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An emerging risk factor for obesity: does disequilibrium of polyunsaturated fatty acid metabolism contribute to excessive adipose tissue development?

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A positive energy balance (energy intake > energy expenditure), in which total fat intake plays an important role, is commonly regarded as a major factor contributing to obesity. Adipose tissue development, i.e. both size (hypertrophy) and number (hyperplasia), is stimulated by high dietary fat intake during early postnatal development, a susceptibility that now appears to continue well into adulthood. Recent human and animal studies suggest that by altering rates of adipocyte differentiation and proliferation, differences in the composition of dietary fat may also contribute to adipose tissue development. At least in rodent models, the relative intake of *n*-6 to *n*-3 PUFA is clearly emerging as a new factor in this development. In these models, higher linoleate intake raises tissue arachidonic acid, which increases prostacyclin production and, in turn, stimulates signalling pathways implicated in adipogenesis. Signalling pathways stimulated by arachidonic acid probably include phospholipase and/or cyclo-oxygenase activation and may be linked as much to relatively low intake of *n*-3 PUFA as to excessive dietary linoleate. One factor potentially contributing to oversight about the apparent role of dietary *n*-6 PUFA (especially excess dietary linoleate) in adipose tissue development is the historical overestimation of linoleate requirements and the enthusiasm for higher intake of 'essential fatty acids'. More research is needed to address whether disequilibration of dietary PUFA intake contributes to the risk of obesity in humans.

Obesity: Dietary fats: *n*-6 Fatty acids: Adipocyte: Linoleic acid: Arachidonic acid

The prevalence of overweight and obesity has increased dramatically over the last two to three decades in major industrialised and urbanised countries, a trend that does not appear to be stabilising. Positive energy balance (energy intake > energy expenditure) is clearly the main reason for this alarming phenomenon which, among other physiopathological consequences, leads to type 2 diabetes, hypertension and elevated risk of CVD.

This energy imbalance is due largely to substantial reduction in energy expenditure worsened by fat or carbohydrate overconsumption. Indeed, longitudinal and cross-sectional studies in rodents and human subjects have shown a tight relationship between hyperenergetic diets, i.e. high-fat diets (HFD) in most cases, and body fat enlargement. However, the role of total dietary fat intake as the main contributor to human obesity is suspect because, despite the dramatic increase in the prevalence of overweight and obesity, no major change in the total amount of ingested fats has occurred in the last two decades[1–3].

The potential importance of the fatty acid composition of dietary fats is emerging as a factor that may be contributing to the risk of obesity[4,5]. We propose that qualitative changes in ingested fats, more specifically the balance of PUFA of the *n*-6 and *n*-3 series (linoleic acid (LA; 18 : 2*n*-6) : α-linolenic acid (LNA; 18 : 3*n*-3) ratio) and their major metabolites (arachidonic acid (ARA) (20 : 4*n*-6) : EPA (20 : 5*n*-3) + DHA (22 : 6*n*-3) ratio), increases the risk of excessive adipose tissue development and should therefore be taken into consideration. A high intake of *n*-6 PUFA and a very high dietary *n*-6 : *n*-3 PUFA ratio have been implicated in the promotion of many diseases, including CVD, cancer, and inflammatory and autoimmune diseases[6–8].

Early nutrition and excessive adipose tissue development

The consequences of exposure to an energy-rich diet on adipose tissue development are well known in rodents[9,10]. In these studies, energy intake during the suckling period was adjusted by manipulating litter size but after weaning, these animals had access to regular laboratory chow. Under these conditions, both increased adipocyte size and number contribute to the

Abbreviations: ARA, arachidonic acid; COX, cyclo-oxygenase; EFA, essential fatty acid; HFD, high-fat diet; IP-R, prostacyclin receptor; LA, linoleic acid; LC, long-chain; LNA, α-linolenic acid; PLA_2, phospholipase A_2.

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increased fat mass observed in the rats raised in small litters[11]. A similar situation has also been reported in newborn baboons fed until weaning on formulas with different energy densities and then fed a monkey chow diet containg 40 % fat as lard for the next 5 years. Compared with normally reared baboons, TAG mass increased in most fat depots of both female and male overfed baboons. Adipocytes from each depot show marked (female) or marginal (male) hypertrophy. Hyperplasia was also significant in some of the fat depots[12]. The importance of the diet is also well illustrated in neonatal pigs in which formula feeding increases both number and proliferation of adipose precursor cells but their ability to differentiate is decreased relative to mature sow-reared pigs[13].

Obese human subjects exhibit a more rapid and earlier increase in both number (hyperplasia) and size (hypertrophy) of adipocytes[14]. As mature adipocytes do not divide *in vivo*, this emphasises a silent 'weightless' event, i.e. overproliferation in infancy of adipose precursor cells as a function of developmental conditions and in response to diet[15]. Another important developmental issue is whether adipose tissue has the potential to expand at any given age. Contrary to widespread opinion, longitudinal studies in human subjects show that adipocyte number continues to increase in adults, more so in obese than in non-obese subjects[14], possibly because adipose precursor cells are still present in middle-aged and elderly individuals of both sexes[16].

Whether the size and self-renewal of adipose precursor cell pools varies as a function of age or in response to diets is presently unknown in humans. This point is important as subpopulations of precursor cells have recently been characterised in the stromal-vascular fraction of human adipose tissue where they are assumed to self-renew and to be responsible for the lasting potential of this tissue to expand in response to chronic energy excess[17]. Moreover, if it occurs at all, the rate of adipocyte disappearance is low, and apoptosis has only been observed under drastic conditions, making questionable its quantitative importance under physiological conditions[15]. Collectively, these observations strongly suggest that adipocyte formation may well be an irreversible process. Thus, preventing or minimising lifestyle pressure on this phenomenon should represent a key public health issue.

Whether fetal programming plays a role in the rising prevalence of obesity remains unclear because although higher birth weight correlates with higher BMI in young adulthood[18], lower birth weight appears to be associated with the central obesity implicated in the metabolic syndrome[19–24]. Between 1 and 16 years of age, a raised BMI doubles the relative risk of becoming a fat adult[25]. Similarly, the BMI of children at 8 years of age is positively predicted by their BMI at 2 years of age[26]. Infants who are at the high end of the distribution for BMI or who grow rapidly during infancy are at increased risk of subsequent obesity[27]. Further studies are needed to explain the apparent link between birth weight and later obesity as well as to gain some insight into the cellular and the molecular mechanisms responsible for differential susceptibility to hyperplasia *v.* hypertrophy. In normal birth-weight infants, what seems clear is that the health impact of accelerated adipogenesis depends on the stage of development; prenatally it has a positive impact on postnatal and long-term health outcomes, whereas from childhood onwards, the reverse is more often the case.

Fat intake and adipose tissue development

A critical issue in linking dietary fats to obesity is whether an HFD enhances adipose tissue development and whether the type of dietary fat plays a differential adipogenic role. Feeding an HFD to young mice or rats for up to 52 weeks increases proliferation of adipose precursor cells, thereby increasing adipocyte number as well as adipocyte size [9,28,29]. In Pima Indians, 2 weeks overfeeding of an HFD doubles the population of the small, insulin-sensitive adipocytes that appear to be recruited from a pool of preadipocytes[30]. This suggests that both processes – hypertrophy and hyperplasia – take place in response to an HFD. Whether these events co-exist or whether hypertrophy preceeds hyperplasia is not clear. However, both in adult rodents and humans, a 'critical' adipocyte size has been postulated to explain the appearance under high-fat feeding conditions of a population of small fat cells in adipose tissue[9,31]. Thus, recruitment of these new adipocytes seems to be linked to paracrine/autocrine signals released from fully mature adipocytes. Indeed, in rodents, angiotensin II arising from angiotensinogen secreted by adipocytes appears to play such an adipogenic role by triggering prostacyclin production in preadipocytes (see below)[32,33].

Studies on the role of individual fatty acids in adipose tissue development have greatly benefited from *in vitro* experiments using preadipocytes derived both from established clonal lines and from primary cultures of rodent and human preadipocytes. As activators/ligands of PPAR β/δ and PPARγ, NEFA act at the preadipocyte stage and do not need to be metabolised to acyl-CoA in order to enhance the formation of TAG-containing adipocytes[34–37]. It should be stressed that preadipocytes do not contain TAG but have the capacity to synthesise complex lipids for membrane biosynthesis[36]. Moreover, preadipocytes are able to resume a limited number of mitoses in response to non-metabolised or metabolised fatty acids by activating PPAR β/δ[38]. In other words, this role of fatty acids appears 'hormonal' and is quite distinct from their role as substrates for TAG synthesis.

Pups from mice fed an LA-rich diet are 40 % heavier at weaning and fat mass is increased compared with pups from those fed a balanced LA/LNA diet, a difference in body weight that is maintained into adulthood[39]. The enhancing effect of LA on fat mass requires cross-breeding for a few generations, possibly suggesting epigenetic transgenerational events. Inclusion of LNA in the isoenergetic diet rich in LA prevented the enhancement of fat mass, which is consistent with our *in vitro* observations but also with studies showing in rodents that *n*-3 PUFA suppress lipogenesis[40] and increase fatty acid β-oxidation[41–44].

At similar energy intakes, 1-month-old rats given an LA-enriched diet for 6 months exhibit both higher body and higher retroperitoneal fat pad weight and increased adipocyte number than rats fed an SFA-enriched diet. However, adipocyte hypertrophy has been reported in young rats fed an LA-enriched diet for 12 weeks compared with rats fed an LNA-enriched diet[45]. Taken together, these observations suggest that *n*-6 and *n*-3 PUFA differentially affect the balance between proliferation of adipose precursor cells and their differentiation to adipocytes.

British Journal of Nutrition

Arachidonic acid, prostacyclin and adipogenesis

Consistent with an indirect contribution of LA in enhancing adipose tissue development, female mice fed an LA-rich diet have higher ARA levels in their milk (+70 %) compared with mice fed a balanced LA/LNA diet (F Massiéra, Guesnet P and Ailhaud G, unpublished results). Moreover, the LA-rich diet decreases *n*-3 long-chain (LC) PUFA by 40 % in mother's milk, thereby inducing an unbalanced ratio between ARA and EPA + DHA for suckling pups (ratio of 1·4 on LA alone *v.* 0·5 on the balanced LA:LNA ratio).

In piglets aged 10 d, 2 weeks of 0·5 % ARA supplementation causes a 27 % increase in body weight, an effect probably due to increased fat mass because there was no difference in body length[46]. Consistent with a role of ARA in adipose tissue development, a significant positive association between plasma ARA level and human infant body weight at 4 months of age has been reported[47], as well as between ARA levels of adipose tissue lipids and BMI in children of Cyprus and Crete[48].

Among LC fatty acids, ARA is the main adipogenic component of fetal bovine serum in Ob17 preadipocytes. ARA appears to be highly adipogenic primarily because it is the precursor of prostacyclin. In contrast, EPA, DHA and the *n*-3 isomer of ARA (20 : 4*n*-3), which do not give rise to prostacyclin, are 3- to 4-fold less adipogenic than ARA and no more potent than SFA and MUFA in stimulating adipogenesis[39].

After secretion from preadipocytes, prostacyclin acts externally via the prostacyclin receptor (IP-R) on the cell surface. Importantly, IP-R is transiently expressed at the preadipocyte stage and prostacyclin synthesis ceases in mature adipocytes[49,50]. Among natural LC PUFA, only ARA, acting through the IP-R–prostacyclin system, seems to be capable of triggering cAMP production and activating the protein kinase A pathway[39] (Fig. 1).

In rodents, both *ex vivo* and *in vivo* exposure of white adipose tissue to (carba)prostacyclin, a stable analogue of prostacyclin also exhibiting a high affinity for IP-R, stimulates the formation of adipocytes within a few hours[51–54]. Moreover, ARA and some of its metabolites generated through cyclo-oxygenase (COX) and lipoxygenase activities are implicated in adipogenesis as activators/ligands of PPARs[37]. Thus ARA is a potent stimulator of adipogenesis that acts through cell-surface IP-R and nuclear PPAR in early and late events of adipogenesis, respectively (Fig. 1). Human preadipocytes in primary culture [52] and adipose precursor cells established from human adipose tissue[55] are also able to differentiate into functional adipocytes

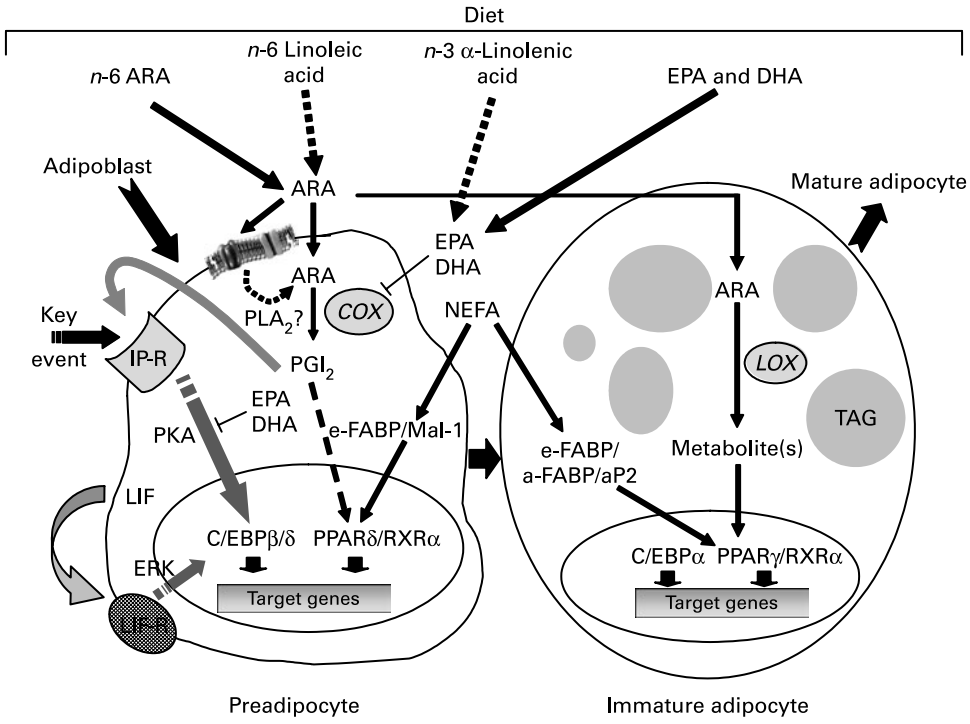

Fig. 1. Redundant pathways and long-chain-fatty acids implicated in adipogenesis[5]. At least two cell-surface receptor/ligand systems (prostacyclin receptor (IP-R)/prostacyclin, and leukaemia inhibitor factor (LIF) receptor/IIF) concur to up-regulate the expression of CCAAT/enhancer binding protein β (C/EBBPβ) and C/EBPδ which in turn up-regulate PPARγ whose expression promotes terminal differentiation or adipogenesis[54]. Arachidonic acid (ARA) provided by the diet or indirectly through linoleic acid metabolism, as well as ARA released from membrane glycerophospholipids via phospholipase A_2 (PLA_2) activity, favours in preadipocytes the synthesis and release of prostacyclin. In addition to LIF which activates the ERK pathway, ARA via prostacyclin plays a unique role in activating the protein kinase A (PKA) pathway by means of IP, and enhances the differentiation process. Furthermore, prostacyclin is assumed to bind to PPAR β/δ. The other NEFA act as activators/ligands of PPARβδ and PPARγ[37]. Upon terminal differentiation, LIF is no longer produced. Production of prostacyclin and other prostaglandins ceases and is accompanied by reduced expression and loss of functional IP. In addition to ARA metabolites synthesised through cyclo-oxygenases (COX) at early step(s), ARA metabolites synthesised through lipoxygenases (LOX) as ligands of PPARγ are also implicated at later step(s). EPA and DHA provided by the diet or arising from α-linolenic acid metabolism inhibit adenylate cyclase and COX activities, presumably altering adipogenesis (see text). 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 NEFA[34,35]. RXR, retinoic acid X receptor.

in the presence of (carba)prostacyclin (F Massiéra, Guesnet P and Ailhaud G, unpublished results), suggesting an ARA-mediated signalling pathway in human preadipocytes similar to that in rodents.

To gain further insight into the contribution of prostacyclin signalling in mouse adipose tissue development before mating and during the gestation/suckling period, wild-type mice and those knocked-out for IP-R were given either an HFD rich in LA but not deficient in LNA (LA diet), or the same iso-energetic diet enriched in LA and LNA (balanced LA/LNA diet)[39]. In contrast to wild-type mice and regardless of whether the diet was rich in only LA or was more balanced in LA and LNA, pups of IP-R knock-out mice had no gain in body weight or fat mass. This suggests that the prostacyclin signalling pathway is a key factor responsible for enhanced fat mass observed in the wild-type pups during the gestation/suckling period in response to an LA-enriched diet. These data also show that the prostacyclin signalling pathway can be down-regulated by decreasing the LA:LNA ratio in the diet.

Inhibition of Ca-independent phospholipase A_2 (PLA_2) β or γ by small interfering RNA prevents ARA availability and impairs late events of adipogenesis[56]. Moreover protection against HFD-induced obesity has been reported in mice knocked out for the gene encoding the pancreatic PLA_2 enzyme, which releases ARA from the sn-2 position of dietary glycerophospholipids, an effect accompanied by a 5-fold decrease in the absorption by intestine of lysoglycerophospholipids[57]. This observation appears at first sight consistent with a decreased postprandial availability of ARA entering adipose tissue and preventing any fat mass gain. Unfortunately, the overall decrease of intestinal fat absorption including TAG prevents drawing any firm conclusions[58]. Thus, it remains to be established whether ARA liberated during phospholipid hydrolysis can actually promote adipogenesis.

Dietary *n*-3 polyunsaturated fatty acids and adipose tissue development

Both the LA intake and the *n*-6 : *n*-3 PUFA ratio affect fat mass size and development. Adult rats fed an HFD enriched with either LNA or EPA/DHA for 4 weeks maintain or have slightly decreased body weight and epididymal fat pad weight[59]. In 5-week-old mice, among five different high-fat semi-purified diets given for 5 weeks, the high LA–low LNA-fed group had the highest while the low LA–high LNA-fed group had the lowest proportion of body fat[60]. Varying the proportions of LA and LNA in the diet alters the synthesis and tissue incorporation of ARA and EPA + DHA[61,62], perhaps through competition between different substrates for δ-6 desaturase or esterification. Interesting observations have been made when rats are given isoenergetic *n*-3, *n*-6/*n*-3 and *n*-6 diets, with LA:LNA ratios of 0·4, 9 and 216, respectively, during the last 10 d of gestation and throughout lactation[63,64]. Under these conditions, the ARA : DHA ratios of milk lipids were 0·7, 2·5 and 8·1, those of serum lipids 1·4, 2·9 and 5·8 and those of adipose tissue lipids 1·1, 4·1 and 6·9, respectively. Of note, in contrast to the other groups, no EPA could be detected in milk, serum and adipose tissue lipids of the *n*-6 group.

At weaning, the body weight and the weight of inguinal adipose tissue were lower in pups of the dams fed the *n*-3 diet compared with pups of the dams fed the other diets. The body weight difference between the *n*-3 and *n*-6/*n*-3 groups was maintained in both males and females at 28 weeks of age. However, beyond 4 weeks of age, the *n*-6 group exhibited a lower body weight than the *n*-3 group, possibly due to an apparent *n*-3 PUFA deficiency.

Various mechanisms could explain the inhibitory role of *n*-3 PUFA in adipose tissue development. First, DHA inhibits adipogenesis[39,65]. It is a strong inhibitor of the synthesis of PG of the 2 series arising from ARA[66]. Second, certain *n*-3 PUFA directly inhibit activity of purified COX-2 and to a lesser extent COX-1, with EPA > DHA > LNA[67]. Since both COX-1 and COX-2 are expressed in preadipocytes[68], dampening of both activities and thus lowering prostacyclin synthesis from ARA by LNA, EPA or DHA cannot be excluded. The down-regulation of the expression of COX genes by *n*-3 PUFA has been recently reported in human endothelial cells, providing a mechanistic basis for the regulation by DHA of COX-2 gene expression and prostacyclin production following stimulation by IL-1. Third, DHA decreases the expression of COX-2 mRNA and COX-2 protein by blocking NF-κB-mediated transcription of COX-2 through multiple signalling pathways[69].

Finally, *n*-3 LC PUFA inhibit cAMP production (EPA > DHA) stimulated by ARA through prostacyclin[39]. They may also inhibit the catalytic subunit of protein kinase A[70]. *n*-3 LC PUFA could have these effects because they control the release of ARA from complex lipids through PLA_2 activities, or because they attenuate the production of eicosanoids (including prostacyclin) by direct inhibition of COX and/or lipoxygenase activity or their expression (Fig. 1). How ARA release is controlled is uncertain owing to the multiplicity of PLA_2 enzymes which are active within and surrounding cells (adipocytes, pericytes, endothelial cells) and to the fact that these enzymes exhibit distinct but intertwined roles[71].

Intake and body content of polyunsaturates in humans

The potentially important role played by an LA-enriched diet in increasing body weight in human subjects was shown a few decades ago[72]. In that key study, two groups of about 400 elderly institutionalised men (mean age 66 years) received, at constant energy intake 40 % of energy as fat as a conventional diet (control group) or as a diet in which LA-enriched oil (experimental group) replaced most of the saturated fats. Good adherence to each diet was checked by analysing the fatty acid composition of lipids from serum and adipose tissue, the composition of which became similar to that of dietary fats after 5 years. Most interestingly, the rate of LA accumulation in adipose tissue was positively associated with weight gain. Moreover, after 5 years, the body weight of the experimental group had increased by 3 % whereas that of the control group had decreased by 2 % (presumably by a decrease in lean body mass). Although adipose tissue mass *per se* was not determined in this study due to the lack of convenient non-invasive techniques at that time, these results are consistent with a fat mass gain due to LA-induced recruitment of adipocyte precursor cells still present in the subcutaneous adipose tissue of elderly men and women[16].

Within 7 weeks of starting formula milks varying widely in LA content (from 1·4 to 43·1 %), the LA content of the subcutaneous fat in infants becomes very similar to their level of LA

British Journal of Nutrition

intake[73]. In contrast, in elderly subjects, when the LA content of dietary fat was raised from 12 to 38 %, more than 3 years were necessary to stabilise the LA content of the body fat[72]. Hence, the responsiveness of fatty acid composition of adipose tissue to differences in LA intake is much greater during the rapid adipose tissue development in infants than in the elderly.

When full-term infants received a formula containing 16 % LA, changing LNA from 0·4 to 3·2 % lowered serum ARA and increased serum DHA, an effect also associated with 13 % lower body-weight gain presumably due to lower fat accumulation[47]. The situation appears different when infants are either breast-fed or fed a formula supplemented with both ARA and DHA. On a long-term basis, the ARA and DHA content of breast milk is more closely related to their dietary intake than to LA and LNA intakes[5]. Importantly, the intake of ARA by mothers has no effect on the ARA content of breast milk *per se* but reduces its EPA and DHA content, thus leading to an increase in both the ARA:DHA ratio[5] and the ARA:(EPA + DHA) ratio. The mean ARA:DHA ratio of mature breast milk is approximately 1·1–1·7 in most Western European countries but is notably higher in the USA (2·0–2·6). In ARA- and DHA-supplemented infant formula in which the ARA:DHA ratio of 1·5–2·0 mimics that of human breast milk, LA and LNA intake have little impact on the blood levels of ARA and *n*-3 LC PUFA, which are thus determined by their actual content in the formula[74]. Thus, in breast-fed infants or those given unsupplemented or LC PUFA-supplemented formula, the levels of ARA and/or the ARA/(DHA+EPA) ratio increase in plasma when *n*-3 PUFA intake is low, i.e. under the nutritional conditions that presently prevail in major industrialised countries. These observations are of interest in the context of the adipogenic effect of ARA and the anti-adipogenic effect of DHA and EPA *in vitro* [39,65] (Fig. 1).

Compared with infants, the situation in adults is different but we hypothesise that *n*-6 PUFA are still unfavourable to controlling adipogenesis because, at least in France in the last four decades, LA and ARA intake have increased by 2·3- and 2·5-fold, respectively, whereas LNA intake has decreased by 40 %[5]. The situation is similar in the USA where not only has LNA intake remained low, but *n*-3 LC PUFA intake is also very low. Under these dietary conditions, the tissue incorporation of ARA increases[75], which favours the formation of 2 series eicosanoids formed from ARA over the 3 series eicosanoids produced from EPA[76].

Because of very low conversion from LA, dietary supply of ARA appears to be the main factor determining the ARA status in humans[77]. Variations in LA dietary intake induce only modest changes in ARA synthesis and in tissue incorporation in humans[78,79]. Epidemiological studies assessing fatty acid intake are in good agreement with these observations and show that intake of ARA but not LA correlates with plasma ARA in humans (Rum & Hornstra[80]; P Astorg, S Bertrais, F Laporte, N Arnault, C Estaquio, P Galan, A Favier and S Hercberg, unpublished results). Both dietary EPA and DHA can reduce ARA in plasma but this requires a higher intake than that generally delivered by Western diets[75]. Meanwhile, a high LA intake associated with a low LNA intake (high LA:LNA ratio) competitively reduces the synthesis of *n*-3 LC PUFA from LNA, thereby increasing the ARA:EPA + DHA ratio[81]. This modulation concerns specifically EPA and DHA synthesis, which are very low in humans[77,81,82].

A steady increase in the LA intake and in the LA:LNA ratio in Western countries during the last decades has been demonstrated using fatty acid biomarkers such as in human breast milk and adipose tissue[5,83–85]. Human milk content of LA and LNA reflects both their short-term intake and their long-term homeostasis, the latter being partly regulated by selective mobilisation of endogenous adipose stores[86]. A factor that may contribute to stimulating adipogenesis is that the LA: LNA ratio in human breast milk is about 30 % higher than in dietary lipids, an effect probably due to the high rate of LNA β-oxidation[62,87].

Time-trend changes in the content of PUFA in human milk and adipose tissue of Western populations can be estimated in the USA where robust data are available[5] (Table 1). Since the 1950s, the content of LA has steadily increased to 15–16 % of total fatty acids in the 1980s, whereas at the same time that of LNA has remained unchanged and is rather low. The corresponding values for the suckling infant are about 8 energy % as LA and 1–1·3 energy % as LNA in total fatty acids of human milk. Thus, the LA:LNA ratio of the milk has also increased to reach mean values of 16–22. Similar temporal variations in LA content of the milk have been reported in other Western countries but they are less dramatic and have taken place more recently[5]. They are most probably ascribable to increasing consumption of margarines and edible oils high in LA and low in LNA, which generally contribute to a significant proportion of total PUFA intake.

Due to insufficient data for EPA in human milk and for all LC PUFA in adipose tissue, it is more difficult to estimate the time trends in the intake of ARA, EPA or DHA (Table 1). Before 1980, only a few investigators reported the ARA content of breast milk or adipose tissue, while no data are available for EPA and only one publication was found for DHA (Table 1; Ailhaud *et al.* [5]). Moreover, the level of EPA in both breast milk and adipose tissue and that of DHA in adipose tissue are rather low, making their determination difficult. Since 1980, only two studies appear to have reported the EPA content in human milk[5]. Nevertheless, since the 1990s, the ARA content of breast milk of US women is within the range of values reported in other Western countries (0·4–0·66 %)[5], but that the content of DHA is about 30–50 % lower (0·2 % in USA *v.* 0·3–0·4 % in Europe, for example)[4].

Such large inter-country differences result from lower seafood consumption in the USA, which provides the main dietary supply of EPA and DHA. A recent estimate of PUFA consumption reports a mean daily intake of EPA + DHA of about 110 mg in the USA[88], whereas it reaches values above 200 mg in other Western countries[89,90]. Thus, the ARA:EPA + DHA ratio is 1·5- to 1·7-fold higher in breast milk of US women compared with breast milk of European women, favouring the impact of the unbalanced LA:LNA ratio.

Overestimation of linoleic acid requirement and arachidonic acid availability

One reason why an absolute or a relative overload of tissue LA or ARA exists and could increase susceptibility to adipogenesis and obesity in humans is because, historically, true dietary requirement for LA seems to have been substantially overestimated. If humans (or animals) were consuming an appropriate amount of LA to meet actual dietary requirements,

British Journal of Nutrition

Table 1. Content of PUFA (% of total fatty acids) in mature milk and adipose tissue in USA during the last 25 years*
(Median values and standard deviations and ranges)

	LA (18:2*n*-6)		LNA (18:3*n*-3)		LA:LNA ratio		ARA (20:4*n*-6)		EPA (20:5*n*-3)		DHA (22:6*n*-3)		ARA:EPA + DHA ratio	
	Median	SD	Median	SD	Median	SD	Median	SD	Median	SD	Median	SD	Median	SD
Human milk†														
Before 1980 (*n* 13)														
Median	10·5	2·9	1·3	0·4	8·9	3·0	0·44	0·17	–		–		–	
Range	5·5–14·5		0·8–1·9		6·3–14·0		0·20–0·57							
1980–1990 (*n* 12)														
Median	15·3	1·4	1·3	0·6	15·7	10·4	0·45	0·18	–		0·18	0·10	3·02	1·09
Range	12·9–17·6		0·4–2·4		7·0–44·0		0·10–0·69		0·0–0·10		0·06–0·30		1·33–4·00	
1990–2005 (*n* 17)														
Median	15·3	2·0	1·1	0·3	16·3	11·0	0·49	0·10	0·09	0·07	0·20	0·06	1·99	0·76
Range	12·7–20·2		0·28–1·5		8·9–58·3		0·24–0·67		0·02–0·30		0·09–0·37		0·80–3·76	
Adipose tissue‡														
Before 1980 (*n* 10)														
Median	9·5	1·4	–		–		–		–		–		–	
Range	7·5–11·4													
1980–1990 (*n* 6)														
Median	15·6	2·3	0·9	0·5	17·0	5·8	0·56	0·17	–		–		–	
Range	12·0–18·5		0·6–1·9		8·5–25·3		0·38–0·70		0·01–0·06		0·10–0·19		2·44–3·45	
1990–2005 (*n* 3)														
Median	15·9	2·3	0·7	0·1	21·7	1·2	0·26	0·14	–		–		–	
Range	14·4–18·5		0·6–0·9		21·0–23·2		0·10–0·38		0·003–0·01		0·10		0·97–3·45	

LA, linoleic acid; LNA, α-linolenic acid; ARA, arachidonic acid.
* *n* Refers to the number of studies.
† Only the data on mature human milks (i.e. at least 1 month of lactation) were considered since LA and long-chain PUFA content varies during the maturation of milk. Before the 1980s, the general use of empty GLC columns did not allow a clear-cut separation and quantification of all PUFA, i.e. LNA (only four studies reported its content) and mainly all long-chain PUFA (only three studies have measured the ARA content with no EPA and DHA determination). Between 1980 and 1990, the quantification of all PUFA was then possible with the use of capillary GLC columns but only two studies have reported data on EPA content.
‡ Adipose tissue of both women and men were considered because no effect of sex on PUFA content was noted. Generally data on long-chain PUFA showed a very low content of *n*-3 long-chain PUFA (adapted from Ailhaud *et al.* [5]).

it would not be logical to argue that even a modest excess LA intake above the level required needed for health could promote sufficient adipogenesis to result in a pathological state (obesity). Therefore, the plausibility of the link between *n*-6 PUFA and risk of obesity implicitly necessitates, as a pre-condition, an absolute or relative dietary overload of these fatty acids compared with physiological requirement.

Evidence has been emerging for a decade now that LA requirements may well have been overestimated. This evidence is fairly solid in rats and, by extrapolation, probably means a similar overestimation has occurred in humans, but this remains to be verified. The currently recommended intake of LA for rats (2 energy %) is based entirely on studies done in animals that were concurrently deficient in *n*-3 fatty acids, i.e. were 'essential fatty acid' (EFA) deficient. The EFA deficiency model leads to an overestimate of the actual dietary requirement for *n*-6 PUFA because it takes more LA to correct or prevent EFA deficiency than to correct specific *n*-6 PUFA deficiency. In other words, *n*-6 PUFA depletion is more severe in the concurrent absence of dietary *n*-3 PUFA than in the presence of dietary *n*-3 PUFA. One reason why both clinical and biochemical symptoms of EFA deficiency are more severe than those of specific *n*-6 PUFA deficiency is that low levels of LNA actually protect against or inhibit the depletion of *n*-6 PUFA when the latter are absent from the diet. Indeed, total dietary fat deficiency has more severe effects on growth and the triene:tetraene ratio than does EFA deficiency, which, in turn, is more severe than specific LA deficiency[91,92].

An abundant literature describes competition between *n*-6 and *n*-3 PUFA at virtually every level including absorption, plasma transport, metabolism through desaturation-chain elongation and on to the COX and lipoxygenase pathways. It is therefore counterintuitive that *n*-3 PUFA would protect against symptoms of *n*-6 PUFA depletion. However, several studies show that low dietary levels of LNA do indeed inhibit the onset of symptoms of *n*-6 PUFA depletion. One of the first such studies to directly address this point showed that when LNA was provided at 10 mg/d, growth of rats that had previously been consuming a totally fat-depleted diet could be restored to normal with 10 mg/d instead of the 20 mg LA/d required in the absence of LNA[93]. Hence, when provided at approximately 0·1 energy % in the diet, LNA reduced dietary LA requirement from about 0·2 to 0·1 energy %. Others have also shown that a modest dietary intake of LNA (>2 energy %) maintains essentially normal growth in rats for 12–15 weeks post-weaning[87,94,95].

Bourre *et al.* [61] published a key study supporting a synergistic rather than antagonistic role of a low intake of LNA in protecting against *n*-6 PUFA depletion. They showed in rats that reproduction, gestation, lactation and subsequent growth of the second generation were supported by as little as 0·02 energy % LA but only in the presence of 0·5 energy % LNA. Their detailed tissue analysis showed that the physiological requirement for LA to sustain normal reproduction, pregnancy and growth of the subsequent generation was much lower than was needed to achieve a plateau in tissue *n*-6 PUFA, whether in brain, liver or elsewhere.

British Journal of Nutrition

Preliminary results of a key experiment needed to verify whether LA requirements have been overestimated because of an absence of *n*-3 PUFA in the EFA deficiency model have recently been reported[96]. In that study, young rats consumed diets containing 0, 0·1, 0·25, 0·5, 1, 2 or 4 energy % LA, all of which also contained 0·5 energy % LNA. There was also an EFA-deficient group (0 energy % LA and 0 energy % LNA). Hence, an LA-deficient group also receiving no *n*-3 PUFA (EFA deficient) could be compared with an LA-deficient group receiving a modest intake of LNA (0·5 energy %). Plasma and tissue levels of *n*-6 PUFA were predictably the lowest in the groups with the lowest LA intake. However, 20 : 3*n*-9 was about two-fold higher in plasma, muscle and liver of the EFA-deficient group than in the 0 energy % LA-deficient group receiving 0·5 energy % LNA. The triene:tetrane ratio was also 30–150 % higher in the EFA-deficient compared with the 0 % LA group[96]. The point is that 0·5 energy % LNA did not exacerbate the effect of even extreme LA deficiency on tissue fatty acid profiles but, indeed, had the reverse effect; it reduced the severity of LA deficiency. At LA intakes < 1 energy %, growth of the rats over the 6-week study period was not significantly different across groups, again emphasising that low LA intake alone is not as severe as EFA deficiency and is not exacerbated by concurrent intake of as much as 0·5 energy % LNA. This recent study directly supports the conclusion of previous studies showing that as much as 0·5 energy % LNA protects against *n*-6 PUFA deficiency and reduces LA requirement[61,92,93,95].

Details of this particular study[96] have been provided because they show that by excluding dietary *n*-3 PUFA in EFA-deficient diets, animal modelling of LA (or *n*-6 PUFA) requirements has been flawed and needs serious re-evaluation. As concluded by Bourre *et al.* [61], we believe that $< 0{\cdot}5$ energy % LA may well be sufficient for normal growth, development and reproduction in rats. A study needs to be done with several groups consuming between 0·0 and 2·0 energy % LA in the presence of some *n*-3 PUFA intake (0·5 energy % LNA should be adequate in rats) so that dietary LA requirement can be correctly estimated. The flawed model of EFA deficiency also appears to have distorted the functional relationship between *n*-6 and *n*-3 PUFA as one of competition when, at physiologically relevant intakes, such competition may be less important or may not exist at all.

Although LA intake clearly appears to be above true LA requirements, a critical point is to assess its effect on tissue ARA in humans, i.e. is tissue ARA coming predominatly from synthesis from LA or from ARA supply from the diet? LA tracer and supplementation studies in healthy adults suggest that about 0·5 % of dietary LA is converted to ARA, or about 100 mg/d[77]. About 100–200 mg/d ARA seems to be present in the diet, at least in North America and Japan[97,98]. However, in foods commonly consumed in the USA in which ARA levels were analysed, its content is about 2-fold higher than previously assessed from food composition tables[99]. This observation favours a significant underestimation of ARA content in foods and is consistent with a daily intake of 500 mg in France estimated from production data[5]. If it were so, ARA intake should be predominant in determining ARA availability.

Concluding remarks

The prevalence of overweight and obesity has escalated dramatically during the last decades both in children and adults. Among dietary factors, profound quantitative and qualitative changes have taken place in the last four decades in the Western industrialised world, particularly the rising intake of *n*-6 and declining intake of *n*-3 PUFA by both humans and domesticated animals. These changes are due first to changes in the feeding pattern of breeding stock, for example, animal feed with a predominance now of maize–soyabean diet, and second to human dietary habits, for example, increased consumption of plant oils (sunflower-seed oil mainly)[5]. Along with an increase in fat consumption and sedentarity, we suggest that this disequilibration in PUFA intake is an emerging risk factor contributing in addition to long-term net positive energy balance.

The presence of a cause–effect relationship between the obesity epidemic and alterations of PUFA composition in the food chain during the last four decades is difficult to assess for various reasons: (i) the shape of the prevalence curves of overweight/obesity depends on the cut-off points for BMI; (ii) food processes and food habits have taken place rather smoothly; (iii) keeping in mind that fat mass enlargement proceeds through adipocyte hypertrophy but is mainly due to an increase in fat cell number, a significant lag time may exist between overproliferation of precursor cells and their differentiation in adipocytes.

As adipose tissue is the major peripheral target organ in which fatty acids enter via chylomicrons or VLDL, we propose that several aspects of disequilibrated PUFA metabolism conspire to stimulate fat cell formation and to increase the prevalence of overweight and obesity:

(1) Low *n*-3 PUFA intake;
(2) Overestimation of LA requirement and, as a direct consequence, the increased LA and ARA content of solid foods in the last decades which has been accompanied by a significant increase in the LA:LNA and ARA:(DHA + EPA) ratios;
(3) Stimulatory role of ARA and prostacyclin in adipogenesis;
(4) Antagonistic role played by ARA[39] and DHA[65] in adipogenesis;
(5) Permanent occurrence of adipose precursor cells throughout life.

Whether exposure of humans for a few generations is required (as it appears to be in rodents) in order to observe the fat mass-enhancing effect of *n*-6 PUFA-enriched diets, this would lead to a transgenerational but possibly reversible situation. As this PUFA disequilibration arises directly from the food chain, a re-evaluation of the agricultural and food policy would be most welcome as one component of a strategy aimed at addressing the ongoing obesity epidemic.

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British Journal of Nutrition

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