

## Dietary modulation of body composition and insulin sensitivity during catch-up growth in rats: effects of oils rich in *n*-6 or *n*-3 PUFA

Gayathri Yepuri<sup>1</sup>, Helena Marcelino<sup>1</sup>, Yasaman Shahkhalili<sup>2</sup>, Olivier Aprikian<sup>2</sup>, Katherine Macé<sup>2</sup>, Josiane Seydoux<sup>3</sup>, Jennifer L. Miles<sup>1</sup>, Jean-Pierre Montani<sup>1</sup> and Abdul G. Dulloo<sup>1\*</sup>

<sup>1</sup>Division of Physiology, Department of Medicine, University of Fribourg, Rue du Musée 5, CH-1700 Fribourg, Switzerland

<sup>2</sup>Nutrition and Health Department, Nestlé Research Center, CH-1000 Lausanne 26, Switzerland

<sup>3</sup>Department of Basic Neurosciences, Faculty of Medicine, University of Geneva, 1 rue Michel Servet 1211, Geneva 4, Switzerland

(Received 12 May 2010 – Revised 23 November 2010 – Accepted 7 December 2010 – First published online 31 January 2011)

### Abstract

The present study investigates whether excessive fat accumulation and hyperinsulinaemia during catch-up growth on high-fat diets are altered by *n*-6 and *n*-3 PUFA derived from oils rich in either linoleic acid (LA),  $\alpha$ -linolenic acid (ALA), arachidonic acid (AA) or DHA. It has been shown that, compared with food-restricted rats refed a high-fat (lard) diet low in PUFA, those refed isoenergetically on diets enriched in LA or ALA, independently of the *n*-6:*n*-3 ratio, show improved insulin sensitivity, lower fat mass and higher lean mass, the magnitude of which is related to the proportion of total PUFA precursors (LA + ALA) consumed. These relationships are best fitted by quadratic regression models ( $r^2 > 0.8$ ,  $P < 0.001$ ), with threshold values for an impact on body composition corresponding to PUFA precursors contributing 25–30% of energy intake. Isoenergetic refeeding on high-fat diets enriched in AA or DHA also led to improved body composition, with increases in lean mass as predicted by the quadratic model for PUFA precursors, but decreases in fat mass, which are disproportionately greater than predicted values; insulin sensitivity, however, was not improved. These findings pertaining to the impact of dietary intake of PUFA precursors (LA and ALA) and their elongated–desaturated products (AA and DHA), on body composition and insulin sensitivity, provide important insights into the search for diets aimed at counteracting the pathophysiological consequences of catch-up growth. In particular, diets enriched in essential fatty acids (LA and/or ALA) markedly improve insulin sensitivity and composition of weight regained, independently of the *n*-6:*n*-3 fatty acid ratio.

**Key words:** Obesity; Thermogenesis; Programming and insulin sensitivity; Weight regain

It has long been known from studies of nutritional rehabilitation in malnourished humans and other mammals that the recovery of body fat occurs at a disproportionately faster rate than that of lean tissue deposition<sup>(1,2)</sup>, independently of the level of dietary energy or protein supplementation<sup>(1,3,4)</sup>. This phenomenon of preferential catch-up fat, with lean tissue recovery lagging, has been demonstrated not only during weight recovery in adults who have lost weight in response to poverty and famine-related malnutrition<sup>(1–4)</sup>, anorexia nervosa<sup>(5)</sup> and disease-related cachexia<sup>(6–8)</sup>, but also during nutritional rehabilitation of undernourished children<sup>(9–12)</sup> and in young adolescents recovering from anorexia nervosa<sup>(13,14)</sup>. In more recent years, there has also been a resurgence of interest into this phenomenon of preferential catch-up fat in infants and younger children, primarily because its occurrence concomitant to hyperinsulinaemia

during catch-up growth<sup>(15–19)</sup> is viewed to be of central importance in the mechanisms by which catch-up growth predisposes to obesity, type 2 diabetes and CVD later in life<sup>(20–22)</sup>. Consequently, approaches that redirect nutrient partitioning from preferential catch-up fat to enhanced lean tissue accretion are of potential value as much as for protection against disease risks associated with catch-up growth after fetal or neonatal malnutrition, as for improving the recovery of lean tissue (and hence functional recovery) after malnutrition at any stage of growth.

However, progress in this field of nutritional energetics and body composition regulation has been limited by the necessity for precise determinations of body composition and concomitant measurements of energy balance in a dynamic state of weight gain. In addition, because differences in the level of energy intake tend to obscure effects due to diet composition

**Abbreviations:** AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; ARASCO, arachidonic acid single cell oil; DHASCO, docosahexaenoic acid single cell oil; HF, high fat; LA, linoleic acid.

\* **Corresponding author:** Dr A. G. Dulloo, fax +41-26-300 9734, email [abdul.dulloo@unifr.ch](mailto:abdul.dulloo@unifr.ch)

*per se*, the impact of various dietary formulations on lean and fat tissue deposition during weight recovery is often difficult to interpret.

In search for approaches that could alter nutrient partitioning during catch-up growth, we have therefore utilised rats as a model of food restriction–refeeding<sup>(23,24)</sup> which, similar to human infants and children showing catch-up growth, exhibit preferential catch-up fat associated with hyperinsulinaemia. In this animal model, both catch-up fat and hyperinsulinaemia can be demonstrated during refeeding on a low-fat (chow) diet in the absence of hyperphagia, and are exacerbated by isoenergetic refeeding on a high-fat (HF-lard) diet<sup>(24,25)</sup>. In an earlier investigation into the influence of dietary fat types on the composition of weight regained, we found that the exacerbation of fat deposition shown with certain dietary fats (lard, olive oil and menhaden fish oil) does not occur with coconut oil (rich in medium-chain fatty acids) or in safflower oil which is rich in *n*-6 PUFA<sup>(26)</sup>. Although diets high in these fatty acids are well known to be more thermogenic and less fattening during spontaneous growth than diets high in long-chain SFA<sup>(27–29)</sup>, an unexpected outcome of our previous study<sup>(26)</sup> is that it revealed that the efficacy of safflower oil in limiting catch-up fat resided in minor part through increased thermogenesis, with the major effect in reducing body fat attributed to a shift in energy partitioning in favour of lean tissue accretion.

Given that *n*-6 PUFA, essentially as linoleic acid (LA), account for more than two-thirds of total fatty acids in safflower oil, the primary objective of the study reported here was to gain further insights into the role of diets rich in the two essential fatty acids, LA and  $\alpha$ -linolenic acid (ALA), as well as their elongated and desaturated products arachidonic acid (AA) and DHA, in the modulation of body composition and whole-body insulin sensitivity during catch-up growth. The following specific questions were addressed: (1) is the dual effect on lean and fat mass obtained with safflower oil also observed with other oils rich in LA, such as maize oil, sunflower oil and grapeseed oil in which LA accounts for >40% of total fatty acids; (2) is there a dose–response relationship between dietary LA content and lean mass accretion during catch-up growth, and what would be the threshold level of dietary LA that would enhance the gain in protein mass during catch-up growth; (3) is the stimulatory effect of LA modified by partial substitution with ALA and hence by the *n*-6:*n*-3 fatty acid ratio; (4) is the enhanced lean mass observed with LA-rich safflower oil mimicked by oils rich in AA (a major metabolite of LA) or by DHA (a major metabolite of ALA); (5) what would be the impact of HF diets rich in these essential fatty acids (LA and ALA) or in their major metabolites (AA and DHA) on blood glucose homeostasis?

## Materials and methods

### General study design

All experiments were performed in male Sprague–Dawley rats (Elevage Janvier, St Bertherin, France). The rats, aged 6 weeks, were adapted to room and cage environments for at least 5 d

before the start of each experiment; they were caged singly in a temperature-controlled room ( $22 \pm 1^\circ\text{C}$ ) with a 12 h light–12 h dark cycle. They were maintained on a commercial pelleted chow diet (Kliba; Provimi-Lacta, Cossonay, Switzerland) consisting, by energy, of 24% protein, 66% carbohydrates and 10% fat, and had free access to tap water. The experiments were conducted after this period of adaptation in rats selected on the basis of body weight being within  $\pm 5$  g of the mean body weight (i.e. between 235 and 245 g). As in previously reported studies from our laboratory<sup>(24–26)</sup>, they were food restricted for 2 weeks at approximately 50% of their spontaneous *ad libitum* daily food intake. At the end of the food restriction period, groups of animals of similar mean body weight were re-fed a fixed ration of the various test diets at a level approximately equal in metabolisable energy content to the normal intake of non-restricted (control) animals, i.e. 85 kcal (355 kJ) of the test diet that corresponds to 28 g of chow daily per animal. During all refeeding experiments, the diets were provided in food containers secured to the back of the cage in a way that prevented food spillage, and each animal consumed the test diet provided during the refeeding period. After 2 weeks of energy-controlled refeeding, all animals were killed, and their carcasses were analysed for body composition as described below. Animals used in the present studies were maintained in accordance with our institute's regulations and guide for the care and use of laboratory animals, and all experimental procedures were performed under conditions approved by the Ethical Committee of the State of Fribourg Veterinary Office.

### Diets

The test oils were incorporated into semi-synthetic and synthetic diets whose nutrient compositions are shown in Table 1. For the semi-synthetic diets, the fatty acid profile of both chow and test oils was analysed by GC as detailed below. The overall fatty acid composition of the HF diets, determined from the fatty acid composition of both test oil and basal mixture, and expressed as SFA, MUFA and PUFA, is presented in Table 2. The energy digestibility of the test diets was determined during preliminary studies, which involved feeding groups of three animals each of the test diets over 5 d (after a period of food restriction as described above), measuring food intake and collecting faeces; any food spilled was collected, dried and weighed, and suitable corrections were made to the gross food intake. From energy density of diets and faeces measured by bomb calorimetry<sup>(30)</sup>, the values for energy digestibility were found to be similar, varying between 86.2 and 86.8% of energy intake. The various food ingredients were purchased from the following sources: ground standard chow (Provimi-Lacta); vitamin-free casein, DL-methionine and choline chloride (Sigma, St Louis, MO, USA); American Institute of Nutrition (AIN)-76 vitamin mixture and AIN 76 mineral mixture were purchased from MP Biomedicals (Cleveland, OH, USA); rapeseed oil, maize oil, sunflower oil, grapeseed oil and safflower oil were purchased from local supermarkets (Migros/Coop, Fribourg, Switzerland); safflower oil and linseed oil were

**Table 1.** Composition of high-fat diets

Semi-synthetic diets				
Diet composition (g/100 g diet)				
Basal mix*				
Chow	59.0			
Casein	10.8			
L-Met	0.1			
Sunflower oil	1.4			
AIN mineral mix	1.7			
AIN vitamin mix	0.5			
Choline chloride	0.1			
Test fat/oil†	26.4			
List of test fat/oils‡				
Lard				
Rapeseed oil				
Maize oil				
Sunflower oil				
Grapeseed oil				
Linseed oil				
Safflower oil – S1				
Safflower oil – S2				
Safflower oil – S3				
ARASCO				
DHASCO				
Nutrient composition (% ME)				
Protein	21.0			
Fat	58.2			
Carbohydrates	20.8			
ME density (kJ/g diet)§	19.97			
Synthetic diets				
Diet composition§ (g/100 g diet)				
Casein	18.0			
L-Cys	0.25			
Mineral mix	4.5			
Vitamin mix	1.3			
<i>tert</i> -Butylhydroquinone	0.08			
Choline bitartrate	0.25			
Cellulose	6.5			
Sucrose	10			
Maize starch	32.1			
Oil mix	27			
Composition of oil mix‡ (g/27g total)				
	Diet 1	Diet 2	Diet 3	Diet 4
Coconut oil	2.59	5.43	12.5	12.9
Maize oil	0.01	0.03	0.80	1.75
Palm olein	0.27	6.27	6.40	5.54
Rapeseed oil	4.38	1.29	1.93	1.54
Linseed oil	0	1.79	0.64	5.30
Safflower oil	19.6	12.2	4.69	0
Nutrient composition‡ (% ME)				
Protein	15.4			
Fat	52			
Carbohydrates	32.6			
ME density (kJ/g diet)§	19.6			

AIN, American Institute of Nutrition; ARASCO, arachidonic acid single cell oil; DHASCO, docosahexaenoic acid single cell oil; ME metabolisable energy.

\* The basal mix provides 50% of total energy content, and its fat content contributes 8.2% of energy content (1.5% from SFA, 2.6% as MUFA, 4.1% as PUFA).

† The added fat was either lard (control diet) or test oils richer in PUFA, and provides 50% of total dietary energy content. S1, S2 and S3 refer to safflower oil purchased from three different commercial sources: S1, Migros, Fribourg, Switzerland; S2, Coop, Fribourg, Switzerland; S3, MP Biomedicals (Cleveland, OH, USA).

‡ The diet ingredients were purchased from companies in Switzerland: casein (Schweizerhall, Basel); L-cysteine (Fluka, Buchs, Switzerland); AIN-93 M mineral mix and AIN-93 M vitamin mix (Socochim, Lausanne); sucrose (Howeg, Bussigny, Switzerland); coconut oil, maize oil, palm olein, safflower oil and rapeseed oils (Sofinol SA, Manno); linseed oil (Sabo, Manno); cellulose (Christ water Technology, Basel). Maize starch and choline bitartrate were purchased from Synopharm (Barsbüttel, Germany).

§ ME density was estimated by computation using values (kJ/g) for the ME content of chow, 13.01; fat/oil, 37.66; carbohydrates, 16.736; protein, 16.736.

**Table 2.** Fatty acid composition of high-fat (HF) diets (as a percentage of dietary energy content) in experiments (a) varying in oils rich in linoleic acid (LA) and/or  $\alpha$ -linolenic acid (ALA), (b) varying in the ratio of safflower oil and linseed oil (S:L), (c) varying in PUFA and SFA, but with MUFA constant, or (d) varying in the ratio of arachidonic acid and docosahexaenoic acid single cell oils (A:D)

	SFA	MUFA	PUFA	<i>n</i> -6 PUFA*	<i>n</i> -3 PUFA*
(a) Study I					
HF-lard	25.1	24.8	8.2	7.3	0.9
HF rapeseed oil	5.5	35.0	17.8	13.4	4.4
HF maize oil	8.1	20.0	30.0	29.1	0.9
HF sunflower oil	7.4	16.2	34.5	33.8	0.6
HF grapeseed oil	7.8	11.9	38.3	37.6	0.7
HF safflower oil – S1	7.5	11.8	38.8	38.0	0.8
HF safflower oil – S2	7.2	9.8	41.1	40.5	0.6
HF safflower oil – S3	6.8	9.4	42.0	41.3	0.6
(b) Study II and IV					
HF S:L (1:0)	6.0	9.1	43.1	41.0	2.10
HF S:L (0:1)	6.0	12.1	40.1	15.5	24.6
HF S:L (1:1)	6.0	10.6	41.6	28.5	13.1
HF S:L (2:1)	6.0	10.1	42.1	32.5	9.6
HF S:L (1:2)	6.0	11.1	41.1	24.5	16.6
(c) Study III					
Diet 1	8.5	10.7	30.8	30	0.75
Diet 2	17.3	10.7	22.0	20	2.0
Diet 3	28.3	10.7	11.0	10	1.0
Diet 4	28.3	10.7	11.0	5.5	5.5
(d) Study V					
HF A:D (0:1)	15.0	18.2	24.8	–	20
HF A:D (1:3)	14.9	17.8	25.7	5	15
HF A:D (1:1)	14.5	16.7	27.1	10	10
HF A:D (3:1)	14.0	15.7	28.3	15	5
HF A:D (1:0)	14.0	14.9	29.1	20	–

\* For the diets in parts (a), (b) and (c), *n*-6 PUFA and *n*-3 PUFA are LA and ALA, respectively. For the diet in part (d), *n*-6 PUFA and *n*-3 PUFA are arachidonic acid and DHA, respectively.

also purchased from MP Biomedicals; the microbial oils arachidonic acid single cell oil (ARASCO) and docosahexaenoic acid single cell oil (DHASCO) were obtained from Martek Bioscience Corporation (Columbia, MD, USA). DHASCO refers to a mixture of an oil extracted from the unicellular alga *Cryptocodinium cohnii* and high-oleic sunflower oil, and contains about 40% of product weight as DHA. ARASCO refers to a mixture of an oil extracted from the unicellular fungus *Mortierella alpina* and high-oleic sunflower oil, and contains about 40% AA by weight. There are no detectable amounts of EPA in ARASCO or DHASCO.

### Body composition analysis

After the animals were killed by decapitation, the skull, thorax and abdominal cavity were incised, and the gut was cleaned of undigested food. The whole carcasses were dried to a constant weight in an oven at 70°C and were subsequently homogenised for the analysis of fat content by the Soxhlet extraction method as described previously<sup>(23,24)</sup>; the dry lean mass (a proxy of protein mass) was determined by subtracting total body fat and body water content from body weight.

### Fatty acid analysis

Total lipids of diet (50 mg), adipose tissue (50 mg) and liver (300 mg) were extracted according to the method of Folch *et al.*<sup>(31)</sup> in the presence of triheptadecanoin as an internal

standard (Avanti, Alabaster, AL, USA). Total lipids of muscle (300 mg) were extracted by the method of Srivastava *et al.*<sup>(32)</sup>, using methanol and chloroform sequentially in the volume ratio of 1:3 and in the presence of diheptadecanoyl-*sn*-glycero-3-phosphocholine as an internal standard (Avanti). The extracted lipids were transesterified to their methyl esters in a 3% H<sub>2</sub>SO<sub>4</sub> methanolic reagent for 3 h at 80°C, extracted with hexane, dried under N<sub>2</sub> and resuspended into hexane. Fatty acid methyl ester separation was performed by automated GLC (Hewlett Packard 6890 series; Waldbronn, Germany) using a flame ionisation detector (280°C) and a BPX 70 column (30 m × 0.22 mm internal diameter × 0.25 μm thickness). A standard mixture (GLC-36 Nestlé; Nu-Check-Prep, Elysian, MN, USA) of fatty acid methyl esters was also injected to identify fatty acid methyl ester peaks. Fatty acid concentrations in diets were determined on a weight basis by comparing GLC peak area to the internal standard. Fatty acid concentrations in tissues were calculated as percentages of total fatty acids by comparing GLC area of each fatty acid to the total fatty acids.

### Glucose tolerance tests

Glucose tolerance tests were performed between days 12 and 14 of refeeding, according to the protocol described previously<sup>(24)</sup>. Food was removed early in the morning (07.00 hours). At 6–7 h later, i.e. in the post-absorptive phase, blood was drained from the tail vein and immediately

followed by an intraperitoneal injection of glucose (2 g/kg body weight). At intervals of 30 min for the next 2 h period, blood samples were taken from the tail vein in heparinised tubes and transferred on ice. The blood samples were then centrifuged, and the plasma was frozen and stored at  $-20^{\circ}\text{C}$  for later assays of plasma glucose and insulin. Plasma glucose was determined using a Beckman glucose analyser (Beckman Instruments, Palo Alto, CA, USA), while plasma insulin was assessed using a rat insulin ELISA kit (Crystal Chem, Inc., Downer's Grove, IL, USA).

### Data analysis and statistics

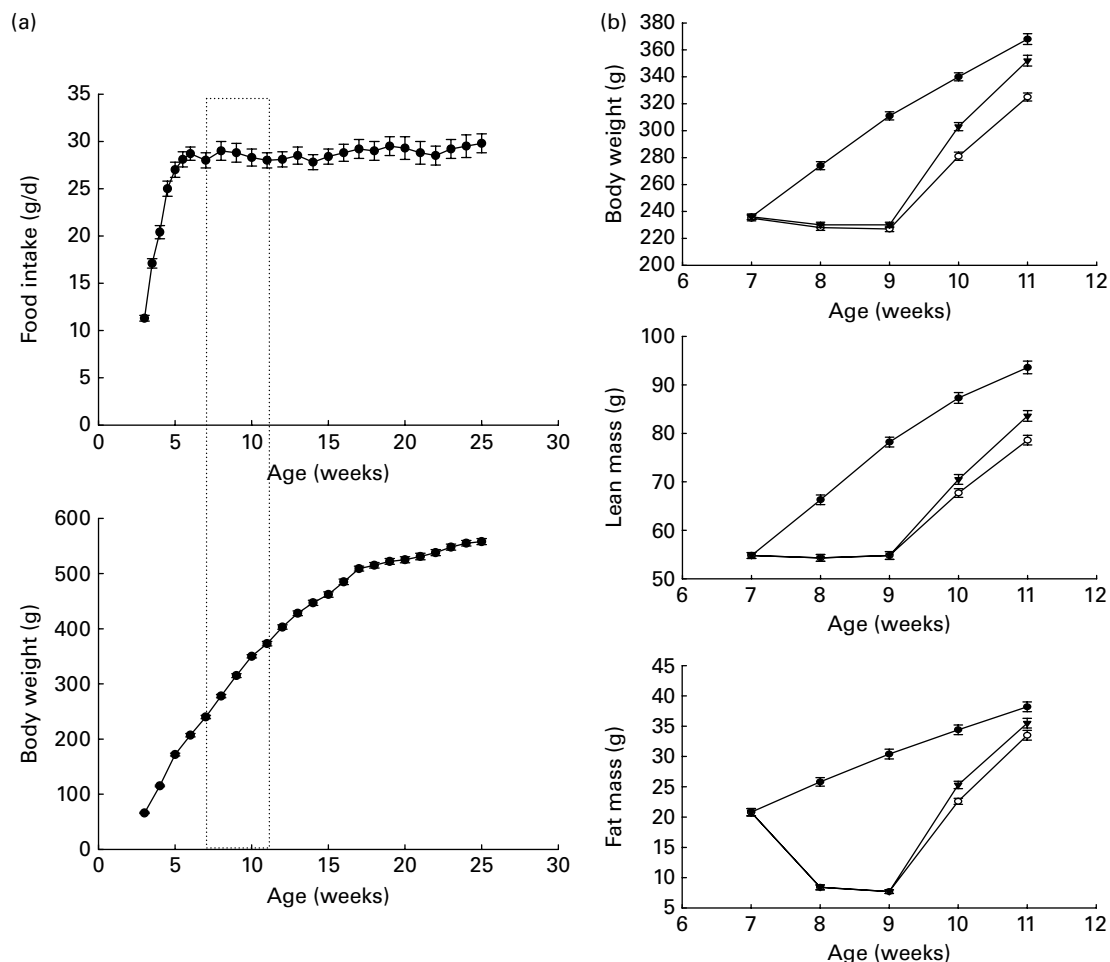
All data are presented as means with their standard errors. In general, the data were analysed by one-way ANOVA, followed by *post hoc* pairwise comparisons using Scheffé's test after ANOVA had established significant differences. Data collected over time (i.e. growth and food intake) were analysed by

ANOVA with repeated-measures design. The statistical treatment of data was performed using the computer software STATISTIK 8 (Analytical Software, St Paul, MN, USA).

## Results

### Rat model of catch-up fat during refeeding after food restriction

As reported previously<sup>(24–26)</sup>, the rats were studied in an age range characterised by a high rate of weight gain for spontaneously growing controls (Fig. 1(a)). During the food restriction period (Fig. 1(b)), the fixed ration diet of 14 g chow daily represented approximately 50% of their spontaneous *ad libitum* daily food intake. The body weights of the food-restricted rats (between 234 and 238 g) were only slightly and non-significantly reduced relative to their weights at the onset of the food restriction period (Fig. 1(b)). Comparison of



**Fig. 1.** (a) Growth profile and daily food intake of male Sprague–Dawley rats maintained *ad libitum* on a standard chow diet between the age of 3 and 25 weeks under conditions of our laboratory. The rectangular dotted line encloses the age range (7–11 weeks) and growth period pertaining to our studies of food restriction and refeeding, and during which the rats show rapid increases in body weight ( $P < 0.001$ ). Within this age range of 7–11 weeks, food intake (28 g chow daily) was not significantly different across time, such that providing 14 g chow daily corresponds to a 50% reduction of *ad libitum* food intake throughout this period. (b) Rat model of food restriction–refeeding: After growth arrest due to food restriction, refeeding *ad libitum* leads to hyperphagia (+10% higher food intake than controls,  $P < 0.01$ ) and catch-up growth in both lean body mass ( $P < 0.01$ ) and fat mass ( $P < 0.001$ ). Prevention of hyperphagia by providing the refeed animals with the same amount of food as controls (i.e. 28 g chow daily) still results in catch-up in fat mass ( $P < 0.01$ ) but not in lean body mass. Values are means, with standard errors represented by vertical bars ((a)  $n$  10; (b)  $n$  6). —●—, Control *ad libitum*; —○—, refeed normophagic; —▼—, refeed hyperphagic.

the body composition of groups of animals at the onset and at the end of the 2-week food restriction period shows significant reductions in body fat ( $-50\%$ ,  $P<0.01$ ) but not in dry lean mass. Refeeding the animals with the same amount of food as controls (i.e. 28 g chow daily) results in greater gain in fat mass ( $P<0.01$ ), but not in lean body mass, relative to controls, which is hence in line with our previous demonstrations of an increased metabolic efficiency directed at catch-up fat in this rat model of energy-controlled refeeding<sup>(23,24)</sup>.

### Study I: screening of oils rich in linoleic acid

In study I, two separate experiments were conducted during which groups of food-restricted animals ( $n$  6–8) were refed isoenergetic amounts of HF diets in which the main source of fat was derived either from safflower oil, sunflower oil, maize oil or from grapeseed oil, i.e. oils in which LA contributed  $>40\%$  of total fatty acids. In each experiment, these HF diets were compared with one group refed isoenergetically on a HF-lard (control) diet.

#### Expt 1: comparison across safflower oils

The specific aim of the first experiment was to test whether safflower oils obtained from three different commercial sources were all equally capable of altering body composition during refeeding. The total  $n-6$  PUFA (essentially LA) content of these three safflower oils varied between 69 and 76%, and LA contributed 38, 40.5 and 41.3% of dietary energy content in HF safflower – S1, HF safflower – S2 and HF safflower – S3, respectively (Table 2, part a). The results obtained for body composition are shown in Table 3. They were all effective in increasing lean mass and reducing body fat relative to the HF-lard diet, but the highest lean mass and lowest fat mass were achieved with the HF safflower diet (S3) with the highest LA content (41.3%). Conversely, the HF safflower oil diet (S1) with the lowest LA content (38%) resulted in a less marked effect on body composition.

#### Expt 2: comparison with other oils rich in linoleic acid

In the second experiment that screened other oils rich in LA, refeeding was conducted with the HF diets made either

from safflower oil (S3), grapeseed oil, sunflower oil or from maize oil, in which LA contributed between 29 and 41% of dietary energy content (Table 2, part a). They were compared with the HF diets rich in lard or in rapeseed oil, both of which contain  $<20\%$  LA. The results, presented in Table 4, indicate that the HF diets made from grapeseed oil or sunflower oil, and to a lesser extent from maize oil, resulted in higher lean mass, lower fat mass and lower fat:lean ratio relative to the HF diets made from lard or rapeseed oil. However, these data also indicate that HF safflower oil was more effective than either grapeseed oil or sunflower oil in increasing lean mass and reducing fat mass, as indicated by the lowest fat:lean ratio obtained with the HF safflower oil diet compared with the other LA-rich diets. Thus, while all HF diets rich in LA had an impact on body composition, their effects varied as a function of their LA content within the range of 29–41%.

### Study II: linoleic acid v. $\alpha$ -linolenic acid

This experiment examined the extent to which the changes in body composition observed with the HF safflower oil diet would persist if the high LA content were reduced at the expense of an increase in ALA. This was achieved by mixing various proportions of safflower oil and linseed oil in HF diet preparations, such that, as shown in Table 2 (part b), the ratio of LA:ALA (and hence the  $n-6:n-3$  ratio) can be markedly varied while maintaining the total PUFA (LA + ALA) content at 40–43% of dietary energy content of the HF diets. The results on body weight and body composition after 2 weeks of isoenergetic refeeding (Table 5) indicate that they were all equally effective in increasing lean mass and in reducing body fat or the fat:lean ratio when compared with the HF-lard control diet. These changes are also reflected in an increase in mass of organs and tissues of the lean body mass (skeletal muscle, heart, liver and kidney) and in a decrease in both epididymal and retroperitoneal fat pad mass (Table 6). The application of ANOVA specifically to groups refed diets high in PUFA shows no significant differences in body composition or in organ/tissue mass across these HF diets. Similarly, a test of glucose tolerance conducted in the groups refed HF-lard, HF safflower oil, HF linseed or a mixture of HF safflower and linseed oils on the last day of this experiment also indicates that refeeding on diets high in LA or

**Table 3.** Effects of isoenergetic high-fat (HF) diets made from safflower oil from various sources on body weight and body composition (Mean values with their standard errors,  $n$  8)

Group	HF diet	Body composition									
		Body weight (g)		Water (g)		Fat (g)		Lean (g)		Fat:lean	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	HF lard	362	5	228	5	56.1 <sup>a</sup>	2.4	78.2 <sup>a</sup>	0.9	0.72 <sup>a</sup>	0.04
2	HF safflower – S1*	354	4	216	4	52.2 <sup>a,b</sup>	2.4	85.3 <sup>b</sup>	2.0	0.62 <sup>a,b</sup>	0.04
3	HF safflower – S2*	364	7	224	7	49.6 <sup>a,b</sup>	2.3	90.2 <sup>b</sup>	1.6	0.55 <sup>b</sup>	0.03
4	HF safflower – S3*	370	5	233	6	45.2 <sup>b</sup>	2.0	91.6 <sup>c</sup>	0.9	0.49 <sup>b</sup>	0.03
ANOVA		NS		NS		$P<0.02$		$P<0.001$		$P<0.001$	

<sup>a,b,c</sup> Mean values with unlike superscript letters were significantly different by a *post hoc* pairwise comparison ( $P<0.05$ ).

\* S1, S2 and S3 refer to safflower oil purchased from three different commercial sources.

**Table 4.** Effects of isoenergetic high-fat (HF) diets from various oils rich in linoleic acid on body weight and body composition (Mean values with their standard errors, *n* 6)

Group	HF diet	Body composition									
		Body weight (g)		Water (g)		Fat (g)		Lean (g)		Fat:lean	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	HF lard	366	3	231	3	57.1 <sup>a</sup>	2.2	77.9 <sup>a</sup>	0.6	0.73 <sup>a</sup>	0.03
2	HF rapeseed	367	5	232	6	56.3 <sup>a</sup>	3.1	79.1 <sup>b</sup>	1.3	0.72 <sup>a</sup>	0.05
3	HF safflower	367	2	232	3	45.0 <sup>a</sup>	1.2	90.2 <sup>b</sup>	0.5	0.50 <sup>b</sup>	0.01
4	HF grapeseed	359	4	220	6	52.7 <sup>a</sup>	3.6	86.3 <sup>a,b</sup>	1.8	0.62 <sup>a,b</sup>	0.06
5	HF sunflower	367	3	230	4	48.5 <sup>a</sup>	2.4	87.5 <sup>b</sup>	1.5	0.56 <sup>a,b</sup>	0.04
6	HF maize	357	3	223	4	53.3 <sup>a</sup>	1.6	80.8 <sup>a,b</sup>	0.9	0.66 <sup>a,b</sup>	0.02
ANOVA		NS		NS		<i>P</i> <0.01		<i>P</i> <0.001		<i>P</i> <0.001	

<sup>a,b</sup> Mean values with unlike superscript letters were significantly different by a *post hoc* pairwise comparison (*P*<0.05).

ALA resulted in similarly lower insulin response than refeeding on the HF-lard diet, with these reductions being significant over the time period of 30–90 min after the glucose load, and when assessed as area under the curve (Table 5). Plasma glucose profile and area under the curve for plasma glucose did not differ across the four groups. Thus, these results suggest that it is the total intake of PUFA precursors (LA + ALA), rather than the specific intake of LA or ALA, that has an impact on body composition and insulin sensitivity during refeeding.

#### Study III: high PUFA v. low MUFA

As indicated in Table 2 (parts a and b), the variation in PUFA content is inversely related to that in MUFA + SFA content, and more specifically related ( $r^2$  0.79; *P*<0.001) to variations in MUFA content given that SFA content is relatively constant. To delineate variations in high PUFA from those lower in MUFA, groups of animals were refed synthetic HF diets which were formulated so that PUFA content varied between 11 and 31%, but with the contribution of MUFA being kept at a low but constant level of about 11% (Table 2, part c). This diet formulation also resulted in a wide range in the ratio of

*n*-6:*n*-3 fatty acids, namely between 1 and 40. The results of refeeding these test diets in isoenergetic amounts for 2 weeks on body weight and body composition are presented in Table 7. Compared with the group refed the diet with low PUFA content (group 4), only the group refed the HF diet with the highest PUFA content (group 1) showed significantly higher final weight (+7–10%), body water (+6%) and lean mass (+8%), and lower body fat (–12 to –17%).

#### Study IV: fatty acid profiling in organs/tissue

To examine the extent to which refeeding diets enriched with LA, ALA or both have an impact on the proportion of these essential fatty acids and their metabolites in key peripheral tissues and organs, a study was conducted to assess the fatty acid composition of the liver, white adipose tissue (epididymal) and skeletal muscle (gastrocnemius). These tissues/organs were harvested from groups of rats refed for 9 d on HF diets enriched either with lard, safflower oil, linseed oil or with a 1:1 mixture of safflower and linseed oils (Table 2, part b), and the fatty acid analysis of these tissues/organs was performed (Table 8). Compared with animals refed the HF-lard diet, those refed either one of the three PUFA-enriched diets

**Table 5.** Effect of high-fat (HF) diets made from different ratios of safflower oil (S) and linseed oil (L) on body weight and body composition, as well as on plasma glucose and insulin in response to a glucose load

(Mean values with their standard errors, *n* 8)

Group	HF diet	Body composition										Glucose tolerance test*			
		Body weight (g)		Water (g)		Fat (g)		Lean (g)		Fat:lean		Glucose (AUC)†		Insulin (AUC)†	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	HF lard	362	3	232	3	51.3 <sup>a</sup>	1.1	78.3 <sup>a</sup>	0.8	0.66 <sup>a</sup>	0.02	5503	360	381 <sup>a</sup>	40
2	HF S:L (1:0)	370	3	239	4	41.3 <sup>b</sup>	0.9	89.6 <sup>b</sup>	0.7	0.46 <sup>b</sup>	0.01	5539	444	180 <sup>b</sup>	46
3	HF S:L (0:1)	369	3	238	4	43.4 <sup>a,b</sup>	2.1	87.7 <sup>b</sup>	1.2	0.50 <sup>b</sup>	0.03	5098	473	201 <sup>b</sup>	22
4	HF S:L (1:1)	363	6	232	6	42.9 <sup>b</sup>	1.6	88.0 <sup>b</sup>	1.5	0.49 <sup>b</sup>	0.02	5706	914	196 <sup>b</sup>	46
5	HF S:L (2:1)	369	5	239	6	40.4 <sup>b</sup>	1.9	89.9 <sup>b</sup>	0.7	0.45 <sup>b</sup>	0.02	–	–	–	–
6	HF S:L (1:2)	369	2	238	3	41.6 <sup>b</sup>	2.1	88.9 <sup>b</sup>	0.6	0.47 <sup>b</sup>	0.02	–	–	–	–
ANOVA		NS		NS		<i>P</i> <0.002		<i>P</i> <0.001		<i>P</i> <0.001		NS		<i>P</i> <0.01	

<sup>a,b</sup> Mean values with unlike superscript letters were significantly different by *post hoc* pairwise comparison (*P*<0.05).

\* Glucose tolerance test was conducted only in groups 1–4.

† AUC refers to the area under the curve for plasma glucose (mg/100 ml per 2 h) or insulin curve (ng/ml per 2 h) above baseline (pre-glucose load) plasma levels.

**Table 6.** Organ/tissue mass after refeeding with high-fat (HF) diets made from lard, safflower oil (S), linseed oil (L) or a 1:1 mixture of these two oils (Mean values with their standard errors, *n* 8)

Group	HF diet	Adipose tissue* (g)		Skeletal muscle† (g)		Heart (g)		Liver (g)		Kidney (g)‡	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	HF lard	6.72 <sup>a</sup>	0.13	2.83 <sup>a</sup>	0.03	0.96 <sup>a</sup>	0.02	11.4 <sup>a</sup>	0.17	1.14 <sup>a</sup>	0.02
2	HF S:L (1:0)	5.61 <sup>b</sup>	0.10	2.99 <sup>b</sup>	0.05	1.01 <sup>a</sup>	0.01	12.3 <sup>b</sup>	0.25	1.21 <sup>b</sup>	0.02
3	HF S:L (0:1)	5.50 <sup>b</sup>	0.26	3.01 <sup>b</sup>	0.04	0.99 <sup>a</sup>	0.01	12.2 <sup>b</sup>	0.16	1.21 <sup>b</sup>	0.01
4	HF S:L (1:1)	5.63 <sup>b</sup>	0.13	2.99 <sup>b</sup>	0.05	1.00 <sup>a</sup>	0.02	12.4 <sup>b</sup>	0.13	1.22 <sup>b</sup>	0.02
ANOVA		<i>P</i> <0.001		<i>P</i> <0.01		<i>P</i> <0.05		<i>P</i> <0.01		<i>P</i> <0.01	

<sup>a,b</sup> Mean values with unlike superscript letters were significantly different by a *post hoc* pairwise comparison (*P*<0.05).

\* Sum of epididymal and retroperitoneal fat pads.

† Sum of gastrocnemius, tibialis anterior and soleus muscles.

‡ Sum of both kidneys.

show a higher proportion of tissue fatty acids as total PUFA at the expense of lower proportions as SFA and MUFA, irrespective of the organ/tissue studied. These increases in tissue PUFA as *n*-6 PUFA or *n*-3 PUFA reflect the content of the diet enriched in LA or ALA, respectively. Thus, relative to the HF-lard group, the groups that consumed LA-enriched diets with safflower oil show increases in tissue LA that account for more than 80% of the increase in *n*-6 PUFA in the liver, muscle and adipose tissue. Similarly, in the groups that consumed the ALA-enriched diets with linseed oil, the increase in tissue ALA accounts for most (78–95%) of the increase in *n*-3 PUFA in adipose tissue and skeletal muscle. In the liver, however, ALA and ALA metabolites accounted for about 50% each for the increase in total *n*-3 PUFA in animals consuming the ALA-enriched diets, with EPA and DHA in turn accounting for about two-thirds of the increase in ALA metabolites. Furthermore, whereas EPA was significantly increased in all three tissues of animals refed the ALA-enriched diets, a significant increase in DHA only occurred in the adipose tissue. Similarly, AA, a main metabolite of LA, was found to be significantly higher only in adipose tissue of the group refed the LA-enriched diet with safflower oil compared with the HF-lard group.

### Study V: high arachidonic acid and DHA oils

To test whether the effect of HF diets based on oils rich in LA and/or ALA on body composition and insulin sensitivity could be mimicked by oils rich in their elongated products (AA or DHA, respectively), refeeding studies were conducted to assess the impact of HF diets based on microbial oils ARASCO and DHASCO (rich in AA) or DHASCO (rich in DHA) or mixtures of ARASCO and DHASCO to yield different ratios of AA:DHA, as indicated in Table 2 (part d). The results of these experiments comparing HF diets containing isoenergetic amounts of either lard, a 1:1 mixture of safflower and linseed oils, ARASCO or DHASCO are presented in Table 9. They indicate that, compared with the HF-lard diet, refeeding either the HF ARASCO or HF DHASCO diet resulted in a marked decrease in body fat by 18–24% and a marginal increase in lean mass (+5%). Another study comparing diets with different proportions of ARASCO and DHASCO showed no significant differences in body composition in animals refed on these diets (Table 10). By contrast, these studies indicate that diets in which the fat was derived primarily from ARASCO resulted in significantly raised plasma glucose concentrations during the test of glucose tolerance compared with the other oils; this being reflected in the area under the

**Table 7.** Effects of various isoenergetic high-fat (HF) synthetic diets (1–4) differing in PUFA and SFA, but with MUFA constant, on body weight and composition\*

(Mean values with their standard errors, *n* 6)

Group†	Diet		Body composition									
			Body wt (g)		Water (g)		Fat (g)		Lean (g)		Fat:lean	
	PUFA (% diet energy)	<i>n</i> -6: <i>n</i> -3 (LA:ALA)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	31	40:1	371	1	239 <sup>a</sup>	2	47.1 <sup>a</sup>	1.5	84.7 <sup>a</sup>	0.4	0.56 <sup>a</sup>	0.02
2	22	10:1	362	2	226 <sup>b</sup>	2	56.7 <sup>b</sup>	2.5	79.2 <sup>b</sup>	0.7	0.72 <sup>b</sup>	0.04
3	11	10:1	364	3	232 <sup>a,b</sup>	3	53.7 <sup>a,b</sup>	1.5	78.1 <sup>b</sup>	1.0	0.69 <sup>b</sup>	0.03
4	11	1:1	361	4	229 <sup>a,b</sup>	5	55.0 <sup>a,b</sup>	1.4	77.3 <sup>b</sup>	0.9	0.71 <sup>b</sup>	0.02
HF lard (positive control)			366	5	234	5	55.3	1.8	78.6	0.9	0.68	0.04
ANOVA			<i>P</i> =0.07		<i>P</i> <0.05		<i>P</i> =0.01		<i>P</i> <0.001		<i>P</i> <0.01	

LA, linoleic acid; ALA,  $\alpha$ -linolenic acid.

<sup>a,b</sup> Mean values with unlike superscript letters were significantly different by a *post hoc* pairwise comparison (*P*<0.05).

\* An additional group refed on a HF-lard semi-synthetic diet was added as a positive control.

† In addition to the groups (1–4) refed on synthetic diets, another group refed isoenergetically on a HF-lard (semi-synthetic) diet has been added as a positive control, and is not included in the statistical analysis of data for the groups (1–4) refed the synthetic diets.



**Table 8.** Fatty acid profile in tissues/organs after 9 d of refeeding with high-fat diets made from lard, safflower oil, linseed oil or a 1:1 mixture of these two oils\*(Mean values with their standard errors, *n* 6)

	Lard		Safflower		Linseed		Mix (1:1)		ANOVA
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
<b>Muscle</b>									
Total SFA	35.0 <sup>a</sup>	0.6	29.3 <sup>b</sup>	1.8	27.7 <sup>b</sup>	1.7	26.4 <sup>b</sup>	0.9	<i>P</i> =0.001
Total MUFA	29.5 <sup>a</sup>	2.7	17.4 <sup>b</sup>	1.7	18.8 <sup>b</sup>	2.0	18.7 <sup>b</sup>	1.3	<i>P</i> =0.001
Total PUFA	35.5 <sup>a</sup>	2.2	53.3 <sup>b</sup>	0.7	53.6 <sup>b</sup>	0.4	54.9 <sup>b</sup>	0.5	<i>P</i> <0.0001
PUFA <i>n</i> -6	27.9 <sup>a</sup>	1.3	46.7 <sup>b</sup>	1.2	30.3 <sup>a</sup>	1.0	38.1 <sup>c</sup>	0.5	<i>P</i> <0.0001
C18:2 <i>n</i> -6 (LA)	18.3 <sup>a</sup>	0.4	34.6 <sup>b</sup>	2.9	22.4 <sup>a</sup>	0.5	29.9 <sup>c</sup>	0.6	<i>P</i> <0.0001
C18:3 <i>n</i> -6	–	–	–	–	–	–	–	–	–
C20:3 <i>n</i> -6	0.4	0.0	0.4	0.0	0.3	0.0	0.3	0.0	NS
C20:4 <i>n</i> -6 (AA)	8.5	1.0	10.4	1.7	7.1	1.0	7.3	0.7	NS
C22:4 <i>n</i> -6	0.7 <sup>a</sup>	0.1	1.2 <sup>b</sup>	0.2	0.3 <sup>c</sup>	0.0	0.4 <sup>c</sup>	0.0	<i>P</i> <0.0001
PUFA <i>n</i> -3	7.6 <sup>a</sup>	0.9	6.6 <sup>a</sup>	0.9	23.3 <sup>b</sup>	0.7	16.9 <sup>c</sup>	0.3	<i>P</i> <0.0001
C18:3 <i>n</i> -3(ALA)	0.9 <sup>a</sup>	0.1	0.5 <sup>a</sup>	0.1	13.3 <sup>b</sup>	2.0	8.3 <sup>c</sup>	0.8	<i>P</i> <0.0001
C20:5 <i>n</i> -3 (EPA)	0.1 <sup>a</sup>	0.0	–	–	1.04 <sup>b</sup>	0.1	0.4 <sup>c</sup>	0.0	<i>P</i> <0.0001
C22:5 <i>n</i> -3	1.2 <sup>a</sup>	0.2	1.1 <sup>a</sup>	0.2	2.6 <sup>b</sup>	0.4	2.1 <sup>b</sup>	0.2	<i>P</i> <0.001
C22:6 <i>n</i> -3 (DHA)	5.4	0.8	5.1	0.8	6.1	0.9	6.0	0.7	NS
<b>Liver</b>									
Total SFA	38.1 <sup>a</sup>	0.5	25.3 <sup>b</sup>	0.7	30.6 <sup>c</sup>	0.5	27.2 <sup>d</sup>	0.6	<i>P</i> <0.0001
Total MUFA	21.5 <sup>a</sup>	1.3	12.9 <sup>b</sup>	0.4	14.8 <sup>b</sup>	0.5	13.7 <sup>b</sup>	0.6	<i>P</i> <0.0001
Total PUFA	40.4 <sup>a</sup>	1.0	61.8 <sup>b</sup>	0.6	54.7 <sup>b</sup>	0.7	59.2 <sup>b</sup>	0.5	<i>P</i> <0.0001
PUFA <i>n</i> -6	33.3 <sup>a</sup>	0.8	58.8 <sup>b</sup>	0.8	31.7 <sup>a</sup>	0.7	44.4 <sup>c</sup>	0.3	<i>P</i> <0.0001
C18:2 <i>n</i> -6 (LA)	14.4 <sup>a</sup>	0.3	35.2 <sup>b</sup>	0.9	18.7 <sup>c</sup>	0.7	27.0 <sup>d</sup>	0.5	<i>P</i> <0.0001
C18:3 <i>n</i> -6	0.2 <sup>a</sup>	0.0	0.7 <sup>b</sup>	0.0	0.2 <sup>a</sup>	0.0	0.3 <sup>c</sup>	0.0	<i>P</i> <0.0001
C20:3 <i>n</i> -6	0.8 <sup>a</sup>	0.1	1.3 <sup>b</sup>	0.1	1.1 <sup>c</sup>	0.0	1.0 <sup>c</sup>	0.1	<i>P</i> =0.0001
C20:4 <i>n</i> -6 (AA)	16.7 <sup>a</sup>	0.6	16.8 <sup>a</sup>	0.5	11.1 <sup>b</sup>	0.6	14.5 <sup>c</sup>	0.5	<i>P</i> <0.0001
C22:4 <i>n</i> -6	0.7 <sup>a</sup>	0.1	2.8 <sup>b</sup>	0.0	0.1 <sup>c</sup>	0.0	0.5 <sup>d</sup>	0.0	<i>P</i> <0.0001
PUFA <i>n</i> -3	7.1 <sup>a</sup>	0.3	3.0 <sup>b</sup>	0.2	22.9 <sup>c</sup>	1.1	14.8 <sup>d</sup>	0.3	<i>P</i> <0.0001
C18:3 <i>n</i> -3 (ALA)	0.4 <sup>a</sup>	0.0	0.3 <sup>a</sup>	0.0	8.8 <sup>b</sup>	0.5	4.8 <sup>c</sup>	0.2	<i>P</i> <0.0001
C20:5 <i>n</i> -3 (EPA)	0.1 <sup>a</sup>	0.0	0.1 <sup>a</sup>	0.0	3.9 <sup>b</sup>	0.4	1.3 <sup>c</sup>	0.1	<i>P</i> <0.0001
C22:5 <i>n</i> -3	0.7 <sup>a</sup>	0.0	0.3 <sup>a</sup>	0.0	3.4 <sup>b</sup>	0.3	2.4 <sup>c</sup>	0.1	<i>P</i> <0.0001
C22:6 <i>n</i> -3 (DHA)	5.2 <sup>a</sup>	0.3	2.2 <sup>b</sup>	0.1	6.2 <sup>c</sup>	0.2	6.0 <sup>c</sup>	0.3	<i>P</i> <0.0001
<b>EWAT</b>									
Total SFA	31.3 <sup>a</sup>	0.3	20.6 <sup>b</sup>	0.5	19.7 <sup>b</sup>	0.4	19.1 <sup>b</sup>	0.2	<i>P</i> <0.0001
Total MUFA	47.9 <sup>a</sup>	0.3	27.2 <sup>b</sup>	0.3	30.0 <sup>c</sup>	0.2	27.5 <sup>b</sup>	0.3	<i>P</i> <0.0001
Total PUFA	20.8 <sup>a</sup>	0.4	52.2 <sup>b</sup>	0.7	50.3 <sup>c</sup>	0.5	53.3 <sup>b</sup>	0.4	<i>P</i> <0.0001
PUFA <i>n</i> -6	19.1 <sup>a</sup>	0.3	50.8 <sup>b</sup>	0.7	22.9 <sup>c</sup>	0.7	37.6 <sup>d</sup>	0.4	<i>P</i> <0.0001
C18:2 <i>n</i> -6 (LA)	18.4 <sup>a</sup>	0.3	48.9 <sup>b</sup>	0.7	22.2 <sup>c</sup>	0.5	36.5 <sup>d</sup>	0.4	<i>P</i> <0.0001
C18:3 <i>n</i> -6	–	–	0.1 <sup>a</sup>	0.0	–	–	0.1 <sup>b</sup>	0.0	<i>P</i> <0.05
C20:3 <i>n</i> -6	–	–	0.2 <sup>a</sup>	0.0	–	–	0.1 <sup>b</sup>	0.0	<i>P</i> <0.01
C20:4 <i>n</i> -6 (AA)	0.4 <sup>a</sup>	0.0	1.1 <sup>b</sup>	0.1	0.5 <sup>a</sup>	0.1	0.6 <sup>a</sup>	0.0	<i>P</i> <0.0001
C22:4 <i>n</i> -6	0.1 <sup>a</sup>	0.0	0.2 <sup>b</sup>	0.0	–	–	0.1 <sup>a</sup>	0.0	<i>P</i> <0.0001
PUFA <i>n</i> -3	1.7 <sup>a</sup>	0.2	1.4 <sup>a</sup>	0.1	27.4 <sup>b</sup>	1.1	15.7 <sup>c</sup>	0.6	<i>P</i> <0.0001
C18:3 <i>n</i> -3 (ALA)	1.6 <sup>a</sup>	0.2	1.3 <sup>a</sup>	0.1	26.1 <sup>b</sup>	1.2	14.6 <sup>c</sup>	0.6	<i>P</i> <0.0001
C20:5 <i>n</i> -3 (EPA)	–	–	–	–	0.4 <sup>a</sup>	0.1	0.3 <sup>b</sup>	0.0	<i>P</i> <0.05
C22:5 <i>n</i> -3	–	–	–	–	0.4	0.0	0.3	0.0	NS
C22:6 <i>n</i> -3 (DHA)	0.1 <sup>a</sup>	0.0	0.1 <sup>a</sup>	0.0	0.3 <sup>b</sup>	0.0	0.4 <sup>b</sup>	0.0	<i>P</i> <0.0001

LA, linoleic acid; AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; EWAT, epididymal white adipose tissue.<sup>a,b,c,d</sup> Mean values with unlike superscript letters were significantly different by a *post hoc* pairwise comparison (*P*<0.05).

\* Values are expressed as a percentage of total fatty acids.

curve for plasma glucose (Tables 9 and 10). Furthermore, unlike the HF diet enriched in LA + ALA, the diets high in ARASCO or DHASCO did not result in a significantly lower plasma insulin response curve below that of the HF-lard group (Tables 9 and 10).

## Discussion

Using the same rat model of restriction–refeeding as in the present study, it has previously been reported<sup>(26)</sup> that, compared with refeeding on a low-fat diet, isoenergetic refeeding on HF diets in which 50% of energy intake was derived from

either lard, olive oil or menhaden fish oil resulted in a higher efficiency of fat deposition and excessive adiposity, effects which, however, were not observed when the dietary fat was derived from safflower oil. In fact, refeeding on the safflower oil diet not only prevented the excessive fat deposition, but also resulted in a higher lean body mass, a dual effect on body composition that is reproduced in our studies here not only with safflower oils from various commercial sources, but also with other oils that are rich either in LA (sunflower, grapeseed and maize), in ALA (linseed) or in various mixtures of LA and ALA (obtained by mixing safflower and linseed oils). Taken together, these findings show that it is the total PUFA

**Table 9.** Effects of high-fat diets made from microbial oils rich in arachidonic acid (AA) (arachidonic acid single cell oil; ARASCO) or DHA (docosahexaenoic acid single cell oil; DHASCO) on body weight and body composition, as well as on plasma glucose and insulin in response to a glucose load (Mean values with their standard errors, *n* 6)

Group	Diet	Diet				Body composition						Glucose tolerance test						
		PUFA (% diet energy)	AA (% diet energy)	DHA (% diet energy)	Body weight (g)	Water (g)		Fat (g)		Lean (g)		Fat:lean		Glucose (AUC)*		Insulin (AUC)*		
						Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean
1	HF lard	8	-	-	361 <sup>a</sup>	3	234 <sup>a</sup>	3	50.5 <sup>a</sup>	1.8	76.8 <sup>a</sup>	1.1	0.66 <sup>a</sup>	0.03	4005 <sup>a</sup>	1014	322 <sup>a</sup>	19
2	HF S:L (1:1)	42	-	-	367 <sup>a</sup>	2	240 <sup>a,b</sup>	2	37.6 <sup>b</sup>	0.8	89.0 <sup>b</sup>	0.5	0.42 <sup>b</sup>	0.01	4329 <sup>a,b</sup>	570	176 <sup>b</sup>	27
3	HF ARASCO	29.4	20	-	367 <sup>a</sup>	2	250 <sup>b</sup>	1	35.8 <sup>b</sup>	0.8	81.4 <sup>b</sup>	0.9	0.44 <sup>a,b</sup>	0.01	9346 <sup>b</sup>	1785	283 <sup>a,b</sup>	26
4	HF DHASCO	24.8	-	20	345 <sup>b</sup>	5	226 <sup>a</sup>	4	40.1 <sup>b</sup>	1.3	78.9 <sup>a,b</sup>	0.9	0.51 <sup>a</sup>	0.01	5373 <sup>a,b</sup>	985	260 <sup>a,b</sup>	33
ANOVA					<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.001		<i>P</i> <0.02		<i>P</i> <0.01	

S, safflower oil; L, linseed oil.

<sup>a,b</sup> Mean values with unlike superscript letters were significantly different by a post hoc pairwise comparison (*P*<0.05).

\* AUC refers to the area under the curve for plasma glucose (mg/100 ml per 2h) or insulin curve (ng/ml per 2h) above baseline (pre-glucose load) plasma levels.

content of the HF diet, i.e. total LA + ALA content, rather than LA, ALA or the ratio of LA:ALA (and hence the *n*-6:*n*-3 fatty acid ratio) that is important in the dual effects of these high PUFA diets in increasing lean mass and in reducing fat mass. Furthermore, our studies demonstrate that these HF diets rich in LA and/or ALA improve whole-body insulin sensitivity, also independently of the LA:ALA (and hence *n*-6:*n*-3 fatty acid) ratio.

*Relationship between PUFA precursors and body composition*

Further analysis of the body composition data using semi-synthetic HF diets indicates the relationships between total PUFA (LA + ALA) content *v.* lean mass and fat mass that are best fitted by quadratic regression models, with threshold values for an impact on body composition corresponding to between 25 and 30% of energy intake as total LA + ALA content.

For the relationship between change in lean mass (*y*) and PUFA intake (*x*), the equation is as follows:  $y = y_0 + ax + bx^2$ , where  $y_0 = 1.078$ ,  $a = -0.235$  and  $b = 0.013$ . For the relationship between change in fat mass (*y*) and PUFA intake (*x*), the equation is as follows:  $y = y_0 + ax + bx^2$ , where  $y_0 = -2.640$ ,  $a = 0.453$  and  $b = -0.019$ . Support for these regression models can also be derived from other data on lean mass or fat mass obtained by iso-energetic refeeding on synthetic diets (study III) in which PUFA varied between 11 and 31% of energy intake: the changes in lean and fat mass relative to a HF-lard diet are close to those predicted by the quadratic regression models. Another feature of this close fit between model prediction and measured values is that, because the synthetic diets were formulated to keep MUFA content low and constant, it reinforces the contention here that these relationships between body composition and dietary fatty acid composition are specifically related to total PUFA content rather than to MUFA content of the diet. In other words, the differential effects on body composition observed with the diets that follow the quadratic models are explained by variations in PUFA and not by variations in MUFA (study III), nor in SFA, which is practically a constant in study I (Expt 2). Furthermore, in the latter study, the HF rapeseed oil with the lowest SFA (5.5%) had no effect on body composition relative to the HF-lard diet high in SFA (25%).

*Impact of PUFA elongation-desaturation products*

To what extent these effects of high-PUFA diets on body composition can be attributed to dietary LA or ALA in their own right or to their elongation-desaturation products is not known. Our analysis of the fatty acid composition of the liver, adipose tissue and skeletal muscle indicates that although most of the increases in PUFA in these key tissue/organs reflect the high dietary LA or ALA content of the diets, some of their respective metabolites were nonetheless increased significantly, with adipose tissue showing significant increases in AA in response to the LA-enriched diets or in EPA and DHA in response to the ALA-enriched diets. Furthermore,

**Table 10.** Effects of high-fat (HF) diets made from different mixtures of microbial oils rich in arachidonic acid single cell oil (A) or docosahexaenoic acid single cell oil (D) on body weight and body composition, as well as on plasma glucose and insulin in response to a glucose load (Mean values with their standard errors, *n* 6)

Group	HF diet	Body composition										Glucose tolerance test			
		Body weight (g)		Water (g)		Fat (g)		Lean (g)		Fat:lean		Glucose (AUC)*		Insulin (AUC)*	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	HF A:D (0:1)	335	7	217	5	42.3	1.5	75.9	1.5	0.55	0.02	4162 <sup>a</sup>	718	297	53
2	HF A:D (1:3)	352	5	231	4	43.5	2.2	77.8	1.2	0.56	0.04	3622 <sup>a</sup>	721	303	56
3	HF A:D (1:1)	349	3	230	3	41.4	1.8	77.7	1.2	0.53	0.03	5341 <sup>a,b</sup>	379	366	35
4	HF A:D (3:1)	350	4	228	3	44.4	1.4	77.3	0.8	0.58	0.02	7443 <sup>a,b</sup>	1600	316	51
5	HF A:D (1:0)	342	5	225	5	41.7	2.3	75.7	1.3	0.55	0.04	12 105 <sup>b</sup>	3114	347	56
ANOVA		NS		NS		NS		NS		NS		P<0.01		NS	

<sup>a,b</sup> Mean values with unlike superscript letters were significantly different by a *post hoc* pairwise comparison ( $P<0.05$ ).

\* AUC refers to the area under the curve for plasma glucose (mg/100 ml per 2 h) or insulin curve (ng/m per 2 h) above baseline (pre-glucose load) plasma levels.

the findings here that isoenergetic refeeding with diets made from ARASCO and DHASCO (high in AA and DHA, respectively) also resulted in an increase (albeit marginal) in protein gain and marked decrease in body-fat gain suggest that at least some of the effects of LA and ALA on body composition might be mediated by their elongated products. It is to be noted that these diets enriched in AA or DHA provided less total PUFA than those rich in LA and/or ALA (25–29% *v.* 30–42%, as shown in Table 2). Nonetheless, the effects of diets made from ARASCO and DHASCO on body composition are predicted by the quadratic model relating total PUFA content to the lean mass and but not to the fat mass. These marked deviations in fat mass reduction by these oils thus underlie the more potent effects of AA and DHA in reducing fat mass than their respective precursors LA and ALA. The findings here of a more pronounced anti-adiposity effect of AA and DHA than the respective precursor during catch-up growth are in line with studies in spontaneously growing rats. These have shown that (1) dietary  $\gamma$ -LA – an elongated and desaturated product of LA and an immediate precursor of AA – in the form of borage oil causes less body fat accumulation than LA in the form of safflower oil<sup>(33)</sup>, and that (2) concentrates of *n*-3 PUFA of marine origin and rich in DHA had more pronounced effects in reducing adiposity in mice than *n*-3 ALA or *n*-6 LA precursors in the form of linseed oil, perilla oil or safflower oil<sup>(34,35)</sup>. Whether AA has more potent effects than its immediate *n*-6 PUFA precursor  $\gamma$ -LA on body composition is not known, but there is increasing evidence that DHA and its immediate *n*-3 PUFA precursor EPA may differ considerably in their effects on both fat mass and lean mass. Indeed, using long-chain *n*-3 PUFA concentrates that differed in the EPA:DHA ratio, it has also been shown that the protective effect of *n*-3 PUFA on adipose tissue accretion in mice was stronger with DHA than with EPA<sup>(35,36)</sup>. These data are consistent with our past study indicating that refeeding on HF diets made from menhaden fish oil, which is high in EPA but relatively low in DHA, failed to reduce body fat accumulation, and, in addition, led to reduced lean body mass accretion relative to HF diets rich in lard or olive oil<sup>(26)</sup>. Thus, the effects of *n*-6 or *n*-3 PUFA on body composition depend not only on total PUFA contents in the diet but also on the proportion of

their specific elongated–desaturated products. However, our findings that most of the increases in PUFA in key tissues/organs reside in increases in LA and/or ALA raise the possibility that these essential fatty acids themselves may be directly involved in the mechanisms by which diets rich in LA and/or ALA improve body composition during catch-up growth.

#### High-PUFA oils and insulin sensitivity

The present studies also provide insights into the effects of HF diets made from oils rich in LA and/or ALA and their main elongation–desaturation products (AA and DHA) in the modulation of blood glucose homeostasis during catch-up growth, a state of hyperinsulinaemia even on a low-fat diet and exacerbated by a HF-lard diet<sup>(24)</sup>. The results obtained during the test of glucose tolerance, indicating that the groups refed on the HF diets enriched in LA and/or ALA show no alterations in plasma glucose but markedly reduced plasma insulin in response to a glucose load, suggest that these diets enriched in essential fatty acids are equally effective in improving insulin sensitivity. By contrast, this test also revealed higher glucose responses and, to a lesser extent, higher insulin responses observed with diets rich in ARASCO. Furthermore, unlike HF diets made from safflower or linseed oils, the diets high in ARASCO or DHASCO did not result in a lower plasma insulin response curve below that of the HF-lard group. Thus, whereas diets very high in ARASCO and DHASCO had a marginal effect on lean mass accretion and a marked effect on body fat, they failed to improve glucose homeostasis, with the diet high in ARASCO resulting in more pronounced hyperglycaemia relative to the other diets in response to a glucose load.

#### Outcome of screening high-PUFA diets

Based on an overall analysis of body composition changes in the context of isoenergetic refeeding on PUFA-enriched HF diets, it can be concluded that of all oils screened, the lowest rate of catch-up fat is observed with diets made from safflower oil, linseed oil, ARASCO oil and DHASCO. However, it is also evident that whereas the lower rate of catch-up fat

with diets made from ARASCO and DHASCO, albeit unaccompanied by an improvement in glucose homeostasis, resides primarily in enhanced thermogenesis, that from diets made from safflower or linseed oils (which are associated with improved insulin sensitivity) resides primarily in an enhanced energy partitioning towards lean mass accretion. Given the high energy cost of protein accretion (and its maintenance) as opposed to low energy cost of fat deposition and maintenance particularly on HF diets, it can be calculated that about two-thirds of the reduction in fat accretion during catch-up fat result from a shift in energy partitioning towards lean body mass, and the remaining one-third resides in enhanced thermogenesis. While an enhanced sympathetic thyroid action on peripheral tissues (via uncoupling protein 1 in brown adipose tissue,  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase in brown adipose tissue, liver or muscle) has often been described in response to feeding on diets high in PUFA<sup>(28,33–40)</sup>, the mechanisms by which high-PUFA diets contributing >25% of energy intake enhance energy partitioning towards lean body mass is a new avenue for further investigations. These mechanisms could implicate increased secretion and/or increased sensitivity to the anabolic effects of numerous hormones known to favour lean tissue accretion (e.g. insulin, insulin-like growth factor-1 and growth hormone, thyroid-stimulating hormone and thyroid hormones, catecholamines and testosterone) or decreased sensitivity to other hormones whose actions lead to reduce lean mass (e.g. myostatin and IL-15). Further studies are also warranted to investigate whether (1) the ability of these diets enriched in essential fatty acids to modulate body composition, shown here during refeeding in male rats after post-weaning food restriction, can also be demonstrated in female rats and in animal models of catch-up growth after intra-uterine or neonatal malnutrition, and (2) whether improvements in body composition during catch-up growth in these models would have a long-term impact on susceptibility to metabolic diseases later in life.

### Conclusions and clinical implications

Infants and children with faltered growth often have poor appetite, particularly under conditions of malnutrition infections. Consequently, the high energy requirements for catch-up growth can only be achieved with energy-dense formulations, which in turn impose a need for a HF content<sup>(41)</sup>, an important factor that promotes excessive adiposity and insulin resistance during catch-up growth. The present study in the rat indicates that HF diets enriched with LA and/or ALA have the potential to limit excessive accretion of body fat while improving lean body mass and insulin sensitivity during catch-up growth. That these improvements occur independently of the dietary LA:ALA ratio and hence in the *n*-6:*n*-3 fatty acid ratio (ranging from <1 to >40) is in line with the view that challenges emphasis placed upon this ratio, rather than in total *n*-3 and *n*-6 PUFA contents for clinical benefits<sup>(42)</sup>. Whether diets providing such high PUFA intake, exceeding 25% of total energy intake, are safe and effective in modulating body composition and metabolic health in humans are issues that must be considered with caution. There is,

however, evidence from trials in non-human primates demonstrating cardiovascular benefits, and no evidence of harm, with LA intakes of 25% of energy for up to 5 years<sup>(43,44)</sup>. In humans, randomised trials with *n*-6 PUFA intakes (mostly as LA) of 11–21% of energy for up to 11 years show reduced risk for CHD and with no evidence of harm<sup>(45)</sup>. It should also be pointed out that the extrapolation of findings from small rodents to humans should also take into account the state of knowledge, indicating that the efficacy of several specific food ingredients that either stimulate thermogenesis (e.g. caffeine, green tea catechins and medium-chain TAG) or that shift nutrient partitioning in favour of lean tissue (e.g. conjugated LA) has been demonstrated at much lower doses in humans (albeit adults) than in laboratory rodents<sup>(27,46,47)</sup>. Further support for this contention, in the context of dietary enrichment with essential fatty acids, can be derived from a recent report<sup>(48)</sup> that supplementation with modest amounts of LA-rich safflower oil resulted in reduced trunk fat and increased lean mass in obese women with type 2 diabetes mellitus. Taken together, the possibility therefore arises that diets enriched with more modest amounts of PUFA as LA and/or ALA than shown here to be effective in improving body composition and insulin sensitivity in rats may have relevance for improving body composition and insulin sensitivity during catch-up growth in humans.

### Acknowledgements

We are grateful to Marie-Francoise Baeriswyl, Aldo Tempini, Florence Blancher, Irina Monnard and Manuel Ribeiro for their excellent technical support. The present study was supported primarily by a research grant from the Swiss National Science Foundation (grant no. 3200b0-113634), and a minor contribution from Nestlé Limited, Switzerland. A. G. D. designed and supervised the study. A. G. D., J.-P. M. and J. S. obtained funding and provided administrative, technical and material support. G. Y., H. M. and J. L. M. performed the animal experiments and contributed to the analysis of data and interpretation of the results. K. M., Y. S. and O. A. contributed to the design of part of the study, and were responsible for the analysis of dietary lipids. G. Y. and A. G. D. wrote the draft of the manuscript, and all the authors critically reviewed the manuscript. We declare that there is no conflict of interest regarding the present study.

### References

1. Keys A, Brozek J, Henschel A, *et al.* (1950) *The Biology of Human Starvation*. Minneapolis, MN: University of Minnesota Press.
2. Dulloo AG, Jacquet J & Montani JP (2002) Pathways from weight fluctuations to metabolic diseases: focus on maladaptive thermogenesis during catch-up fat. *Int J Obes Relat Metab Disord* **26**, Suppl. 2, S46–S57.
3. Barac-Nieto M, Spurr GB, Lotero H, *et al.* (1979) Body composition during nutritional repletion of severely undernourished men. *Am J Clin Nutr* **32**, 981–991.

4. Jackson AA (1984) Nutritional adaptation in disease and recovery. In *Nutritional Adaptation in Man*, pp. 111–126 [K Blaxter and JC Waterlow, editors]. London: John Libbey.
5. Mitchell PB & Truswell AS (1987) Body composition in anorexia nervosa and starvation. In *Handbook of Eating Disorders. Part I: Anorexia and Bulimia Nervosa*, pp. 45–77 [PJV Beaumont, GD Burrows and RC Casper, editors]. Amsterdam: Elsevier.
6. van Eys J (1985) Nutrition and cancer: physiological inter-relationships. *Annu Rev Nutr* **5**, 435–461.
7. Streat SJ, Beddoe AH & Hill GL (1987) Aggressive nutritional support does not prevent protein loss despite fat gain in septic intensive care. *J Trauma* **27**, 262–266.
8. Kotler DP, Tierney AR, Culpepper-Morgan JA, *et al.* (1990) Effect of home total parenteral nutrition on body composition in patients with acquired immunodeficiency syndrome. *J Parenter Enteral Nutr* **14**, 454–458.
9. Ashworth A (1969) Growth rates in children recovering from protein-calorie malnutrition. *Br J Nutr* **23**, 835–845.
10. MacLean WC & Graham GG (1980) The effect of energy intake on nitrogen content of weight gained by recovering malnourished infant. *Am J Clin Nutr* **33**, 903–909.
11. Graham GG, MacLean WC Jr, Brown KH, *et al.* (1996) Protein requirements of infants and children: growth during recovery from malnutrition. *Pediatrics* **97**, 499–505.
12. Martins PA, Hoffman DJ, Fernandes MT, *et al.* (2004) Stunted children gain less lean body mass and more fat mass than their non-stunted counterparts: a prospective study. *Br J Nutr* **92**, 819–825.
13. Schmidt MH & Klein M (1997) Determination of body fat in treatment of anorexia nervosa. *Z Kinder Jugendpsychiat Psychoter* **25**, 27–34.
14. Mika C, Herpertz-Dahlmann B, Heer M, *et al.* (2004) Improvement of nutritional status as assessed by multifrequency BIA during 15 weeks of refeeding in adolescent girls with anorexia nervosa. *J Nutr* **134**, 3026–3030.
15. Ong KKL, Ahmed ML, Emmett PM, *et al.* (2000) Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ* **320**, 967–971.
16. Levy-Marchal C, Jaquet D & Czernichow P (2002) Long-term metabolic consequences of being born small for gestational age. *Semin Neonatol* **9**, 67–74.
17. Soto N, Bazaes RA, Pena V, *et al.* (2003) Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. *J Clin Endocrinol Metab* **88**, 3645–3650.
18. Jaquet D, Deghmoun S, Chevenne D, *et al.* (2005) Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth. *Diabetologia* **48**, 849–855.
19. Ibanez L, Ong K, Dunger DB, *et al.* (2006) Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. *J Clin Endocrinol Metab* **91**, 2153–2158.
20. Dulloo AG, Jacquet J, Seydoux J, *et al.* (2006) The thrifty ‘catch-up fat’ phenotype: its impact on insulin sensitivity during growth trajectories to obesity and metabolic syndrome. *Int J Obes* **30**, Suppl. 4, S23–S35.
21. Morrison JL, Duffield JA, Muhlhauser BS, *et al.* (2010) Fetal growth restriction, catch-up growth and the early origins of insulin resistance and visceral obesity. *Pediatr Nephrol* **25**, 669–677.
22. Meas T (2010) Fetal origins of insulin resistance and the metabolic syndrome: a key role for adipose tissue? *Diabetes Metab* **36**, 11–20.
23. Dulloo AG & Girardier L (1993) Adaptive role of energy expenditure in modulating body fat and protein deposition during catch-up growth after early undernutrition. *Am J Clin Nutr* **58**, 614–621.
24. Crescenzo R, Samec S, Antic V, *et al.* (2003) A role for suppressed thermogenesis favoring catch-up fat in the pathophysiology of catch-up growth. *Diabetes* **52**, 1090–1097.
25. Dulloo AG & Girardier L (1992) Influence of dietary composition on energy expenditure during recovery of body weight in the rat: implications for catch-up growth and obesity relapse. *Metabolism* **41**, 1336–1342.
26. Dulloo AG, Mensi N, Seydoux J, *et al.* (1995) Differential effects of high-fat diets varying in fatty acid composition on the efficiency of lean and fat tissue deposition during weight recovery after low food intake. *Metabolism* **44**, 273–279.
27. St-Onge MP & Jones PJ (2002) Physiological effects of medium-chain triacylglycerols: potential agents in the prevention of obesity. *J Nutr* **132**, 329–332.
28. Mercer SW & Trayhurn P (1987) Effect of high fat diets on energy balance and thermogenesis in brown adipose tissue of lean and genetically obese ob/ob mice. *J Nutr* **117**, 2147–2153.
29. Shimomura Y, Tamura T & Suzuki M (1990) Less body fat accumulation in rats fed a safflower oil diet than in rats fed a beef tallow diet. *J Nutr* **120**, 1291–1296.
30. Miller DS & Payne PR (1959) A ballistic bomb calorimeter. *Br J Nutr* **13**, 501–508.
31. Folch J, Lees M & Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.
32. Srivastava NK, Pradhan S, Mittal B, *et al.* (2006) An improved, single step standardized method of lipid extraction from human skeletal muscle tissue. *Anal Lett* **39**, 297–315.
33. Takahashi Y, Ide T & Fujita H (2000) Dietary gamma-linolenic acid in the form of borage oil causes less body fat accumulation accompanying an increase in uncoupling protein 1 mRNA level in brown adipose tissue. *Comp Biochem Physiol B Biochem Mol Biol* **127**, 213–222.
34. Ikemoto S, Takahashi M, Tsunoda N, *et al.* (1996) High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. *Metabolism* **45**, 1539–1546.
35. Ruzickova J, Rossmeisl M, Prazak T, *et al.* (2004) Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue. *Lipids* **39**, 1177–1185.
36. Flachs P, Horakova O, Brauner P, *et al.* (2005) Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. *Diabetologia* **48**, 2365–2375.
37. Matsuo T, Komuro M & Suzuki M (1996) Beef tallow diet decreases uncoupling protein content in the brown adipose tissue of rats. *J Nutr Sci Vitaminol (Tokyo)* **42**, 595–601.
38. Matsuo T, Shimomura Y, Saitoh S, *et al.* (1995) Sympathetic activity is lower in rats fed a beef tallow diet than in rats fed a safflower oil diet. *Metabolism* **44**, 934–939.
39. Takeuchi H, Matsuo T, Tokuyama K, *et al.* (1995) Serum triiodothyronine concentration and Na<sup>+</sup>, K<sup>(+)</sup>-ATPase activity in liver and skeletal muscle are influenced by dietary fat type in rats. *J Nutr* **125**, 2364–2369.
40. Clarke SD (2000) Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. *Br J Nutr* **83**, Suppl. 1, S59–S66.

41. Prentice AM & Paul AA (2000) Fat and energy needs of children in developing countries. *Am J Clin Nutr* **72**, Suppl. 5, 1253S–1265S.
42. Deckelbaum RJ & Calder PC (2010) Dietary *n*-3 and *n*-6 fatty acids: are there 'bad' polyunsaturated fatty acids? *Curr Opin Clin Nutr Metab Care* **13**, 123–124.
43. Wolfe MS, Parks JS, Morgan TM, *et al.* (1993) Childhood consumption of dietary polyunsaturated fat lowers risk for coronary artery atherosclerosis in African green monkeys. *Arterioscler Thromb* **13**, 863–875.
44. Wolfe MS, Sawyer JK, Morgan TM, *et al.* (1994) Dietary polyunsaturated fat decreases coronary artery atherosclerosis in a pediatric-aged population of African green monkeys. *Arterioscler Thromb* **14**, 587–597.
45. Harris WS, Mozaffarian D, Rimm E, *et al.* (2009) Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation* **119**, 902–907.
46. Westerterp-Plantenga MS (2010) Green tea catechins, caffeine and body-weight regulation. *Physiol Behav* **100**, 42–46.
47. Steck SE, Chalecki AM, Miller P, *et al.* (2007) Conjugated linoleic acid supplementation for twelve weeks increases lean body mass in obese humans. *J Nutr* **137**, 1188–1193.
48. Norris LE, Collene AL, Asp ML, *et al.* (2009) Comparison of dietary conjugated linoleic acid with safflower oil on body composition in obese postmenopausal women with type 2 diabetes mellitus. *Am J Clin Nutr* **90**, 468–476.