β-Oxidation of linoleate in obese men undergoing weight loss[1–3]

Stephen C Cunnane, Robert Ross, Jody L Bannister, and David JA Jenkins

ABSTRACT

Background: In animals, the whole-body content and accumulation of linoleate can be measured and compared with its intake to determine linoleate β-oxidation. This method can also provide quantitative information about the β-oxidation of linoleate in humans.

Objectives: The objectives of the study were to *1*) use the whole-body fatty acid balance method to quantify whole-body concentrations of linoleate in humans, *2*) estimate the distribution of linoleate between adipose and lean tissue, and *3*) assess the effect of weight loss on linoleate stores and β-oxidation in obese humans.

Design: Nine healthy obese men underwent supervised weight loss for 112 d (16 wk). Magnetic resonance imaging data and fatty acid profiles from fat biopsies were both used to determine linoleate stores in adipose and lean tissue and in the whole body. Linoleate β-oxidation was calculated as intake − (accumulation + excretion).

Results: Mean weight loss was 13 kg and linoleate intake was 24 ± 6 mmol/d over the study period. Whole-body loss of linoleate was 37 ± 18 mmol/d, or 28% of the level before weight loss. Combining the intake and whole-body loss of linoleate resulted in linoleate β-oxidation exceeding intake by 2.5-fold during the weight-loss period.

Conclusions: All dietary linoleate is β-oxidized and at least an equivalent amount of linoleate is lost from the body during moderate weight loss in obese men. The method studied permits the assessment of long-term changes in linoleate homeostasis in obese humans and may be useful in determining the risk of linoleate deficiency in other conditions.   *Am J Clin Nutr* 2001;73:709–14.

KEY WORDS   Adipose tissue, linoleate, magnetic resonance imaging, obesity, weight loss, exercise, β-oxidation

INTRODUCTION

Linoleate (18:2n−6) is the main polyunsaturated fatty acid (PUFA) in the diet and in the body. Despite an extensive history of research into linoleate metabolism and its role in nutrition and health, little is known in humans about linoleate partitioning toward β-oxidation. In weight-stable adults, the proportion of linoleate in fatty acids in adipose tissue reflects habitual linoleate intake (1–3). Thus, β-oxidation is probably the main route of dietary linoleate utilization; however there is little quantitative evidence supporting this assumption.

During food restriction resulting in a weight loss of 10–25 kg in obese humans, the percentage of linoleate in adipose tissue remains unchanged compared with values before weight loss (4–6). This suggests that during energy deficit β-oxidation of linoleate occurs at a rate similar to that of other long-chain fatty acids. Nevertheless, linoleate depletion from the body can exceed that of other long-chain fatty acids in weight-cycling animals despite adequate linoleate intake (7). An energy deficit caused by fat malabsorption or disease that generates the need for parenteral nutrition leads to a risk of linoleate deficiency and possible depletion of long-chain n−6 PUFA, eg, arachidonate (20:4n−6; 8, 9). Thus, there are 2 mechanisms by which food restriction and weight loss incur a potential risk of linoleate deficiency: *1*) reduced linoleate intake because mammals are unable to synthesize linoleate except from the small amounts of dietary hexadecadienoate (16:2n−6) present in some common edible green vegetables (10), and *2*) increased linoleate β-oxidation because it may exceed linoleate intake during weight loss. Hence, it would be beneficial to assess the risk of linoleate deficiency with weight loss in humans and to determine the significance of increased linoleate β-oxidation as a risk for linoleate deficiency.

In animals, β-oxidation of linoleate relative to intake can be assessed by using the whole-body fatty acid balance method. With this method, β-oxidation is determined as the difference between linoleate accumulation and intake (7). This method has not been used to determine linoleate utilization in living animals or humans but, in principle, this should be possible. This study therefore had the following 3 objectives: *1*) to use the whole-body fatty acid balance method to quantify whole-body concentrations of linoleate in humans, *2*) to estimate the distribution of linoleate between adipose and lean tissue, and *3*) to assess the effect of weight loss on linoleate stores and β-oxidation in obese humans. Obese subjects undergoing weight loss were chosen because it was anticipated that this would cause readily measurable changes in whole-body linoleate.

709

Fatty acid profiles of 4 plasma lipid classes were measured to determine whether linoleate changes in blood after weight loss were proportional to those in adipose tissue or to weight loss itself. If so, they could potentially serve as a substitute for the time consuming and expensive magnetic resonance imaging (MRI)–based measurements of changes in whole-body linoleate.

METHODS

Weight-loss regimen

This study was done in obese men undergoing voluntary but supervised weight loss induced by a combination of food restriction and exercise. Subjects were healthy and had no disease conditions known to influence metabolism of PUFA. Each subject provided written consent and all procedures received ethical approval by both the University of Toronto and Queen's University. The inclusion criteria were as follows: a body mass index (BMI; in kg/m^2) >27, weight stability ±2 kg in previous 6 mo, no use of prescription drugs known to affect body weight, and consumption of <2 alcoholic beverages/d. At entry, the mean (±SD) age of all subjects was 47 ± 8 y.

Basal energy requirements were calculated and a weight-maintenance diet was prescribed for 2 wk to obtain baseline data. The weight-maintenance requirements were then reduced by 238 MJ/d (1000 kcal/d) for the 112-d (16-wk) study period. Food items were self-selected and a maximum of 30% fat was permitted in the diet during the weight-loss period. Food intake records were maintained daily throughout the study period and were reviewed weekly between each subject and a registered dietitian. The exercise regimen was either aerobic (treadmill, stationary cycling, or stair-stepping 5 times/wk) or resistance training (weight training plus sit-ups 3 times/wk). The energy cost of the exercise was ≈700 kJ/d (11).

Analysis of linoleate intake

Complete daily food intake records for the 112-d study period were obtained from each participant. Total intake of energy, protein, carbohydrate, fat, PUFA, and linoleate were analyzed for each subject at the University of Toronto by using a program based on US Department of Agriculture data (NUTRIPUT, version 2.02; US Department of Agriculture, Washington, DC).

Adipose tissue biopsies and plasma fatty acid analysis

Before and after weight loss, needle biopsies of subcutaneous fat were obtained from the anterior abdominal wall of each subject undergoing weight loss. Fat biopsies were obtained by suction into a 10-mL syringe through an 18-gauge needle without local anesthetic. The samples (5–20 mg) were stored in chloroform at –20°C for fatty acid analysis. After all the biopsies were collected, the chloroform was evaporated under nitrogen, and fatty acids in triacylglycerols were extracted into diethyl ether and partitioned against a 0.9% saline solution. The ether phase was removed and evaporated and the fatty acids were trans-methylated by using 14% boron trifluoride in methanol.

Fasting forearm venous blood samples (10 mL in tubes containing EDTA) were obtained immediately before and at the end of the weight-loss period. Plasma total lipids were extracted into chloroform:methanol (2:1, vol:vol) and separated into total phospholipids, cholesteryl esters, triacylglycerols, and free fatty acids by thin-layer chromatography (12). After saponification, fatty acid methyl esters from each lipid fraction were prepared by using boron trifluoride in methanol. Capillary gas chromatography was used to analyze adipose and plasma fatty acid methyl esters by using a 30-m capillary column (Durabond 23; J & W Scientific, Folsom, CA) with automated sample injection and a 2-phase temperature ramp (model 5890; Hewlett-Packard, Palo Alto, CA). The percentages of fatty acids in adipose tissue and cholesteryl esters were corrected for relative response factors by comparison with a standard reference fatty acid mixture (NuChek Prep, Elysian, MN).

Magnetic resonance imaging and quantification of linoleate stores

Each subject undergoing weight loss had 2 sets of transverse MRI scans consisting of 41 slices covering the full length of the body (1.5 T whole-body scanner; General Electric, Milwaukee). The imaging was done at Kingston General Hospital, Kingston. One set of scans was obtained just before weight loss and the other after weight loss. Slices were 10-mm thick and were positioned every 40 mm down the full length of the body with arms extended. Whole-body adipose and lean tissue volumes were determined from the cross-sectional areas of each scan as described previously (11, 13). These MRI measurements of lean and adipose tissue volume in living humans have been validated by us (13) and others (14) in comparison with the lean and fat composition of dissected human cadavers. The intraobserver CV for the in vivo MRI measurements was 2.1%. MRI data for skeletal muscle volumes differed from cadaver measurements by 1.3% (13).

Conversion of the MRI-based adipose tissue volumes and percentage fatty acid composition data to actual fatty acid mass (in mol) in total adipose tissue required that the adipose tissue volumes be corrected for fatty acid density and water content in adipose tissue. The fat biopsies obtained for fatty acid analysis were too small for reliable measurements of fat density or water content. For this purpose, larger fat samples (100–400 g) from 5 different individuals not involved in the weight-loss study were obtained from the Cosmetic Surgery Institute (Toronto). Five 2–3-g weighed replicates of each individual sample were used for density measurements by volume displacement at 37°C. Four additional 2–3-g replicates from the same 5 individuals were used to determine the water content of adipose tissue by freeze-drying to constant weight over 7 d.

The percentage of linoleate in abdominal subcutaneous adipose tissue is reported to be the same as at other body sites, including visceral fat (15, 16); thus, the linoleate concentrations obtained in the single biopsy represented adipose tissue linoleate concentrations throughout the body. After the conversion factor to obtain fatty acid mass from adipose volume as measured by MRI was determined (*see* Results), the actual amount of linoleate in whole-body adipose tissue stores (in mol) was determined by multiplying the fatty acid mass in adipose tissue by the percentage of linoleate in the biopsies.

Linoleate excretion

To calculate linoleate balance and β-oxidation, a measure of linoleate excretion in feces was also needed. Fecal samples were not collected from the subjects undergoing weight loss so average linoleate excretion values were obtained from fecal samples stored from a different study with similar subjects. In that study, 3-d pooled fecal collections were obtained from 12 middle-aged adults who had all consumed the same metabolic diet over 4 wk.

TABLE 1
Fatty acid composition in subcutaneous adipose tissue total lipids and plasma cholesteryl esters before and after weight loss in obese men[1]

	Adipose tissue		Cholesteryl esters	
	Before	After	Before	After
	% by wt of total fatty acids			
Palmitate	27.0 ± 1.9	26.2 ± 1.8	14.0 ± 1.6	14.1 ± 1.8
Stearate	4.5 ± 1.0	5.0 ± 1.3	1.4 ± 0.2	1.1 ± 0.7
Oleate	54.0 ± 2.1	54.3 ± 2.6	23.0 ± 1.7	25.1 ± 2.0[2]
Linoleate	12.7 ± 1.6	12.0 ± 1.3[2]	52.5 ± 3.3	50.4 ± 5.1
Arachidonate	0.7 ± 0.1	0.8 ± 0.6	8.3 ± 1.1	8.2 ± 1.3
α-Linolenate	1.1 ± 0.2	1.2 ± 0.6	0.6 ± 0.2	0.6 ± 0.2
Docosahexaenoate	<0.1	0.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.1

[1] $\bar{x} \pm$ SD; $n = 9$.
[2] Significantly different from before weight loss, $P < 0.05$ (Student's *t* test).

The diet provided 20% of energy as fat and 25 mmol linoleate/d, which was similar to the diet of the subjects undergoing weight loss in the present study.

A 2-stage fecal lipid extraction method was used (17). Two freeze-dried, homogenized 2-g replicates/subject were acidified and 20 mL heptane:diethyl ether:ethanol (1:1:1) containing heptadecanoic acid as an internal standard were added, vortexed for 5 min, and centrifuged at $2500 \times g$ at 4°C for 10 min. The supernate was decanted and filtered. The centrifuged fecal pellet was reextracted twice into 20 mL heptane:diethyl ether:ethanol:distilled water (1:1:1:1), centrifuged at $200 \times g$ at 20°C for 10 min, decanted, and filtered. The lipid extracts were combined and rotary evaporated to dryness at 30°C. The dried fatty acid extracts were methylated and analyzed by capillary gas chromatography and corrected for relative response factors as described for plasma fatty acids. Fecal linoleate was quantified relative to the internal standard.

Whole-body linoleate balance

In animal studies, linoleate intake, excretion, and whole-body accumulation can all be measured directly (7). In the present study, linoleate intake and the loss of linoleate stores from adipose tissue were measured directly in each person undergoing weight loss. Lean tissue linoleate was estimated from lean tissue volume as measured by MRI (13) and literature values indicating 4% fat weight and 20% linoleate in total fatty acids of lean tissue (18–21). Thus, total lean tissue weight, when multiplied by 0.04, gave total lean tissue fatty acids. This value multiplied by 0.20 gave linoleate in kilograms, which was converted to moles. As shown elsewhere for muscle, heart, and liver (18, 22), lean tissue concentrations of linoleate and long-chain n−6 PUFAs were assumed not to change with weight loss in the present study.

Conversion of linoleate to long chain n−6 PUFAs is inhibited by fasting and weight loss (23), and long-chain PUFAs undergo relatively low β-oxidation (24), so the whole-body content of the long-chain n−6 PUFA derived from linoleate was assumed to remain constant during weight loss. Hence, linoleate β-oxidation was determined from a simplified balance equation excluding the n−6 long-chain PUFA:

$$\text{Linoleate } \beta\text{-oxidation} = \text{intake} - [\text{whole-body accumulation (loss)} + \text{excretion}] \quad (1)$$

Data analysis

All data are expressed as means ± SDs. Unpaired Student's *t* tests were used to determine whether BMI affected fecal linoleate excretion and to determine possible effects of type of exercise on adipose tissue fatty acid composition. Paired *t* tests were used to determine the significance of effects of weight loss on linoleate β-oxidation, the percentage of linoleate in adipose tissue, and adipose tissue density at different temperatures. GRAPH PAD INPLOT version 4.0 (Graph Pad Software, San Diego) was used for regression analyses.

RESULTS

Body weight

Body weight loss was 13.0 ± 4.9 kg; 27% of the loss was of initial adipose tissue and 3% was of initial lean tissue. The mode of exercise did not significantly affect the results so data for both aerobic and resistance exercise were combined.

Adipose and lean tissue linoleate

The percentage of linoleate decreased significantly in subcutaneous fat after weight loss (**Table 1**). Besides linoleate, the only 2 other n−6 PUFAs that were consistently present in adipose tissue were dihomo-γ-linolenate (20:3n−6) and arachidonate. They totaled <1% of adipose tissue fatty acids and their proportions did not change significantly with weight loss in either group. n−3 PUFAs were present in adipose tissue biopsies but their content in the body was not quantified in this study.

The density of subcutaneous fat was 0.82 ± 0.01 kg/L ($n = 5$). The actual proportion of fatty acids in adipose tissue was 88.3 ± 3.5% ($n = 5$). Multiplying the fat density by the percentage of fatty acids in adipose tissue provided the factor 0.724, which was used to correct adipose tissue volume for the total mass of adipose fatty acids. Correction of adipose tissue volumes for fatty acid mass and multiplication by the percentage of linoleate in the biopsies gave the total mass of linoleate in adipose tissue. There was 14.7 mol linoleate in adipose tissue before weight loss and 10.5 mol after weight loss (**Table 2**). Using published values of 4% fat in lean tissue and linoleate at 20% of lean tissue fatty acids (18–21), we estimated that there was ≈1.76 mol of linoleate in lean tissue, which accounted for ≈12% of whole-body linoleate before and ≈16% after weight loss (Table 2). During weight loss, >98% of the decrease in whole-body linoleate came from adipose tissue. Both the decrease in mass and percentage of linoleate in adipose tissue correlated significantly with the decrease in adipose tissue occurring with weight loss (**Table 3**).

Linoleate intake, excretion, and β-oxidation

Linoleate intake was 24 ± 6 mmol/d, or 3.2% of energy intake (Table 2). Linoleate intake was significantly positively correlated with total energy intake ($r = 0.65$, $P < 0.01$) and with total fat intake ($r = 0.88$, $P < 0.0001$). Linoleate excretion was 0.8 mmol/d, or 4.0 ± 2.4% of daily linoleate intake. Linoleate excretion was not affected by differences in BMI. Average daily linoleate β-oxidation during the weight-loss period was calculated by subtracting the loss of whole-body linoleate from total linoleate intake after first deducting linoleate excretion. Over the 112-d study period, 6.77 ± 2.63 mol (60 mmol linoleate/d) was β-oxidized. Thus, during the weight-loss

TABLE 2
Whole-body linoleate and linoleate balance in obese men during a 16-wk period of supervised weight loss[1]

Whole-body linoleate (mol)	
Adipose tissue	
Before weight loss	12.92 ± 2.94
After weight loss	8.81 ± 2.43
Change	-4.11 ± 1.79[2]
Lean tissue	
Before weight loss	1.76 ± 0.11
After weight loss	1.69 ± 0.07
Change	-0.07 ± 0.05
Total[3]	
Before weight loss	14.68 ± 2.71
After weight loss	10.50 ± 2.25
Change	-4.18 ± 2.03[2]
Linoleate balance	
Intake (mmol/d)	24 ± 6
Excretion	
(mmol/d)	0.8 ± 0.5
(% of linoleate intake)	4.0 ± 2.4
Whole-body accumulation (mmol/d)	-37 ± 18
Disappearance[4]	
(mmol/d)	60 ± 23
(% of linoleate intake)	250 ± 96

[1] $\overline{x} \pm$ SD; $n = 9$.
[2] $P < 0.05$ (Student's t test).
[3] Sum of the change in adipose and lean tissue linoleate.
[4] Equivalent to β-oxidation: intake − (accumulation + excretion).

period, β-oxidation of linoleate exceeded available linoleate (intake – excretion) 2.5-fold.

Adipose tissue linoleate decreased in direct proportion to the loss of adipose tissue (Table 3). Linoleate β-oxidation was also significantly positively correlated with weight loss and with loss of adipose tissue (Table 3). Linoleate β-oxidation was not significantly correlated with linoleate intake, the percentage of linoleate in adipose tissue, or the change in the percentage of linoleate in adipose tissue (data not shown).

Plasma fatty acid profiles

Before weight loss, the percentage of linoleate in adipose tissue was correlated with the percentage of linoleate in plasma cholesteryl esters ($r = 0.67$, $P < 0.001$; Table 1). Weaker positive correlations between the percentage of linoleate in adipose tissue and the percentage of linoleate in plasma free fatty acids ($r = 0.47$, $P < 0.05$), total phospholipids ($r = 0.52$, $P < 0.02$), and triacylglycerols ($r = 0.42$, $P < 0.07$) were also observed (data not shown). Weight loss did not significantly change the concentration of any of these lipid classes in plasma. After weight loss, there was no longer a significant correlation between the percentage of linoleate in adipose tissue and in any of the plasma lipid fractions, including the cholesteryl esters. The change in percentage of linoleate in plasma cholesteryl esters was significantly positively correlated with total weight loss ($r = 0.79$, $P < 0.01$), total adipose tissue loss ($r = 0.83$, $P < 0.006$), and total adipose tissue loss of linoleate ($r = 0.80$, $P < 0.01$).

DISCUSSION

We showed here that the whole-body linoleate content and its distribution between lean and adipose tissue can be determined in

living humans. We estimated that 6.5% of whole-body linoleate concentrations in these obese men was in lean tissue (Table 2). The proportion of dietary linoleate utilized by lean tissue could not be estimated by using the present method. With respect to weight loss, the main observation was that obese men undergoing voluntary weight loss induced by a combination of food restriction and exercise β-oxidized 2.5-fold more linoleate than they consumed. Our present data show that when exercise accompanies moderate food restriction, adipose tissue linoleate is depleted slightly but significantly more rapidly than are other long-chain fatty acids measurable in adipose tissue. Exercise is well known to increase fat oxidation (25) and linoleate is readily β-oxidized (24, 26–28), especially during high intensity exercise of short duration (29).

Even after correction for a possible 30% underreporting of fat intake (30), linoleate β-oxidation was still more than twice that of linoleate intake. Thus, our data show that during moderate weight loss, dietary linoleate is completely β-oxidized as a fuel and whole-body linoleate losses are at least equivalent to what was consumed and β-oxidized from the diet. Because only 28% of whole-body linoleate stores were lost during the study period and subjects continued to consume linoleate, they were presumably not at imminent risk of linoleate deficiency. Nevertheless, the present method provides an approach to assessing this risk either with longer-term weight loss or in clinical situations characterized by weight cycling or in which wasting is a risk, eg, in patients with cystic fibrosis (9), with malabsorption (8), or who need total parenteral nutrition (31).

Despite β-oxidizing all of the linoleate consumed for 16 wk and a 28% decrease in whole-body linoleate content, plasma fatty acid profiles did not reflect this significant change in linoleate homeostasis. In the 4 main plasma lipid classes, serum linoleate was not significantly affected by weight loss nor was the classic fatty acid marker of dietary linoleate deficiency, eicosatrienoate (20:3n−9), increased significantly after weight loss. This may have been due in part to the adequate linoleate intake during the study period. It may also have been that plasma fatty acid profiles are not sensitive to significant changes in linoleate metabolism when linoleate intakes are adequate. The significant positive correlation between changes in plasma cholesteryl linoleate and weight loss or adipose tissue loss suggests that this fraction of plasma linoleate could potentially substitute for the MRI-based adipose tissue measurements in estimating linoleate β-oxidation during weight loss involving food restriction and exercise.

TABLE 3
Change in adipose tissue linoleate and linoleate β-oxidation in relation to weight loss in obese men[1]

	r	P
Change in adipose tissue linoleate as a function of the reduction in adipose tissue (9.0 ± 4.5 kg[1]) during weight loss		
(ΔLinoleate, -4.11 ± 1.79 mol)	0.97	<0.0001
(ΔLinoleate, -0.7 ± 0.8%)	0.74	<0.02
Change in linoleate β-oxidation (6.77 ± 2.63 mol) as a function of the change in body weight (kg) or the change in adipose linoleate (mol)		
(ΔBody weight, -12.9 ± 4.9 kg)	0.82	<0.02
(ΔAdipose tissue linoleate, -4.11 ± 1.79 mol)	0.83	<0.005

[1] $\overline{x} \pm$ SD.

The possible effect of underreporting of food intake on whole-body linoleate has been discussed but other potential sources of error deserve comment. Fecal linoleate was measured from samples that were not from the subjects undergoing weight loss but the commonly observed 4% excretion of fat (and linoleate) was observed (32, 33). Although not measured in the subjects undergoing weight loss, values in the expected range for fat density and percentage of water in adipose tissue (34) were obtained from other obese subjects undergoing elective surgery. Skeletal muscle linoleate decreases slightly during exercise without weight loss (18) but we estimate that this change would not have significantly affected changes in whole-body linoleate in the present study because >98% of the whole-body reduction in linoleate with weight loss was from adipose tissue.

Calculating linoleate balance while excluding long-chain n−6 PUFAs and eicosanoids was not a significant source of error. Less than 3% of linoleate intake accumulates in the adult human body even when it is consumed at ≤38% of total fat intake (35). About 1% of dietary linoleate is converted to arachidonate in adult humans under normal dietary conditions (36) and an energy deficit inhibits the desaturation and chain elongation enzymes that are required to convert linoleate to longer-chain n−6 PUFAs (23). Thus, negligible linoleate should disappear through this route during weight loss. Under no circumstances reported does eicosanoid excretion by humans exceed 1 mg/d (37), which represents <0.0002% of the linoleate intake in this study. Thus, our estimate of linoleate β-oxidation during weight loss appears to be robust and consistent with the relevant literature (18, 27, 32, 38).

One important limitation to this method is that MRI equipment and the necessary software to obtain whole-body lean and adipose tissue volumes are not widely available for research purposes. Lean tissue biopsies are also more difficult to obtain than are adipose biopsies. Lean tissue will contain a higher proportion of the whole body content of linoleate and other PUFAs in normal- or underweight individuals than in obese individuals, making whole-body estimates of these fatty acids less reliable as the proportion of adipose tissue is reduced. Our data from plasma fatty acid profiles show that weight loss and adipose tissue loss were significantly correlated with a decreasing proportion of linoleate in plasma cholesteryl esters (Table 3). Thus, measurement of the decrease in plasma cholesteryl linoleate may be an accurate but simpler indirect measure of linoleate β-oxidation during weight loss in obese individuals.

REFERENCES

1. Garland M, Sacks FM, Colditz GA, et al. The relation between dietary intake and adipose tissue composition of selected fatty acids in US women. Am J Clin Nutr 1998;67:25–30.
2. Field CJ, Angel A, Clandinin MT. Relationship of diet to the fatty acid composition of human adipose tissue structural and stored lipids. Am J Clin Nutr 1985;42:1206–20.
3. van Staveren WA, Deurenberg P, Katan M, Burema J, de Groot LCP, Hoffmans MDA. Validity of the fatty acid composition of subcutaneous fat microbiopsies as an estimate of the long-term fatty acid composition of the diet of separate individuals. Am J Epidemiol 1986;123:455–63.
4. Phinney SD, Tang AB, Johnson SB, Holman RT. Reduced adipose tissue 18:3n−3 with weight loss by very low calorie diets. Lipids 1990;25:798–806.
5. Rossner S, Walldius G, Bjorvell H. Fatty acid composition in serum lipids and adipose tissue in severe obesity before and after six weeks of weight loss. Int J Obes 1989;13:603–12.
6. Hudgins LC, Hirsch J. Changes in abdominal and gluteal adipose-tissue fatty acid compositions in obese subjects after weight gain and weight loss. Am J Clin Nutr 1991;53:1372–7.
7. Chen Z-Y, Menard CR, Cunnane SC. Accumulation of polyunsaturates is decreased by weight cycling: whole-body analysis in young, growing rats. Br J Nutr 1996;75:583–91.
8. Jeppesen PB, Christensen MS, Hoy C-E, Mortensen PB. Essential fatty acid deficiency in patients with severe fat malabsorption. Am J Clin Nutr 1997;65:837–43.
9. Parsons HG, O'Loughlin EV, Forbes D, Cooper D, Gall DG. Supplemental calories improve essential fatty acid deficiency in cystic fibrosis patients. Pediatr Res 1988;24:353–6.
10. Cunnane SC, Craig K, Brookes S, et al. Synthesis of linoleate and α-linolenate by chain elongation in the rat. Lipids 1995;30:781–3.
11. Ross R, Rissanen J, Pedwell H, Clifford J, Shragge, P. Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men. J Appl Physiol 1996;72:787–95.
12. Cunnane SC, Wolever TMS, Jenkins DJA. Oleic and linoleic acids account for most of the free fatty acid response to glucose. Nutrition 1993;9:423–9.
13. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher, D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic imaging and computerized tomography. J Appl Physiol 1998;85:115–22.
14. Abate N, Garg A, Pesock RM, Stray-Gundersen J, Grundy SM. Relationships of generalized and regional adiposity to insulin sensitivity. J Clin Invest 1995;96:88–98.
15. Calder PC, Harvey DJ, Pond CM, Newsholme EA. Site-specific differences in the fatty acid composition of human adipose tissue. Lipids 1992;27:716–20.
16. Malcolm GT, Bhattacharyya AK, Velez-Duran M, Guzman MA, Oalmann MC, Strong JP. Fatty acid composition of adipose tissue in humans: differences between subcutaneous sites. Am J Clin Nutr 1989;50:288–91.
17. Jeejeebhoy KN, Ahmed S, Kozak G. Determination of fecal fats containing both medium and long chain triglycerides and fatty acids. Clin Biochem 1970;3:157–63.
18. Andersson A, Sjodin A, Olsson R, Vessby B. Effects of physical exercise on phospholipid fatty acid composition in skeletal muscle. Am J Physiol 1998;274:E432–8.
19. Clandinin MT, Chappell JE, Heim T, Swyer PR, Chance GW. Fatty acid utilization in perinatal de novo synthesis of tissues. Early Hum Devl 1981;5:355–66.
20. Borkman M, Storlein LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty acid composition of skeletal muscle phospholipids. N Engl J Med 1993;328:238–44.
21. Waskell L, Koblin D, Canova-Davis E. The lipid composition of human liver microsomes. Lipids 1982;17:317–20.
22. Jones PJH, Toy B, Cha M. Differential fatty acid accretion in heart, liver and adipose tissue of rats fed beef tallow, fish oil, olive oil and safflower oil at three levels of energy intake. J Nutr 1995;125:1175–82.
23. Brenner RR. The oxidative desaturation of unsaturated fatty acids in animals. Mol Cell Biochem 1974;3:41–52.
24. Gavino GR, Gavino V. Rat liver mitochondrial carnitine palmitoyl transferase activity towards long chain polyunsaturated fatty acids and their CoA esters. Lipids 1991;26:266–71.
25. Calles-Escandon J, Goran MI, O'Connell M, Sreekumaran Nair K, Danforth E Jr. Exercise increases fat oxidation at rest unrelated to changes in energy balance or lipolysis. Am J Physiol 1996;270:E1009–14.

26. Leyton J, Drury PJ, Crawford MA. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. Br J Nutr 1987; 57:383–93.

27. Jones PJH, Pencharz PB, Clandinin MT. Whole body oxidation of dietary fatty acids: implications for energy utilization. Am J Clin Nutr 1985;42:769–77.

28. Bjorntorp J. Rates of oxidation of different fatty acids by isolated rat liver mitochondria. J Biol Chem 1968;243:2130–3.

29. Mougios V, Kotzamanidis C, Koutsari C, Atsopardis S. Exercise-induced changes in the concentration of individual fatty acids and triacylgylcerols of human plasma. Metabolism 1995;44:681–8.

30. Blundell JE. What foods do people habitually eat? A dilemma for nutrition, an enigma for psychology. Am J Clin Nutr 2000;71: 3–5.

31. Mascioli EA, Lopes SM, Champagne C, Driscoll DF. Essential fatty acid deficiency and home total parenteral nutrition patients. Nutrition 1996;12:245–9.

32. Marshall MW, Judd JT, Matusik EJ Jr, Church J, Canary JJ. Effect of low fat diets varying in P/S ratio on nutrient intakes, fecal excre-tion, blood chemistry profiles and fatty acid profiles of adult men. J Am Coll Nutr 1986;5:263–79.

33. Jones PJH, Pencharz PB, Clandinin MT. Absorption of ^{13}C-labeled steariuc, oleic and linoleic acids in humans: applications to breath tests. J Lab Clin Med 1985;105:647–52.

34. Hirsch J, Farquhar JW, Ahrens EH Jr, Peterson ML, Stoffel W. Studies of adipose tissue in man. A microtechnic for sampling and analysis. Am J Clin Nutr 1960;8:499–511.

35. Dayton S, Hashimoto S, Dixon W, Pearce ML. Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. J Lipid Res 1966;7:103–11.

36. Emken EA, Adlof RO, Gulley RM. Dietary linolenic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young, male adults. Biochim Biophys Acta 1994; 1213:277–88.

37. Hansen HS. New biological and clinical roles for the n−6 and n−3 fatty acids. Nutr Rev 1994;52:162–7.

38. Cunnane SC, Anderson MJ. The majority of dietary linoleate in growing rats is oxidized or stored in visceral fat. J Nutr 1997;127:146–52.