–0.42%), and these values have remained constant during the past 25 years. It is important to note that the different DHA levels among countries lead to large differences in the ratio of ARA to DHA, which ranges from 1.1 to 1.4 (Spain, UK, Netherlands, France) up to 2.0–2.6 (Canada, USA). Regarding these data, two important issues remain unsettled with respect to adipose tissue development in animals and humans. First, considering the antithetic role of ARA and DHA in adipogenesis (see Section 3), how important are the ARA and DHA levels of adipose tissue in favouring the development of this organ and, second, how important is their rate of biosynthesis as well as their supply from exogenous sources and endogenous fat stores.

4.3. Lactation period and PUFA composition of infant formulas

The LA content of infant formulas has also widely varied during the last decades (Table 4). In the early 20th century, fats in infant formulas were composed of a mixture of cow's milk fat and butterfat and, thus, contained only 0.8–1.0% of LA. Unsaturated vegetable oils were added to improve the low absorption of butterfat [90]. The development of edible oils rich in oleic acid, such as high-oleic safflower oil and olein-fractions of animal and vegetable oils, gave the opportunity to mimic the human milk fatty acid profile, especially regarding the level of monounsaturated fatty acids (35%). Between 1980 and 1995, several infant formulas differing in their content of LA and LNA were manufactured and marketed in Western countries (Table 4) [68,73,76,80,82,111,151–159,234–251]. At that time, several committees of nutrition and pediatrics did not acknowledge the essentiality of the *n*-3 PUFAs and many infant formulas were particularly poor in LNA. For instance, some formulas provided LA within the range of 9–22%, either with low amounts of LNA (cow milk fats and corn oil) or sometimes with sufficient amount of LNA (blends of soybean and rapeseed oils). Other manufacturers used corn oil as the sole source of fats, hence with a LA content of more than 50% of total fatty acids, leading to a very high LA/LNA ratio. Since 1995, formula manufacturers have limited the LA content and have changed that of LNA in order to match the current ratio found in human milk. At the present time, infant formulas contain LNA from rapeseed and soybean oils, with a LA/LNA ratio ranging between 5 and 15. However the LA content in formulas is still high and greatly variable, ranging between 10% and 30%, i.e. between the adequate level and the highest level reported in breast milks in Western countries (Table 5) [252–255].

Currently, in infant formulas, the fat content is within the ranges presently found in human milk. The minimum fat content recommended is 4.4 g/100 kcal or 2.8 g/dL and the maximal content is fixed at 6.0 g/100 kcal or 4.5 g/dL [85,256]. Generally, most infant formulas (starter and “follow on”) provide between 3.0 and 3.9 g/dL.

Table 4

Range of linoleic acid (LA) levels, range of LA to α -linolenic (LNA) ratios and fat sources used in term infant formulas marketed in Western countries during the last two decades^a

	Linoleic acid	LA/LNA ratio	Fat sources
Infant formulas marketed between 1980 and 1995	9.0–22.0 (up to 57.0)	6.3–61.0 (up to 120)	Cow milk fat, corn; cow milk fat, soybean, rapeseed, coconut Corn oil
Main infant formulas marketed after 1995	8.9–26.0	8.5–21.7	High-oleic safflower or sunflower, coconut, palm, soybean, rapeseed

^a LA content is expressed as % of total fatty acids. Total lipid content was in the range of 2.5–3.6 g/100 mL of prepared milk. Adapted from Refs. [68,73,76,80,82,111,151–159,234–251] and from Guesnet (unpublished results).

Table 5

Range of the linoleic acid (LA) levels, range of LA to α -linolenic (LNA) ratios and fat sources used in term infant formulas marketed in 2005^a

	Linoleic acid	LA/LNA ratio	Fat sources
<i>Starter and follow-up infant formulas</i>			
Milupa	10.1–15.9 (0.37–0.54)	5:1–10.8:1	Palm, coconut, rapeseed, sunflower, high-oleic palm
Nutricia	10.0–13.0 (0.32–0.39)	5.3:1–5.6:1	Palm, coconut, rapeseed, sunflower
Wyeth SMA	16.1–17.8 (0.47–0.58)	10.5:1–10.7:1	Palm, coconut, high-oleic safflower or sunflower, soybean
Nestlé	13.7–18.5 (0.43–0.67)	7.8:1–10.8:1	Palm, palm olein, rapeseed, corn, sunflower, safflower, soybean
Abbott Ross	14.9–29.3 (0.55–1.055)	7.7:1–18.3:1	Palm olein, coconut, high oleic safflower or sunflower, soybean
Mead Johnson	14.6–17.5 (0.51–0.61)	10.0:1	Palm olein, coconut, high-oleic sunflower, soybean
Blédina SA	15.2–18.0 (0.50–0.63)	9.0:10.5	Palm, rapeseed, coconut, sunflower

^a LA content is expressed as % of total fatty acids (g/100 mL). Total lipid contents were in the ranges 2.5–3.6 g/100 mL prepared milk. Abbott Ross, Nestlé, Nutricia, Mead Johnson, and Milupa have developed formulas enriched in long-chain PUFAs (ARA, 0.10–0.55% of total fatty acids; DHA, 0.2–0.4%). Adapted from Refs. [252–255].

4.4. Recommended dietary intakes for $n-6$ and $n-3$ fatty acids for infants and adults

During the perinatal life, PUFAs ensure normal growth and skin physiology, as well as adequate development of brain and visual functions. However, important clinical data (based on physiological and biochemical parameters) are still lacking in order to determine the minimal recommended intake for healthy individuals and the only studies conducted have been carried out with $n-6$ PUFA alone i.e. deficient in $n-3$ PUFA (4).

4.4.1. Newborn infants and composition of infant formulas

Clinical studies suggested that the minimal LA intake that avoids the appearance of biochemical and physiological symptoms of PUFA deficiency in the newborn infant is 1–1.7% energy [100,257]. Minimal LA intakes of 2.7–4.5%, corresponding to the lower LA content found in breast milk in Western countries, are recommended (Table 6) [5,85–88,256,258–261]. To avoid the inhibition of $n-3$ long-chain PUFA synthesis and excessive $n-6$ long-chain PUFA and eicosanoid synthesis, the maximum intake of LA should not exceed 12%, this value corresponding to the higher content found in human breast milk. However, an intake of 15% is considered as safe as there is no clinical data demonstrating that this level is a risk [256,262]. For an optimal neural development (vision), recommended intake of LNA is 0.4–0.75% of energy, with a ratio value of LA to LNA comprised between 5 and 10. An upper limit of 1.5% LNA is also advocated, although adverse effects beyond this level have not been so far reported. The main long-chain derivatives, DHA and ARA, are considered as being conditionally essential in newborns to support growth, brain development and maturation of visual acuity [4,263]. This is of particular importance when dealing with milk replacers containing only the precursor fatty acids (LA and LNA), because DHA and ARA are not incorporated in the tissue phospholipids of bottle-fed infants to levels similar to those fed breast milk [264–266]. The introduction of DHA in the formula entirely corrects these differences in DHA levels. Moreover, randomized studies have shown that this supplementation may favour the maturation of visual activity [267]. However, it raises the question of the optimal balance between PUFA of the $n-6$ and $n-3$ series, which can be addressed by co-supplementing with ARA, in order to match the balance found in breast milk in Western countries (ARA/DHA ratio of 1.1–1.3, with ARA and DHA representing between 0.1% and 0.5% of energy) (Table 6).

The recommended PUFA content could also be expressed as % of total fatty acids for an easier comparison between manufacturers for the fatty acid composition of infant formulas marketed in Western countries (Table 7) [5,85–88,256,258–260,262]. A minimal LA content of 6–8% of total fatty acids is recommended because it is the minimum concentration found in human milk and 10% represent an adequate intake. Of note, an upper value of 35% has been recommended by an American expert panel, considering that corn oil-based infant formulas have been consumed without reported manifest adverse effects *although childhood overweight and obesity have never been so far taken into consideration* [262]. Of note also, the latter infant formulas were poor in $n-3$ PUFA and therefore were inducing a poorer sensitivity of the photoreceptor cells and visual acuity in low birth-weight premature infants [268].

Table 6

Recommended intakes of polyunsaturated fatty acids (PUFA) to cover requirements for newborn term infant and adult man

	Ref.	Linoleic acid	α -linolenic acid	Long-chain (LC) PUFA Docosahexaenoic acid, DHA Arachidonic acid, ARA
Newborn infant in % energy (Min-Max)				
• FDA 1980	[258]	>2.7	–	–
• CNR 1990	[259]	> 3.3	> 0.5	–
• International Society ESPGAN 1991	[85]	4.5–10.8	(0.5–1.5)	DHA: 0.15–0.5; ARA: 0.2–0.5
• United Kingdom BNF, 1992	[86]	4.0 (3.0–12.0)	0.4	DHA: 0.2; ARA: 0.2
• EEC, 1991 1996	[87,88]	2.7–10.8	0.45	DHA: 0.15–0.4; ARA: 0.1–0.25
• France AFSSA 2001	[260]	2.0–4.5	(0.45–1.5)	DHA: 0.1–0.4; ARA: 0.1–0.4
• International Society ISSFAL, 1999–2004 ^c	[5,261]	5.0 (–12)	0.75	DHA: 0.17; ARA: 0.25
• LSRO-ASNS 1998	[256]	3.3–21	(0.7–2.3)	–
Adult man in g/day (% energy)^a				
• Australia NHMRC and NHF, 1992–2001 ^b	[271,272]	12.0 (6.0%)	2.0 (1.0)	–
• United Kingdom BNF, 1992	[86]	12.0 (6.0%)	2.0 (1.0)	–
• Japan, 1996	[271]	10.0–12.0 (5.0–6.0%)	2.0 (1.0)	–
• France AFSSA, 2001	[271]	10.0 (4.0%)	2.0 (0.8)	DHA 0.12 (0.05)
• Germany, USA, Canada, 2002	[234]	14.0–15.0 (7.0%)	1.35 (0.68)	DHA 0.135 (0.07)
• International Society ISSFAL, 1999–2004 ^c	[5,261]	4.44 (2.0%)	1.7–2.4 (0.7–1.0)	DHA 0.22 (0.1)
Safe ranges				
Lower limit in g/day (% energy)				
• United Kingdom BNF, 1992 1999	[86,271]	2.7–8.0 (1–3%)	(0.5)	–
Upper limit in g/day (energy)				
• United Kingdom BNF, 1992 ^d	[86]	26.0 (10%)	6.0 (2.5)	All <i>n</i> –3 LC PUFA 4.0 (2.0)
• International Society ISSFAL, 1999–2004 ^c	[5,261]	6.67 (3%)	–	–
• France AFSSA, 2003	[276]	–	–	All <i>n</i> –3 LC PUFA 2.0 (1.0)

^a For an adult man, the recommendations were based on a 2200–2500 kcal diet. To calculate the PUFA requirements of adult, pregnant and lactating woman, the energy intake to take into account were 1800, 2050 and 2250, respectively [273]. BNF, British Nutrition Foundation; CNR, Canadian Nutrition Recommendations; ISSFAL, International Society for the Study of Fatty Acids and Lipids; EEC, European Economic Community; ESPGAN, European Society of Pediatrics Gastroenterology and Nutrition; FDA, Food and Drug Administration; LSRO – ASNS, Life Sciences Research Office – American Society for Nutritional Sciences; NHF, National Heart Foundation of Australia; NHMRC, National Health and Medical Research Council. For newborn infant until 6 mo-old, the energy intake varied from 360 to 650 kcal per day and infant formulas should contain between 3.3 and 6.5 g/100 kcal.

^b The recommendations were for total *n*–6 PUFA.

^c Values are only recommendations for adequate intakes, considered as the average intake for a group of healthy adult and newborn.

^d The upper safe limit proposed by the British Nutrition Foundation's Task Force in 1992 is suggested by the adverse effect of high LA intake (>10% of energy) on HDL-cholesterol and gallstones formation in man [86].

Table 7

Recommended intake of polyunsaturated fatty acids (PUFA) for term infant formulas in Western countries (expressed as % of total fatty acids)

Fatty acids	Minimum intake ^a	Adequate intake ^b	Upper limits ^c
Linoleic acid (LA, 18:2 <i>n</i> –6)	7.0–8.0 (0.25 mg/dL)	10.0 (0.35 g/dL)	22.0 (35.0 ^d) (0.77–1.20 g/dL)
α -Linolenic acid (LNA, 18:3 <i>n</i> –3)	1.0 (0.035 g/dL)	1.50 (0.05 g/dL)	3.0 (0.1 g/dL)
18:2 <i>n</i> –6:18:3 <i>n</i> –3 ratio	–	5	15
Very long-chain PUFA			
Docosahexaenoic acid (DHA, 22:6 <i>n</i> –3)	–	0.35	1.0
Arachidonic acid (ARA, 20:4 <i>n</i> –6)	–	0.50	1.0

See abbreviations in Table 6. In infant formulas, the minimum fat content recommended is 4.4 g/100 kcal or 2.8 g/dL and the maximum content is fixed at 6.0 g/100 kcal or 4.5 g/dL [85,256]. The average content of 3.5 g/dL was applied to calculate the concentrations of PUFA in g/dL.

^a FDA 1980 [258], EEC, 1991, 1996 [87,88], France AFSSA 2001 [260], LSRO-ASNS 1998 [256].

^b Simopoulos et al. [5].

^c BNF [86], ESPGAN [85], EEC [87,88].

^d Wharton [262], LSRO-ASNS 1998 [256].

4.4.2. Healthy adults and *n*–6 fatty acids

It has been demonstrated in newborn infants and animal species that a dietary intake of 1–2% energy as LA is necessary to prevent or correct the symptoms which specifically result from a deficiency in *n*–6 PUFA [257,269]. With such intakes, the ratio of mead acid (C20:3 *n*–9) to arachidonic acid in phospholipids remains low, suggesting adequate intake of *n*–6 PUFA. However, whether the plateau value of arachidonic acid incorporation in all tissue phospholipids is considered as optimal, the *n*–6 PUFA intake should be 3- to 4-times higher [270]. It is of note that the recommended levels have largely varied according to different committees and, the values for LA in healthy individuals of Western populations range within 4–7% of dietary energy, corresponding to a daily intake of 10–15 g in the adult man (Table 6) [5,86,271–273]. Lower safe limits (1–3% of total energy) and upper safe limits (10%) have also been proposed. Upper safe limits mainly consider the possible adverse effects of high LA intakes in enhancing lipid peroxidation, reduction of *n*–3 long-chain fatty acid bioconversion and alterations of cholesterol metabolism (reduction of HDL-cholesterol and formation of gallstones) [86,274].

International workshops on the dietary intakes for PUFA have recently suggested a reduction in LA and ARA intakes. The purpose of these limitations is to avoid possible adverse effects due to accretion of ARA and excessive production of its oxygenated derivatives (eicosanoids) which would then compete with those of the *n*–3 series. Although no precise recommendation has been established for ARA [5,261], the recommended dietary intake of LA for a healthy adult man is 2–3% of energy, i.e. 4.4 to 6.7 g/day, which is considered as being the amount needed to maintain the normal nutritional state throughout the adult life (“adequate intake”) (Table 6). An upper limit of LA intake of 3–4% has also been suggested though experimental data remain necessary to determine the physiological threshold [4]. For adults, pregnant and lactating women, the recommendations are established on the basis of their respective energy intake, i.e. 1800, 2050 and 2250 kcal, respectively [273].

4.4.3. Healthy adults and *n*–3 fatty acids

n–3 PUFA are essential nutrients owing to the crucial role of its metabolic end-product DHA (Fig. 2), especially in regard to the maturation of visual and brain functions [264]. Official recommendations have been given to cover the requirements of total *n*–3 PUFAs and DHA in adults (Table 6). The recommended daily intakes for LNA range from 0.68% to 1% of energy intake, corresponding to 1.35–2.4 g/day for an adult man. A minimal requirement (0.5–0.7%) and an upper limit (2.5%) have been also proposed [86,261]. Moreover, the ratio of LA to LNA in the diet should range between 10/1 and 5/1. Clinical studies using high intakes of LNA or stable isotope tracers have demonstrated that the endogenous conversion of LNA to DHA is strongly limited and insufficient to cover the DHA requirement of tissue phospholipids per se [275,276]. Therefore, specific intakes of DHA are recommended for adults, essentially based on the amounts found in Western diets (0.05–0.10% energy) (Table 6). These recommendations are crucial for pregnant and lactating women in terms of neonatal benefits to meet the *n*–3 requirements of the fetus and newborn infant (0.3% of energy, i.e. 300 mg DHA/day).

4.5. Concluding remarks

Although breast feeding 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 higher energy intake of formula-fed infants [277]. However, and unfortunately, the fatty acid composition of fats in breast milk and infant formulas has never been taken into consideration with respect to adipose tissue development despite the results of animal studies.

Interestingly, differences are observed between school-age American and European children in regard to the prevalence of overweight (approximately 32% versus 19%) and obesity (approximately 7.5% versus 4%). It is of note that, comparisons of energy intake and prevalence of overweight and obesity in the late eighties 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 (most likely of the *n*–6 series) are 1.5-fold higher in American than in French infants, also favouring a role of *n*–6 PUFAs in promoting excessive adipose tissue development (M.F. Rolland-Cachera, personal communication, INSERM Unit 557, Paris, France).

The US food industry has recently advertised the use for *term* infants of formulas supplemented with DHA and ARA intended for *preterm* infants despite the fact that (i) no clear-cut benefit to brain and eye development has been demonstrated with this fatty acid supplementation in normal term infants [80,278] and (ii) 5 day-old piglets supplemented for 2 weeks with ARA exhibit a 27% increase in body weight with no change in body length [279].

Compared to well-controlled animal studies, characterization of the pre- and early determinants of the child development and health is ongoing but represents a serious challenge. In particular, it may not bring any clue regarding the importance of $n-6$ versus that of $n-3$ PUFAs as we have to face the paucity of data on these fatty acids in food composition tables. Nevertheless, changes over time in PUFA content, if any, can be traced in the food chain and displayed in parallel to the increasing prevalence of overweight and obesity in the last decades, as discussed below.

5. Lipids in the food chain

5.1. General considerations

In the last half century, physiological and biochemical effects of polyunsaturated fatty acids have been thoroughly investigated and are now better understood. As discussed above (see Section 3), changes in food practices are revealed by changes in the fatty acid (FA) composition of breast milk and adipose tissue lipids. Not surprisingly, various factors have changed over time, i.e. animal feed and food processing, leading to significant changes in the fatty acid composition of ingested fats. However, although high levels of $n-6$ PUFAs have been questioned in regard to the development of atherosclerosis [280,281], asthma [282], inflammation and immunity [283,284] their relationships with adipose tissue development have never been considered.

Therefore, a fair reappraisal of fatty acid intake for any given population should take into account changes in the use of plant-derived lipids as well as changes in the lipid composition of animal feed, which in turn have led to changes in the fatty acid composition of the most consumed foodstuffs (meat, eggs. . .). Considering the wealth of available data, in order to estimate these changes with minimal uncertainties and thus to assess their importance, we have purposely focused on deciphering the *evolution of fat consumption in France from 1960 to 2000*, at a time where an epidemic of overweight and obesity in children and adults was already taking place in parallel to that observed in other parts of the world.

In France and other countries, the lack of consistency and agreement between different studies as well as the lack of data before 1980 make comparisons difficult to establish. Nevertheless, the prevalence of overweight among French pre-school children (9–10 years; BMI between 20 and 25), assumed to be ~3% in 1965, increased from 4.7% in 1980 to 10.8% in 1996 whereas that of obese children (BMI > 25) increased in parallel from 0.4% to 1.9%, i.e. a 5-fold increase.

Any attempt to evaluate the consumption of fatty acids raises important methodological problems. Both total lipid consumption and the fatty acid composition of the various ingested lipids have to be estimated. Production data obtained from professional organizations (producers of oil, meat, milk, etc.) have to be cross-examined with consumer data obtained from food questionnaires which are used in cohort studies and often lack reliability [285,286]. As a few examples, the definition of each category product in consumption surveys remains somewhat loose, i.e. by pooling foodstuffs with rather different lipid content such as “cheese” and “cold cuts (cold cooked meats)”. Within the “meat” category is pooled the lipid composition of different cuts from different animal species which may differ in their fatty acid composition. Moreover, the fatty acid composition of fats from animal or plant origin used for cookies, biscuits and ready-made dishes, is not described accurately and thus difficult to assess. Owing to these obvious difficulties, we have deliberately favoured production data after correction first for the corresponding exported and imported amounts, and second for the overestimated consumption as some lipids are not consumed in toto (frying and cooking oils, left-over fat from meat and cold cuts) [285,287]. Another important reason to favour production data in lieu of consumer data is that, during that 1960–2000 period, the part devoted to raw foodstuffs has decreased whereas that of ready-to-use products of the food industry has significantly increased, the fatty acid composition of which is unfortunately ill-defined [285,288].

We have restricted our discussion to the main agreed upon changing trends in human consumption, then those in animal feed. To sum-up, our aim has been to assess for the 1960–2000 period the changes which have occurred with respect to the quality of consumed fatty acids by the French population. Indeed, we assume that the results obtained are representative of most, if not all, of the Western countries and may explain, at least in part, the similar trends already observed in urbanized parts of fast-growing heavily populated countries such as China and Brazil.

Lipid consumption in France includes animal lipids (predominantly in the Northern part of the country [285]) and plant lipids. Lipids from terrestrial animals represent more than 60% of lipid consumption in the 1960–2000 period [285,288]. In addition to saturated and monounsaturated fatty acids, they represent a significant supply of polyunsaturated fatty acids. In fact, animal fats are the major if not only source of long-chain PUFAs as $\Delta 6$ and $\Delta 5$ desaturases are almost exclusive to the animal world (Fig. 2) [289]. Of note, the fatty acid composition of animal fats will depend upon that of animal feed whether the species are mono-gastric [290,291] or polygastric [292,293]. For milks, meats and eggs, the evaluation of FA composition of their lipids in 1960 is based upon the fatty acid composition of lipids of the animal feed which was actually given to domestic animals at that time [290–294].

5.2. Overall consumption changes

The caloric consumption has increased significantly from 1960 to 1980. Both consumption of animal products and plant oils has steadily increased and has reached a plateau in 1980. According to INSEE (Annuaire Statistique de la France), plant oil consumption was 5 kg/year/inhabitant in 1950 and went up to 11 kg in 1985 and 14 kg in 1996 (2.8-fold increase within 46 years). Consumption of meats and meat-derived goods has doubled from 1950 to 1985, and slightly decreased thereafter (–4%) until 1996. Red meat consumption has regularly decreased since 1980 whereas consumption of white meat and fish has at the same time rose steeply [285,288]. Since the sixties, and even more so since the eighties, the relative importance of plant oils in the total lipid intake has been growing.

5.3. Quantitative aspects of lipid consumption

According to our calculations, the total daily fatty acid intake went up from 75 g in 1960 up to 104 g in 2000 whereas the contribution of animal fats went down from 70% to 61% but still remains predominant. Table 8 summarizes the daily average intake of fatty acids per adult.

5.3.1. Plant and derived lipids

The most dramatic increase in triglyceride consumption for the 1960–2000 period has been that of plant oils and margarines (consumed directly or included within processed foods) which increased from 19 g/day to

Table 8
Daily average intake of fatty acid per adult in France

Fatty acid source	1960 (FA: g/day)	2000 (FA: g/day)	Change in g/day (fold increase)
Vegetable oils	19	37	18 (1.9)
Milk	27	33	6 (1.2)
Fish	1	3	2 (3)
Poultry	1	3	2 (3)
Pork	11	12	1 (1.1)
Beef, lamb, veal	6	5	–1 (0.8)
Rabbit, horse, goose	1	0	–1 (0)
Eggs	3	3	0 (1)
Other foodstuffs	6	8	2 (1.3)
Total ^a	75	104	29 (1.4)

^a Adapted from Refs. [285,288,295–299] for the total ingested amounts and from Refs. [290,291,294,296,297,301,303] for the fat content of the different sources.

37 g/day in parallel that of total plant originated fatty acid consumption – that includes triglycerides and structural (complex) lipids from all vegetal foodstuffs – which increased from 22 g in 1960 to 41 g/day in 2000, i.e. a 1.9-fold increase [295].

5.3.2. Milk and derived lipids

A rise has also been observed despite similar butter and milk intake over this 40 year period. The largest part of the increase is accounted by higher consumption of cheese, ultra-fresh products and so-called industrial milk-derived lipids (cookies, ready-cooked meals). Milk-derived lipids represent a fatty acid intake of 33 g in 2000 compared to 27 g/day in 1960, i.e. a 1.2-fold increase [285,288,296].

5.3.3. Meat lipids

Daily consumption of meat and cold cuts has increased 1.6-fold from 1960 to 2000, i.e. from 123 to 203 g [285,288,297,298]. Surprising at first sight, lipid consumption from these foodstuffs has only slightly increased (~17%, from 18 to 21 g/day). This apparent paradox is due to the significant decrease over decades of the fat content of meat and cold cuts. For instance, the mean adiposity of pork carcasses went down from 40% in 1960 to 20% in 2000. This decrease has offset the fact that pig fat (12 g/day) appears as the major lipid contributor, much above lipids from other species (beef, lamb, veal) (5 g/day) which showed no significant range over this 40 year-long period, and well above poultry lipids despite the latter's rise (3 g in 2000 versus 1 g/day in 1960).

5.3.4. Other animal lipids

Egg fatty acids account for a 3.3 g intake/day in 2000 (versus 2.6 g in 1960) [288]. Fish consumption has doubled over that period and this trend continues. However the contribution of fish to fat intake is difficult to assess for various reasons: fish species, type of presentation (fresh, frozen, canned), origin (trawled or farmed). Daily intake is estimated to be 3 g of fish and other seafood lipids/day in 2000 and to have increased approximately 3-fold during the 1960–2000 period due to an increase in fish consumption, and the fat content of consumed fish [285,288].

Regarding meat from other monogastric herbivores, it is almost no longer consumed. From 1960 to 2000, annual consumption went down from 3 kg to less than 0.3 kg for horse meat, 300–80 g for goose meat, and 6 kg to less than 2 kg for rabbit meat [297,299].

5.4. Qualitative trends

5.4.1. Plant lipids

The potent increase of plant lipid consumption has been in France due, in the sixties, to the increased consumption of peanut oil and in the eighties, to that of sunflower oil, [300]. Thus, a shift has taken place from peanut oil containing approximately 31% of PUFAs to sunflower oil containing 65% of PUFAs (almost exclusively of the *n*–6 series for both oils). In 1976, oils containing more than 2% LNA were prohibited in France for cooking, which drastically curtailed rapeseed and soybean oil consumption. As shown in Table 9, sunflower oil, which is very rich in LA, accounts for 31% of oils in 1981 and 50% in 1998. Olive oil consumption, rich in oleic acid and rather poor but balanced in PUFAs of both series, has steadily increased up to 10%. Clearly, among fatty acids from plant lipids, LA remains the largest consumed fatty acid (42%) compared to LNA (1%) and also to the monounsaturated oleic acid (33%) and saturated fatty acids (18%) (Table 10). Strikingly, in 1960, LA represented only 24% of total fatty acids (versus 42% in 2000) consumed from plant

Table 9

Relative contribution of the main plant lipids and margarines consumed in the last 20 years in France (expressed as %)

Year	Olive	Peanut	Sunflower	Soybean	Rapeseed	Coconut	Palm
1981	3	26	31	11	8	8	9
1991	5	11	49	7	13	4	9
1998	10	7	50	2	9	5	9

Table 10

Changes in the fatty acid profile of main plant lipids and margarines consumed in France between 1960 and 2000 (as % of total fatty acids)^a

Year	Palmitic acid (C16:0)	Oleic acid (C18:1)	Linoleic acid (C18:2 <i>n</i> –6)	α -linolenic acid (C18:3 <i>n</i> –3)	<i>n</i> –6/ <i>n</i> –3
1960	12	51	24	1.2	20
2000	10	33	42	1.3	33

^a Adapted from Refs. [295,300] and Karleskind A. “Manuel des corps gras” Tec & Doc Lavoisier Paris, 1992.

oils, the consumption of which has nearly doubled during that period of time (see Section 5.3.1.), whereas the LA/LNA ratio increased from 20 to 33. Importantly, the biochemical modifications of the fatty acids of plant oils as a consequence of hydrogenation (including the production of saturated and/or *trans*-unsaturated isomers during this process) cannot be firmly assessed whereas 25–30% of plant lipids are consumed as margarines and industrial products [295].

5.4.2. Animal lipids

5.4.2.1. Milk and derived lipids. As is the case for plant lipids, the increase in milk-derived lipids (see Section 5.3.2.) has been accompanied by dramatic qualitative changes with respect to *n*–6 PUFAs, with a LA/LNA ratio of 2.0 in 1960 up to 9.1 in 2000 (Table 11). Dramatic changes in stock breeding methods over this period are responsible for this phenomenon. Cows, which traditionally give birth in the Spring, were progressively driven to do so in Autumn under pressure from dairy industries wanting to smooth their output throughout the year. As a consequence, this transfer of calving period resulted in deep changes of animal feed. Corn silage (associated to soybean oilcake supplementation) has become the main cow feed in lieu of grazing. Therefore, for the same quantity of ingested fatty acids by the cow, a corn-based diet will contribute daily to 250 g of LA and 5 g of LNA compared to 60 g of LA and 250 g of LNA for a grass-based diet! Moreover, these dramatic changes in animal feed have been accompanied by a 15% increase of milk fat content during the 1960–2000 period. According to our consumption model, despite the fact that complex biohydrogenation mechanisms of fatty acids are taking place in the rumen [301,302], changes in the cow feed have triggered large changes in the milk fatty acid composition, i.e. the content of saturated fatty acids (C12:0 to C16:0) increased whereas that of unsaturated fatty acids (oleic acid but also *trans*-vaccenic acid, conjugated linoleic acids and α -linolenic acid) declined sharply over that period. Overall, the average content of LNA went from 1% to 0.5% whereas the LA/LNA ratio increased more than 4-fold. In parallel, and quite importantly, the *n*–6 fatty acid content increased, leading also to a 3-fold increase in the *n*–6/*n*–3 ratio, consistent with similar changes observed for breast milk (see Section 4.2.2.).

5.4.2.2. Meat, egg and cold cut lipids. Diets, breeding conditions and the genetic background of domestic animals have changed during the 1960–2000 period, leading to changes in the nutritional profile of meats which now have a lower lipid content.

It is difficult to reconsider accurately the fatty acid profile of pigs raised in the sixties as many changes have occurred since then, i.e. animal feed and breeding conditions (building type, slaughtering age, ...). Dietary changes from mixed diets (lactoserum, bran, secondary cereals, potatoes, cabbage, ...) to cereal-based diet, in which corn grains are predominant, have also occurred. As an example, as the fatty acid composition of

Table 11

Overall changes in France of the fatty acid composition of consumed lipids from terrestrial animal species (milks, eggs, meats) (expressed as % of total fatty acids)^a

Year	Palmitic acid (C16:0)	Oleic acid (C18:1)	Linoleic acid (C18:2 <i>n</i> –6)	α -Linolenic acid (C18:3 <i>n</i> –3)	Arachidonic acid (C20:4 <i>n</i> –6)	Long-chain <i>n</i> –3 PUFAs	<i>n</i> –6/ <i>n</i> –3 PUFAs
1960	25	33	5.5	2.4	0.4	0.5	2.0
2000	29	31	6.2	0.6	0.7	0.2	9.1

^a Adapted from Refs. [285,288,296–298] for the total ingested amounts and from Refs. [290,291,294,296,301,302] for the fat content and the fatty acid profile of the different sources.

adipose tissue and muscle lipids of pigs is a fair reflection of its diet, inclusion of 5% cooked flax seeds as a source of LNA leads within 75-day feeding to 3-fold increase of LNA (1–3% of total fatty acids), a 2-fold increase of EPA (0.2–0.4%) and to a decrease of both LA (from 3.5% to 0.9%) and ARA (1.3–1%), accompanied by a 3-fold decrease of the ratio of the $n-6/n-3$ PUFAs (294).

Avian diets are lipid-rich and strongly influence the fatty acid composition of eggs and poultry meat. These animals have the ability to easily synthesise very long-chain PUFAs, mainly DHA if LNA is the main PUFA in the diet e.g. in the sixties, or mainly ARA nowadays when LA is provided by corn and soybean and becomes predominant PUFA of the diet [291,294].

Cattle meat production methods are still very diverse, but irrespective of sex, age or race, the meat fatty acid profile always reflects that of the animal feed. In France, production methods combine either grazing-based diets with some supplementations or corn silage diets. Traditional diet supplementation with flax seeds are still a fairly widespread practice. Besides production systems for young bovines or milk cows converted to meat production, which are based on corn, cereal and soybean supplementation, the lipid composition of these meats is unlikely to have changed significantly over the 40 year period.

Altogether, lipids from meats and eggs represent a fatty acid intake of approximately 20 g/day. As PUFAs of $n-3$ and $n-6$ series are both substrates of $\Delta 6$ and $\Delta 5$ desaturases (Fig. 2), the proportion of ARA versus that of $n-3$ very long chain PUFAs (EPA, $n-3$ DPA and DHA) depends upon the respective LA and LNA levels whereas the total level of these very long-chain PUFAs remains unchanged. Table 11 shows clearly that, due to the changes in animal diets, LNA and $n-3$ very long-chain PUFA intake have much decreased whereas that of ARA has increased ~ 2 -fold and that the $n-6/n-3$ PUFAs ratio has increased more than 4-fold.

5.4.2.3. Fish lipids. Over the 1960–2000 period was observed a significant increase in fish consumption combined with a change in the makeup of the species consumed, an increase in the consumption of farmed fish and a move away from fresh to processed fish [285,288,303]. Fish lipids are a very important source of $n-3$ very long-chain PUFAs. Their contribution to the intake of these fatty acids has gone from 51% of the total very long-chain $n-3$ fatty acids (EPA + $n-3$ DPA + DHA) in 1960 up to 86% in 2000.

5.5. Combined effects of qualitative and quantitative changes in dietary lipids

Despite methodological shortcomings discussed above and despite difficulties in re-evaluating the animal lipid composition which occurred in the sixties, it can be stated that changes in fatty acid intakes are much greater than is usually believed. The amount of ingested lipids has gone up to 1.4-fold in 40 years, that of $n-6$ PUFAs 2.5-fold and that of ARA 2.3-fold (Table 12). The increase of $n-6$ fatty acid intake is due to (i) a combination of increased consumption of plant oils (sunflower oil mainly) and (ii) a significant shift in animal feed where a corn-soybean diet became predominant and was associated with a change in modes of breeding. Of note, the increased fish consumption offsets the lower contribution of $n-3$ long-chain PUFAs

Table 12
Main changes in fatty acid consumption between 1960 and 2000 in France

	Current intake g/day/adult (in 2000)	“ANC” ^a recommendations	40 years evolution (fold change)	Plant lipid-related increase (g/day)	Animal lipid-related increase (g/day)
Total FA	104	81	1.4	+18.8	+10.1
Palmitic acid (C16:0)	22		1.4	+1.3	+5.0
Oleic acid (C18:1)	33		1.2	+2.3	+2.2
Linoleic acid (C18:2 $n-6$)	21	10	2.5	+11.9	+1.0
Arachidonic acid (C20:4 $n-6$)	0.5		2.3		+0.3
Total $n-6$ PUFAs	22		2.5	+11.9	+1.3
α -Linolenic acid (C18:3 $n-3$)	0.9	2	0.6	+0.3	-0.9
$n-6/n-3$ PUFAs	12		2.9		
LA/LNA	23	5	4.2		

^a ANC, “Apports Nutritionnels Conseillés”, i.e. recommendations for intake.

of terrestrial animals due to changes in animal feed. In this respect, some categories of different populations are consuming small quantities of fish, both in France and other countries, particularly in USA. We must stress that, during this period, the increase of fish consumption was higher in France than in other European countries where it increased more slowly and even decreased sometimes during the same decades [285].

5.6. Concluding remarks

In France, most recent surveys [118,286,304,305], have used food composition tables which were established earlier than changes in animal feed and not well substantiated by analytical data. From all the studies reported herein, it appears that lipid consumption has much increased in the last 40 years but mainly before 1980. However, the main changes have been qualitative with a 4.2-fold increase in the LA/LNA ratio (Table 12) due to a large increase in the content of LA, the precursor of ARA known to be strongly adipogenic in vitro and to favour adipose tissue development (see Section 3). As a consequence of increased LA and decreased LNA intake, a 2.9-fold increase in the $n-6/n-3$ ratio of PUFAs has been observed, consistent with an imbalance between ARA and EPA/DHA in humans (see Section 4). It should be stressed that consumption surveys indicate large disparities in France (285,286,304) according to age, region and social class but, at the whole population level, fish consumption in 1960 represented 14% of total $n-3$ PUFA and 51% of long-chain PUFA supply whereas, four decades later, the corresponding values are 45% and 86%, respectively. Therefore, in order to keep to the main recommendations advised by nutrition committees [306], changing food habits both on a quantitative and qualitative basis appear necessary. If it were so, as domestic animals are presently a main source of lipid intake, changes in food habits should concern in the first place the feeding pattern of breeding stock.

6. Discussion

The prevalence of overweight and obesity has escalated dramatically during the last decades both in children and adults. This recent epidemic is not attributable to genetic factors as it occurred over a short period where substantial changes in population gene pools cannot be seen. Among environmental factors, physical inactivity associated to increased caloric intake, i.e. in practice to high-fat diets, plays an important role. However, in addition to a positive energy balance, our results pointed out the antithetic properties of polyunsaturated fatty acids of the $n-6$ and $n-3$ series [57] in promoting mouse adipose tissue development. Therefore we aimed herein at comparing the changes in the fatty acid composition of ingested food lipids by infants and adults which have taken place in parallel to the increasing prevalence of overweight and obesity over the last decades. The data indicate a significant increase in the $n-6$ PUFA content and in the LA/LNA ratio of breast milk, formula milk (except in the last 10 years for the LA/LNA ratio) and most consumed foods over the last decades. It should be stressed that changes in the lipid chain processed, foods and human food habits have taken place rather smoothly and that it is difficult to relate these alterations at a precise time where obesity epidemics began to “explode”. A tight relationship is even more difficult to establish if one keeps in mind that the shape of prevalence curves of obesity depends on the cut-off points for BMI and that, above all, a significant lag time may exist between hyperplasia of precursor cells and their differentiation into adipocytes, that is between a so-far undetectable biological event and its translation into a visible phenomenon, i.e. adipose tissue development as a consequence of differentiation of precursor cells. It is known that fat mass enlargement is mainly due to an increase in the fat cell number. Fat cell formation takes place from precursor cells which remain abundant throughout life, although early infancy appears as a very important period in that respect. Among various effectors favouring adipogenesis, fatty acids behave as activators/ligands of cell surface and nuclear receptors. Clearly, ARA through prostacyclin production plays (in an autocrine/paracrine manner) a major adipogenic role in addition to one (and possibly more) redundant pathways of adipogenesis (Fig. 1). Therefore, ARA, supplied by the diet or released from endogenous stores, in addition to its production arising from linoleic acid metabolism, appears as a remarkable “booster” of adipogenesis.

Available data indicate that varying the proportion of α -linolenic, and consequently the LA/LNA ratio, alters the production of ARA and prostaglandins that include prostacyclin. They also indicate that in

rodents, under isoenergetic conditions, a high LA content associated with a low LNA content in dietary lipids leads during the gestation/suckling period can lead to a dramatic increase in fat mass which is observed after weaning and is maintained at the adult age. These results are striking in that *PUFAs of n-6 and n-3 series are not equipotent in promoting adipose tissue development*. Thus it appears that limiting LA intake to adequate levels, and maintaining a fine balance between LA and LNA intake, promote an acceptable adipose tissue development. In humans, US epidemiological data indicate a 2-fold increase of the adiposity indices of 6–11 month-old infants between the 1976–1980 and the 1988–1994 period. These observations strongly suggest that qualitative more than quantitative changes in macronutrients such as fats have occurred in the meantime and have remained unnoticed. In this respect, during the last decades, a significant increase of LA content is observed in breast milk of women living in the major industrialized countries of the Western world whereas the LNA content has not changed in a parallel way, leading to a continuous increase in the LA/LNA ratio. Of note, a relative stability of ARA content in breast milk has been observed but it cannot be excluded that its synthesis from LA is increasing during the suckling period and afterwards. In contrast to ARA, milk DHA content is changing widely, probably because of sea food consumption by mothers. This has led to large differences of the ARA/DHA ratio of breast milk which has in the last 10 years greatly increased in USA, Canada and Australia but has decreased in Europe (Table 3). In infant formulas, the LA content is still high and greatly variable (Tables 4 and 5) but, noticeably, the LA/LNA ratio has been significantly decreased since 1995. However, we assume from animal studies that a fine balance between ARA (and possibly DGLA) and DHA (and possibly EPA) is important in situ to modulate fat cell formation. If it were so, the levels of LA and LNA as well as the LA/LNA and ARA/DHA ratios should be taken into consideration in the fatty acid composition of lipids of breast milk and infant formulas for the regulation of fat mass. In any case, considering that LA requirements have been grossly overestimated, the LA content of infant formulas should be reconsidered according to previous recommendations of nutrition committees.

Compared to early infancy where in the last decades, qualitative changes may have played a major role, a combination of events has concurred to promote excessive adipose tissue development in adults. First in the last 40 years, at least in France, the daily fatty acid intake has increased 1.4-fold. Second, among fatty acids, a disproportionate increase in the consumption of LA (2.5-fold), ARA (2.3-fold) and no change in that of total *n-3* PUFAs can be estimated. During the same period, the consumption of LNA has decreased by 40%, leading to a 2.9-fold and a 4.2-fold increase in the ratios of *n-6/n-3* PUFAs and LA/LNA, respectively, despite the increase in fish fat intake (Table 12). In elderly men (mean age of 66 years) fed for 5 years a diet in which the major modification was to substitute saturated fatty acids (conventional diet) by LA (experimental diet) the mean body weight of control subjects (389 men) decreased whereas that of the experimental group (393 men) increased, strongly suggesting an increase in body fat content even at a late age upon LA supplementation [121].

Regarding ARA, another putative role in favouring adipose tissue development could be through the modulation of 2-arachidonoylglycerol (2-AG) production arising from the hydrolysis of arachidonic acid-containing glycerophospholipids. 2-AG is the predominant putative endogenous ligand of the brain cannabinoid receptors which stimulate food intake and lipogenesis in liver [307]. Of interest, 2-AG levels are elevated in mice fed safflower oil (high in LA but deficient in LNA) and are reduced upon DHA-rich fish oil supplementation [308]. Thus changing the *n-6/n-3* ratio alters the levels of this agonist of the cannabinoid receptors implicated centrally in energy balance. Clearly, further experiments should shed some light on this new and exciting avenue of research. To sum up, owing to the presence of adipose precursor cells throughout life and to the fact that adipocytes once formed exhibit little or no turnover in the body [309,310], these quantitative and qualitative changes observed in ingested lipids, associated with increased sedentary, will inevitably lead to fat cell formation and to an increase in the prevalence of overweight and obesity, leading in turn to the metabolic syndrome. The significant change in the composition of the various fatty acids can be traced to changes in human food habits but, quite importantly, also in the feeding pattern of breeding stock. Whether prevention of obesity appears as a critical issue to avoid difficult if not insurmountable health problems to solve in the future, the status of lipids should therefore be re-evaluated from the very beginning of the food chain in which the intricacy of the agricultural and food industry policies is now becoming quite obvious.

Acknowledgements

The authors wish to thank Dr. R. Arkowitz for careful review and Ms. G. Oillaux for outstanding secretarial assistance. The key scientific contribution over the years of Drs. R. Négrel, P. Grimaldi, E. Amri and C. Dani and that of many other distinguished investigators, is gratefully acknowledged. Dr. P. Guesnet wishes to dedicate his contribution to Dr. R.G. Jensen who inspires his studies on milk fat composition.

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