British Journal of Nutrition (2004), **92**, 391–399
DOI: 10.1079/BJN20041217

The effect of six different C18 fatty acids on body fat and energy metabolism in mice

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(Received 19 November 2003 – Revised 29 March 2004 – Accepted 14 May 2004)

We studied the effects of five high-fat semi-purified diets varying at a 4 % (w/w) level in either stearic, oleic, linoleic, α-linolenic, or γ-linolenic acid on body fat and energy metabolism in BALB/c mice. A diet containing caprylic, capric, lauric, and myristic acid was used as a reference diet and a diet with 4 % conjugated linoleic acid (CLA) was used as a positive control as it is known to effectively lower body fat in mice. The diets were fed for 35 d. Body fat was significantly lower in the CLA group than in the other groups but was not significantly different among the non-CLA groups. Among the non-CLA groups, the linoleic acid group tended to have the highest and the α-linolenic acid group the lowest proportion of body fat. In energy-balance studies, the percentage of energy intake that was stored in the body was significantly lower in the CLA group compared with the other dietary groups. The percentage of energy intake eliminated in excreta was highest in the stearic acid group followed by the γ-linolenic acid group. These results were reflected in apparent fat digestibility, which was lowest in the stearic acid group. The percentage of energy intake expended as heat was highest in the CLA-fed mice. The results of the present study suggest that body fat and energy accretion in mice fed diets containing different C18 fatty acids is by far the lowest with CLA and that linoleic acid produced the highest fat intake and energy accretion.

C18 fatty acids: Conjugated linoleic acid: Body composition: Energy metabolism

The type of fat in the diet may affect body fat and energy metabolism. Animal studies suggest that medium-chain fatty acids (St-Onge & Jones, 2002) and fish oils (Soria *et al.* 2002) have a body fat-lowering effect. Long-chain fatty acids with varying degrees of unsaturation may differently affect body fat and energy metabolism, but the reported effects are not consistent. Studies in chickens indicate that diets high in linoleic acid lower body fat compared with more saturated fats such as lard and tallow (Pinchasov & Nir, 1992; Sanz *et al.* 1999, 2000*a*,*b*). Similar results have been reported in studies with rats (Shimomura *et al.* 1990; Dulloo *et al.* 1995; Takeuchi *et al.* 1995) and mice (Mercer & Trayhurn, 1987). However, there are also studies in rats (Awad *et al.* 1990; Hill *et al.* 1992, 1993; Su & Jones, 1993; Shillebeer & Lau, 1994; Okuno *et al.* 1997) and mice (Ikemoto *et al.* 1996) that did not show such a differential effect.

Studies in human subjects also suggest that the type of fat in the diet may affect energy metabolism. Jones & Schoeller (1988) reported that a diet with a high PUFA:saturated fatty acids (SFA) ratio tended to increase the thermogenic effect of food compared with a diet with a low PUFA:SFA ratio. Furthermore, the results of these studies suggest that with a high intake of PUFA there is an increased contribution of fat oxidation to the thermogenic effect of food whereas the contribution of carbohydrates is decreased. BMR was not affected by the fat type (Jones & Schoeller, 1988), but in another study polyunsaturated fat increased BMR (van Marken Lichtenbelt *et al.* 1997).

In the studies mentioned, the metabolic effects of various types of fat differing in fatty acid composition have been studied. In the present study, we were interested in specific fatty acids, particularly the C18 fatty acids, i.e. stearic, oleic, linoleic, α-linolenic, and γ-linolenic acid. We used a diet with a PUFA:SFA ratio of 1·00 as a reference diet. In addition, we used conjugated linoleic acid (CLA) as a positive control as it is known to effectively

Abbreviations: CLA, conjugated linoleic acid; SFA, saturated fatty acids.

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lower body fat in mice (West *et al.* 1998; Terpstra *et al.* 2002). High-fat, semi-purified diets varying in the type of C18 fatty acids at a 4 % (w/w) level were prepared and fed to mice for a period of 35 d. Body composition was measured and the energy balance was studied.

Materials and methods

The experimental protocol was approved by the animal experiments committee of the Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

Animals

Male mice (*n* 96, 5 weeks old; BALB/c–Charles River) were purchased from The Broekman Institute, Schoolstraat 21, 5711 CP, Someren, The Netherlands and housed in a temperature-controlled (21°C) animal room with a 12 h light–dark cycle (light on 06.00–18.00 hours). On arrival, the mice were placed in individual polycarbonate cages with a wire-mesh bottom. A polyethylene pipe with a diameter of 50 mm and a length of 140 mm was added to the cages as environmental enrichment. The mice were fed a commercial rodent diet (Hope Farms, 3440 AB Woerden, The Netherlands) for a period of 2 d and then the control diet (Table 1) for a period of 8 d. The mice were divided into eight groups of twelve animals balanced for body weight. One group was killed to collect pre-experimental values on body composition. The remaining seven groups were used for the feeding trial. Mice that had been spilling food during the 8 d pre-experimental period were allocated to the group that was killed at the beginning of the study. Thus, the experimental groups comprised only mice that did not spill any food.

Diets

We used high-fat (23·5 %, w/w) semi-purified diets (Table 1) containing 4 % (w/w) of different types of C18 fatty acids, i.e. stearic acid (18 : 0), oleic acid (18 : 1*n*-9), linoleic acid (18 : 2*n*-6), CLA (18 : 2*n*-6 and 18 : 2*n*-7), α-linolenic acid (18 : 3*n*-3), and γ-linolenic acid (18 : 3*n*-6). Various sources of natural available oils (Table 2) were added to the diets so that the diets varied in the different types of C18 fatty acids at a level of 4 g/100 g diet. Using these various oils (Table 2) and diets with 23·5 % total fat, we could only incorporate a maximum of 4 g of a specific fatty acid/100 g diet while keeping the composition of the other fatty acids constant. The different types of C18 fatty acids were added at the expense of caprylic (8 : 0), capric (10 : 0), lauric (12 : 0), and myristic (14 : 0) acid in the reference diet. The fatty acid compositions of the various oils were determined and the amounts of the oils that had to be added were calculated. Then, the fatty acid composition of the diets was determined to verify the final fatty acid composition (Table 2). Total lipids in the diets were extracted according to the official methods of analysis of the Association of Official Analytical Chemists (Horwitz, 1975) and the fatty acid composition was determined as described by Metcalfe *et al.* (1966).

Table 1. Composition of semi-purified diets*

Ingredient	g/kg	Metabolisable energy (%)
Casein	200	16·4
Total fat (oil blends)	235	43·3
Total carbohydrates	472	38·6
Maize starch	234	19·1
Dextrose	239	19·5
Cellulose	34	
$CaCO_3$	12	
$NaH_2PO_4.2H_2O$	15	
$MgCO_3$	1	
KCl	1	
$KHCO_3$	7	
Mineral premix†	10	0·8
Vitamin premix†	12	1·0
Total	1000	100

* The diets contained a calculated amount of 20·46 kJ metabolisable energy/g.
† The compositions of the vitamin and mineral premixes have been described previously (Terpstra *et al.* 1988).

The semi-purified diets were prepared by Research Diets Services (Hoge Maat 10, 3961 NC Wijk bij Duurstede, The Netherlands). The air-dry diets were stored at 4°C and, every other day, two parts of air-dry diet were mixed with one part water containing 5 g psyllium/l in a Kitchen Aid kitchen machine (model K5SS/PKM5; Kitchen Aid Europe, Brussels, Belgium). A dough-like-form diet was obtained which was facilitated by the $KHCO_3$ and psyllium and which prevented the mice from spilling the diets in the cages. The freshly prepared diets were fed to the mice in heavy glass containers that could not be tipped. In this way, food consumption could be measured accurately. Animals were offered the diets for *ad libitum* consumption and had free access to tap water. All excreta were also collected quantitatively throughout the 35 d experiment.

Chemical analysis

At the end of the study, the mice were killed by cervical dislocation and weighed after the bladder had been emptied. The carcass was cut in pieces and dried in a forced hot air oven at 60°C for a period of 3 d. The dried carcasses were weighed to calculate the percentage of water, homogenised in a coffee grinder and stored in airtight glass containers. Excreta were dried, homogenised, and stored in the same way.

Total lipids in the dried, homogenised carcasses and excreta were extracted according to the official methods of analysis of the Association of Official Analytical Chemists (Horwitz, 1975). About 1 g dried material was added to a Majonnier flask and 2 ml ethanol was added to moisturise the material. Subsequently, 10 ml HCl (8 mol/l) was added, the contents were gently mixed, and the flask was placed in a water-bath of 80°C for 30–40 min. The tubes were cooled down, 10 ml ethanol (96 %) and 25 ml diethyl ether were added and the tube was vigorously shaken for 1 min. Then, 25 ml petroleum ether (boiling point between 40 and 60°C) was added and the tube was again vigorously shaken for another 1 min. The fat-containing upper layer was decanted in a 150 ml round-bottomed flask. The extraction procedure was

Table 2. Amounts of various oils added to the diets and the measured fatty acid composition of the diets

Diets…	Control	Stearic acid	Oleic acid	Linoleic acid	CLA	α-Linolenic acid	γ-Linolenic acid
Oils added to the diets (g/100 g diet)							
Linseed oil	0·60	0·60	0·60	0·60	0·60	8·98	0·60
Sunflower seed oil (60 % linoleic acid)	13·12	13·18	12·67	19·41	13·11	11·32	
Olive oil	1·63	2·17	7·56		0·95	0·27	4·64
Coconut oil	7·37	2·44	2·36	2·62	2·46	1·66	1·87
Palm oil	0·57	0·21	0·15	0·73	1·13	1·13	0·56
Stearic acid-rich fat	0·21	4·90	0·16	0·14	0·21	0·14	0·26
Clarinol G-80 (CLA)*					5·04		
Gammanol E-25 (primrose oil)							15·57
Total oils added (g/100 g diet)	23·50	23·50	23·50	23·50	23·50	23·50	23·50
Total fat measured (g/100 g diet)	24·07	25·22	24·28	24·41	24·35	23·91	23·95
Fatty acid composition of the diets†							
8 : 0 (Caprylic)	0·59	0·21	0·20	0·22	0·20	0·13	0·17
10 : 0 (Capric)	0·44	0·16	0·15	0·17	0·16	0·11	0·15
12 : 0 (Lauric)	3·25	1·13	1·08	1·19	1·15	0·71	0·88
14 : 0 (Myristic)	1·32	0·49	0·46	0·51	0·51	0·33	0·38
16 : 0 (Palmitic)	2·05	2·13	1·94	1·81	1·99	1·88	1·99
16 : 1*n*-7 (Palmitoleic)	0·03	0·04	0·08	0·02	0·03	0·02	0·06
18 : 0 (Stearic)	1·08	4·64	0·85	0·88	0·94	0·86	0·95
18 : 1*n*-9 (Oleic)	4·88	5·24	8·68	4·96	4·94	4·82	4·87
18 : 2*n*-6 (Linoleic)	8·51	9·10	8·75	12·62	8·60	8·76	8·46
18 : 2*n*-6 and *n*-7 (CLA)	0·00	0·00	0·00	0·00	3·55	0·00	0·00
18 : 3*n*-3 (α-Linolenic)	0·28	0·30	0·32	0·27	0·28	4·22	0·37
18 : 3*n*-6 (γ-Linolenic)	0·00	0·00	0·00	0·00	0·00	0·00	3·68
Other fatty acids	0·43	0·46	0·50	0·46	0·53	0·43	0·54
Unidentified fatty acids	0·01	0·06	0·05	0·06	0·25	0·44	0·27
Total SFA	39·9	37·3	20·7	21·1	22·1	18·4	20·5
Total MUFA	21·9	22·5	39·9	22·0	22·2	22·1	22·4
Total PUFA	39·1	40·1	40·2	56·8	53·2	59·4	57·0
PUFA:MUFA:SFA ratio	0·39:0·21:0·39	0·37:0·22:0·40	0·20:0·39:0·40	0·21:0·22:0·56	0·22:0·22:0·53	0·18:0·22:0·59	0·20:0·22:0·57
PUFA:SFA ratio	1·00	0·93	0·50	0·38	0·42	0·31	0·35

CLA, conjugated linoleic acid; SFA, saturated fatty acids.
*Clarinol G-80 contained 37 % *cis-9,trans*-11 CLA and 38 % *trans*-10, *cis*-12 CLA in the form of triacylglycerols.
†Total fatty acid contents were calculated as follows: total fat measured × 0·95.

repeated twice with 15 ml diethyl ether and 15 ml petroleum ether and the lipid extract was evaporated to dryness under N_2 in a water-bath of 40°C. The round-bottomed flasks with the lipids were dried overnight at 60°C and the total lipids were measured gravimetrically.

For the determination of the ash content, about 0·5 g dried, homogenised carcass was added to a small porcelain crucible and put in an oven that was programmed as follows: 1 h at 200°C, 2 h at 300°C, 3 h at 400°C, and 10 h at 500°C.

The protein content of the dried carcasses was determined with the macro Kjeldahl method.

Bomb calorimetry

The gross energy content in the dried, homogenised carcasses, excreta, and diet was determined with a bomb calorimeter (IKA Calorimeter C4000 Adiabatic; IKA Analysetechnik, Grißheimerweg 5, D-79423, Heitersheim, Germany). As a thermochemical standard, benzoic acid was used (BHD Limited, Poole, Dorset, UK). The total amount of energy that was lost as heat (heat production or energy expenditure) was calculated with the formula:

Energy lost as heat = energy in food – energy in excreta – energy stored in body.

Energy stored in the body was determined as total energy at the end of the 35 d feeding period minus the energy in the body at the beginning of the 35 d feeding period. The same procedure was used to calculate the water, protein, fat and ash retention.

Statistical analysis

The data of the seven dietary groups were statistically analysed with a one-way ANOVA with diet as factor. When ANOVA indicated a significant diet effect for a certain variable, Dunnett's *t* test was used to identify diets which were different compared with the control. The SPSS® statistical software package version 10.1 (Chicago, IL, USA) was used for all the statistical analyses.

Results

Body weight and body composition

The mice fed the stearic acid diet tended to have the highest food intake (Table 3) whereas body-weight gain tended to be the highest in the reference group. Fat deposition was significantly lower and water deposition significantly higher in the mice fed CLA when compared with the other dietary groups (Table 3 and Fig. 1). As a consequence, the amount and proportion of body fat were also significantly lower in the CLA group compared with the other groups whereas the amount and proportion of body water was significantly higher. The deposition, amount and proportion of body fat in the dietary groups other than the CLA group were not significantly different. However among the non-CLA groups, the group fed linoleic acid tended to have the highest deposition and amount of body fat and the lowest deposition and amount of body water. When the mice in all the groups were taken together, there was a significant negative correlation between the proportion of body water and the proportion of body fat (r −0·986; $P<0·01$). Protein and ash depositions were not significantly different among all the various groups and the same was true for the amount and proportion of body protein and ash.

Body composition was determined by measuring separately the amount of water, fat, protein and ash in the carcasses. These four body components added up to about 98–99 % of the weight of the carcasses. The remainder may partially represent losses but also accounts for the amount of glycogen in the body, which was not measured separately. The recoveries in the various groups were similar.

Energy balance

The percentage of energy in the food that was stored in the body was significantly lower in the CLA group compared with the other groups. Essentially no energy was stored in the CLA group. This low efficiency of energy storage was reflected in a significantly lower total energy and fat content of the body. The percentage of energy in the food stored in the body was not significantly different between the non-CLA groups. However, among the non-CLA groups, the mice in the linoleic acid group tended to have the highest percentage of energy in the food that was stored in the body, which is in line with the high amounts of body energy and body fat. When all the mice were taken together, there was a positive correlation between the proportion of body fat and the percentage of energy in the food stored in the body and there was a negative correlation between the proportion of body fat and the percentage of energy in the food expended as heat (Fig. 2).

The percentage of energy in the food that was eliminated into the excreta was significantly higher in the stearic acid group compared with the other groups. The other groups were not significantly different, but the α-linolenic acid group tended to have a higher percentage of energy in the food eliminated in the excreta than did the other non-stearic acid groups. Furthermore, higher percentages of energy in the food eliminated in the excreta were predominantly the result of an increased energy excretion in the form of fat and reflected differences in the apparent fat digestibility. The stearic acid-fed mice had a significantly lower apparent fat digestibility than all the other groups and the γ-linolenic acid group had a lower apparent fat digestibility than the other non-stearic acid groups.

Energy expenditure was calculated as the difference between the energy intake and the energy stored and eliminated in the faeces. The highest relative energy expenditure was calculated in the CLA-fed mice, but this effect only reached statistical significance when compared with the stearic acid group, which had the lowest relative energy expenditure. This low energy expenditure in the stearic acid group, however, was associated with a high excretion of energy in the faeces. As a result, the energy stored in the stearic acid group was, for instance, similar to that in the oleic acid group which had a higher energy expenditure but a lower energy excretion in the faeces (Table 3 and Fig. 2).

Table 3. Body composition and energy balance in mice fed high-fat semi-purified diets containing various C18 fatty acids for a period of 35 d†
(Mean values and pooled standard errors of the mean)

Diets...	Control	Stearic acid	Oleic acid	Linoleic acid	CLA	α-Linolenic acid	γ-Linolenic acid	Pooled SEM	*P* value
Food intake (g dry food/35 d)	113·5	120·5*	114·0	113·3	112·3	114·3	115·7	1·629	0·017
Food intake (g dry food/d)	3·2	3·4*	3·3	3·2	3·2	3·3	3·3	0·05	0·010
Initial body weight (g)	20·7	20·7	20·4	20·7	20·5	20·4	20·4	0·31	0·956
Final body weight (g)	26·1	25·7	25·3	26·0	25·7	25·7	25·1	0·42	0·713
Apparent fat digestibility (%)	96·6	86·2*	96·7	96·7	96·7	96·7	92·4	0·22	<0·001
Metabolised energy (%)	93·3	88·6*	93·8	93·5	92·9	94·0*	91·6*	0·19	<0·001
Body composition									
Fat (g)	2·95	2·75	2·71	3·12	1·17*	2·56	2·63	0·169	<0·001
Water (g)	16·69	16·65	16·32	16·47	18·03*	16·78	16·34	0·245	<0·001
Protein (g)	5·13	5·09	5·01	5·10	5·21	5·12	4·96	0·078	0·425
Ash (g)	0·89	0·86	0·88	0·87	0·92	0·87	0·85	0·020	0·210
Recovery (%)	98·6	98·6	98·4	98·4	98·5	98·5	98·8	2·875	0·960
Water:protein ratio (g/g)	3·26	3·28	3·26	3·23	3·47	3·29	3·32	0·074	0·361
Fat (%)	11·4	10·8	10·9	12·2	4·6*	10·1	10·6	0·58	<0·001
Water (%)	65·1	65·7	65·7	64·5	71·2*	66·3	65·9	0·50	<0·001
Protein (%)	20·0	20·1	20·2	20·0	20·6	20·3	20·1	0·41	0·958
Ash (%)	3·5	3·4	3·2	3·4	3·6	3·4	3·4	0·13	0·497
Change in body composition									
Weight gain (g)	5·38	5·04	4·83	5·27	5·26	5·31	4·76	0·269	0·588
Fat deposition (g)	0·95	0·75	0·75	1·12	−0·84*	0·59	0·67	0·166	<0·001
Water deposition (g)	2·83*	2·77	2·66	2·59	4·35*	3·12	2·68	0·201	<0·001
Protein deposition (g)	1·34	1·29	1·24	1·32	1·44	1·34	1·17	0·062	0·146
Ash deposition (g)	0·27	0·23	0·19	0·24	0·30	0·25	0·23	0·034	0·366
Energy balance (kJ)									
Intake	2542	2666	2566	2465	2509	2558	2597	36·4	<0·009
Storage	63	55	53	69	0·0*	49	45	7·1	<0·001
Expenditure	2311	2311	2355	2236	2343	2356	2335	35·8	0·241
In excreta	169	300*	159	160	165	153	217	5·8	<0·001
In excreta as fat‡	38	167*	36	36	36	36	84	3·0	<0·001
In fat-free excreta	131	133	122	124	129	117	133	4·0	0·049
Energy in whole body									
Initial body energy§ (kJ)	167	167	165	161	165	164	163	2·875	0·960
Final body energy‖ (kJ)									
Measured‖	230	222	217	236	165*	214	209	8·1	<0·001
Calculated¶	230	222	218	236	160	211	209		
Percentage of energy intake that is:									
Stored in the body	2·5	2·1	2·1	2·8	0·0*	1·9	1·7	0·28	<0·001
Expended as heat	90·9	86·7*	91·8	90·7	93·4*	92·1	89·9	0·37	<0·001
Lost in excreta	6·6	11·3*	6·2	6·5	6·6	6·0	8·3*	0·19	<0·001
Lost in excreta as fat	1·5	6·3*	1·4	1·5	1·5	1·4	3·2	0·11	<0·001
Lost in fat-free excreta	5·2	5·0	4·8	5·0	5·1	4·6*	5·1	0·13	0·026

CLA, conjugated linoleic acid.
* Mean value was significantly different from that of the control group ($P<0·05$).
† The data of the seven dietary groups (n 12 mice per group) were statistically analysed with a one-way ANOVA with diet as factor. When ANOVA indicated a significant diet effect, Dunnett's t test was used to identify diets which were different compared with the control on the variable involved. The various C18 fatty acids varied in the diets at a level of 4% (w/w).
‡ Energy in excreta as fat was calculated on the basis of the amount of measured fat in the excreta, given 1 g fat has a gross energy content of 39·8 kJ (McLean & Tobin, 1987).
§ Calculated from a regression line describing the relationship between body weight and total body energy in the twelve mice that had been killed at the beginning of the study.
‖ Measured with a bomb calorimeter.
¶ Calculated on basis of the body composition, given that 1 g animal fat contains 39·8 kJ and 1 g protein contains 23·7 kJ gross energy (McLean & Tobin, 1987).

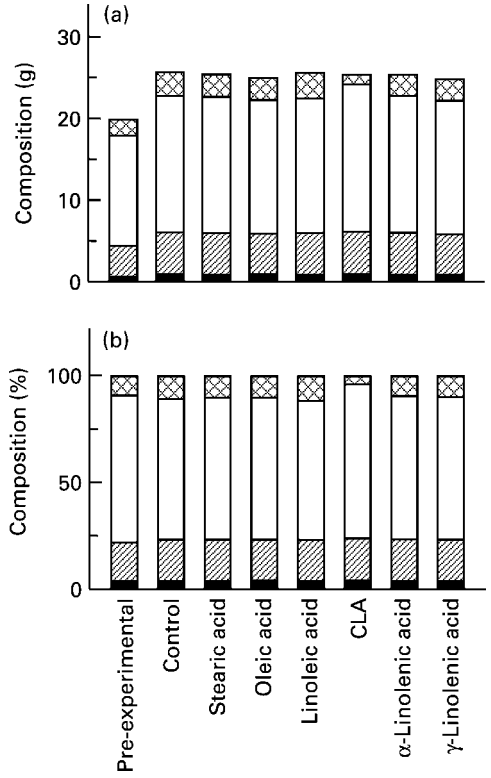

Fig. 1. Absolute whole-body composition (a) and relative whole-body composition (b) of mice fed for 35 d high-fat semi-purified diets varying at a 4 % level in different types of C18 fatty acids. The composition of the mice that were killed at the beginning of the experiment (pre-experimental group) is also given. Values are means (*n* 12 per group). The results of the statistical analyses of the data are given in Table 3. Statistical analysis (ANOVA) of the data for relative whole-body composition showed that there was a significant ($P<0{\cdot}05$) effect of conjugated linoleic acid (CLA) on body fat and body water. When ANOVA indicated a significant diet effect, Dunnett's *t* test was used to identify diets which were different compared with the control on the variable involved. (▨), Fat; (□), water; (▨), protein; (■), ash.

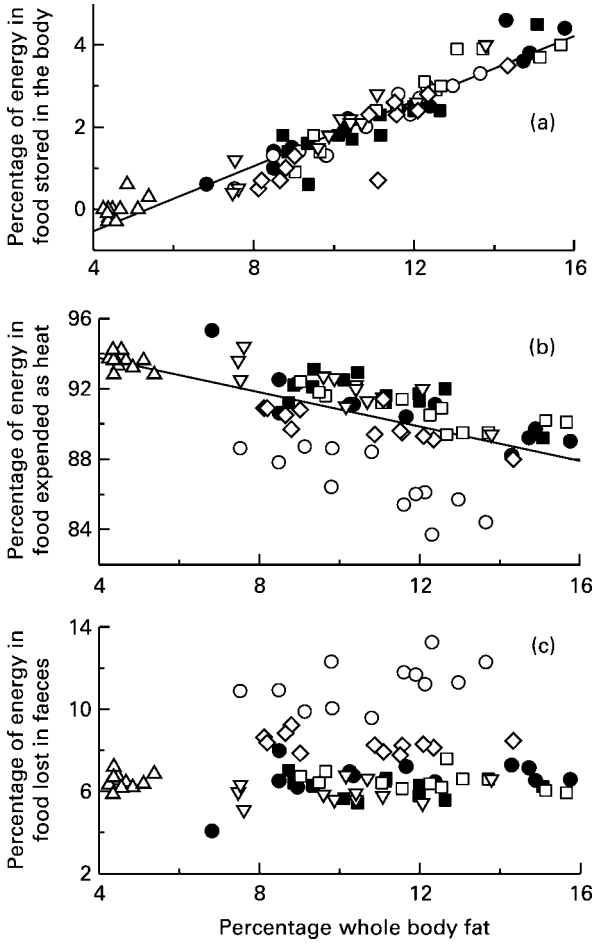

Fig. 2. Correlation between the percentage of body fat and the percentage of energy in the food stored in the body (a), expended as heat (b), and lost in the faeces (c). When all the mice were taken together, there was a positive correlation ($y = 0{\cdot}3968x - 2{\cdot}1296$; r 0·9487, $P<0{\cdot}001$) between the proportion of body fat and the percentage of energy in the food stored in the body and a negative correlation ($y = -0{\cdot}4872x + 95{\cdot}6991$; r −0·6164, $P<0{\cdot}001$) between the proportion of body fat and the percentage of energy in the food expended as heat. (●), Control diet; (○), stearic acid diet; (■), oleic acid diet; (□), linoleic acid diet; (Δ), conjugated linoleic acid diet; (V), α-linolenic acid diet; (◇), γ-linolenic acid diet.

Discussion

We studied the effects of various C18 fatty acids on body fat and energy metabolism. Incorporation of CLA in the diet resulted by far in the lowest amount of body fat and efficiency of energy storage compared with the other dietary groups. This body fat-lowering effect of CLA has been well documented in a large number of studies in mice (West *et al.* 1998; Terpstra *et al.* 2002). However, in those studies the lowering effect of CLA on body fat was demonstrated when CLA was incorporated in the diet at a level of 1 g/100 g diet. In the present study we found that adding 4 g CLA/100 g diet resulted in a proportion of body fat of about 5 %, which is similar to that reported earlier by Terpstra *et al.* (2002). These findings suggest that adding more than 1 g CLA/100 g diet does not further lower body fat. The level of 5 % of body fat probably is the minimum amount reflecting the structural lipids in the body after all the fat depots are depleted.

The major changes in body composition due to feeding CLA were a decrease in body fat and an increase in body water. CLA feeding tended to increase the water:protein ratio in the body, indicating an increase in hydration of the fat-free body mass. As mentioned earlier, CLA feeding may have depleted all storage fat, including that in the lean body mass. A decrease in cellular fat will increase cellular water. If one assumes that the composition of the lean body mass is constant and contains 73 % water, then the relationship between the percentage of whole body fat and percentage of whole body water can be described by the equation:

Percentage body fat = 100 − (1/0·73) % body water, or Percentage body fat = −1·37 % body water + 100 (Pace & Rathbun, 1945).

The percentage water in the lean body mass, however, may slightly differ in animal species (Kodama, 1971; Sheng & Huggins, 1979; Rogers & Webb, 1980) and can also be affected by the amount of whole body fat as seen in the present study.

Linoleic acid was the only dietary variable that tended to raise the amount of body fat and it could be speculated that

the high levels of linoleic acid in our diets have interfered with the fat-lowering properties of CLA. However, as far as we know, there is no evidence for an interaction, if any, between linoleic acid and CLA. There were no significant differences in body fat between the non-CLA groups, but the mice fed the α-linolenic acid diet tended to have the lowest amount of body fat and the linoleic acid group the highest. Similar results have been found in other studies with mice. Ikemoto *et al.* (1996) reported that feeding perilla oil, rich in α-linolenic acid, resulted in a numerically lower group mean amount of white adipose tissue than did the feeding of safflower-seed oil, which is rich in linoleic acid. In addition, Takahashi & Ide (2000) observed that rats fed perilla oil had a significantly lower percentage of epididymal and peri-renal fat than rats fed safflower-seed oil. There are, however, also studies in mice (Hamura & Kudo, 2001) and rats (Hill *et al.* 1993; Takeuchi *et al.* 1995) that did not show such a differential effect on body fat of perilla or linseed oil, both of which are rich in α-linolenic acid *v*. safflower-seed oil or maize oil, which are rich in linoleic acid.

The mice fed γ-linolenic acid tended to have the second lowest amount of body fat among the non-CLA groups. Takada *et al.* (1994) reported that rats fed oil extracted from fungi containing 25 % γ-linolenic acid had a significantly lower proportion of body fat than rats fed soyabean oil containing 53 % linoleic acid. In addition, the rats fed on the diet rich in γ-linolenic acid had a higher activity of carnitine palmitoyl transferase, an enzyme involved in fatty acid oxidation. The studies of Takahashi *et al.* (2000) similarly showed that rats fed borage oil, which is rich in γ-linolenic acid, had a significantly lower amount of epididymal and peri-renal fat than rats fed safflower-seed oil. Further, Phinney *et al.* (1993) observed in obese Zucker rats, but not their lean counterparts, that feeding blackcurrant oil containing 70 % γ-linolenic acid resulted in a significantly lower percentage of body fat than did feeding soyabean oil containing 55 % linoleic acid.

The tendency towards differences in body fat in the mice fed the various C18 fatty acids may be related to differences in the rate of oxidation of these fatty acids. Studies in human subjects (DeLany *et al.* 2000) indicated that the rate of oxidation of SFA increased with decreasing chain length and that the rate of oxidation of the C18 fatty acids was positively correlated with the number of double bonds. Similarly, *in vitro* studies indicated that the order of the maximum rate of oxidation was linoleate > oleate > stearate (Björntorp, 1968). Other studies in human subjects showed that the order of the rate of oxidation was oleate > linoleate > stearate (Jones *et al.* 1985*a*) and studies in rats showed that the oxidation rate was in the order of α-linoleate > oleate > linoleate > stearate. Whole-body fatty acid balance analysis in rats showed that 75·5 % of the linoleic acid consumed was oxidised and 18·75 % was stored whereas 84·9 % of the α-linolenic acid was oxidised and 10·9 % was stored (Cunnane & Anderson, 1996). Further, Kabir & Ide (1996) reported that feeding rats on diets with linseed or perilla oil, which are rich in α-linolenic acid, resulted in an increased fatty acid oxidation in the liver compared with feeding a diet with safflower-seed oil which is rich in linoleic acid. However, studies of Jones (1994) indicated that α-linolenic, linoleic and oleic acid were oxidised at similar rates in rats fed a diet containing these fatty acids in equal proportions. Thus, most of the studies suggest that α-linolenic acid has the highest oxidation rate among the C18 fatty acids, or at least results in the highest oxidation rate of fatty acids, which may explain the low amount of body fat in the α-linolenic acid-fed mice in the present study. Furthermore, the literature data suggest that stearic acid may have the lowest rate of oxidation, but the results for the other C18 fatty acids are not consistent. The oxidation rates of various fatty acids have also been discussed extensively by Jones (1994).

The effect of a dietary fatty acid on body fat and energy metabolism may also be associated with the capacity of a fatty acid to be stored and mobilised. There are indications that α-linolenic and oleic acid are less efficiently stored in the adipose tissue than linoleic and stearic acids (Lin *et al.* 1993; Yeom *et al.* 2002) and that the rate of mobilisation from the adipose tissue is higher for α-linolenic acid than for linoleic acid (Raclot *et al.* 1997). A lower storage capacity and a higher mobilisation rate of a fatty acid may result in a higher oxidation rate.

The effect of fatty acids on body fat and energy metabolism may be due to differences in the rate of oxidation of the fatty acids *per se*, but there is also evidence that fatty acids may influence the transcription of genes involved in fat metabolism. In particular, the *n*-3 family of PUFA appear to have the ability to enhance thermogenesis and to reduce body-fat deposition (Clarke, 2000). These fatty acids may act as ligands for transcription factors and up regulate the transcription of mitochondrial uncoupling proteins, which results in less energy deposition and more energy dissipation. Furthermore, they may induce genes encoding proteins involved in fatty acid oxidation while simultaneously down regulating the transcription of genes encoding proteins involved in lipid synthesis.

Apparent fat digestibility was considerably lower in the mice fed the stearic acid diet than in the mice in the other groups. Studies in rats (Carroll, 1958; Carroll & Richards, 1958) also showed that stearic acid fed as non-esterified fatty acid or as triacylglycerol is poorly absorbed compared with oleic acid. Similarly, studies in human subjects with stable-isotope-labelled fatty acids (Jones *et al.* 1985*b*) indicated that the absorption of stearic acid (78 %) was considerably lower than the absorption of oleic and linoleic acid (98–99·9 %). The absorption of stearic acid is also affected by the position of stearic acid in the triacylglycerol molecule; stearic acid is better absorbed when it is positioned in the sn-2 position than in the 1, 3 position of the triacylglycerol molecule (Bracco, 1994).

Thus, the results of the present study indicate that the type of fatty acid in the diet may affect body fat and energy metabolism. Incorporation of CLA in the diets resulted in significantly lower body fat than the other C18 fatty acids. Body fat of the non-CLA groups did not significantly differ, but the α-linolenic acid-fed mice tended to have the lowest amount of body fat and the mice in the linoleic acid group tended to have the highest amount of body fat. Further studies with larger inclusion

levels of specific fatty acids should be done to define more clearly their effects on energy metabolism.

Acknowledgements

We acknowledge Loders Croklaan B.V., Hogeweg 1, 1521 AZ Wormerveer, The Netherlands for donating the CLA (Clarinol G-80) and primrose oil (Gammanol E-25) preparations. We thank Jan van der Kuilen for his excellent technical assistance. The study was supported by Nutreco Agriculture R&D, Boxmeer, The Netherlands.

References

Awad AB, Bernardis LL & Fink CS (1990) Failure to demonstrate an effect of dietary fatty acid composition on body weight, body composition and parameters of lipid metabolism in the mature rat. *J Nutr* **120**, 1277–1282.

Björntorp P (1968) Rates of oxidation of different fatty acids by isolated rat liver mitochondria. *J Biol Chem* **243**, 2130–2133.

Bracco U (1994) Effect of triglyceride structure on fat absorption. *Am J Clin Nutr* **60**, Suppl., 1002S–1009S.

Carroll KK (1958) Digestibility of individual fatty acids in the rat. *J Nutr* **64**, 399–410.

Carroll KK & Richards JF (1958) Factors affecting digestibility of fatty acids in the rat. *J Nutr* **64**, 411–424.

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.

Cunnane SC & Anderson MJ (1996) The majority of dietary linoleate in growing rats is β-oxidized or stored in visceral fat. *J Nutr* **127**, 146–152.

DeLany JP, Windhauser MM, Champagne CM & Bray GA (2000) Differential oxidation of individual dietary fatty acids in humans. *Am J Clin Nutr* **72**, 905–911.

Dulloo AG, Mensi N, Seydoux J & Girardier L (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 fat intake. *Metabolism* **44**, 273–279.

Hamura M & Kudo S (2001) Effects of conjugated linoleic acid (CLA) on abdominal fat accumulation: comparison with other dietary oils. *J Oleo Sci* **50**, 129–132.

Hill JO, Lin D, Yakubu F & Peters JC (1992) Development of dietary obesity in rats: influence of amount and composition of dietary fat. *Int J Obesity* **16**, 321–333.

Hill JO, Peters JC, Lin D, Yakubu F, Greene H & Swift L (1993) Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats. *Int J Obesity* **17**, 223–236.

Horwitz W (1975) *Official Methods of Analysis of Official Analytical Chemists*, 12th ed., p. 225, Benjamin Franklin Station, DC: Association of Official Analytical Chemists.

Ikemoto S, Takahashi M, Tsunoda N, Maruyama K, Itakura H & Ezaki O (1996) High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. *Metabolism* **45**, 1539–1546.

Jones PJH (1994) Dietary linoleic, α-linolenic, and oleic acids are oxidized at similar rates in rats fed a diet containing these acids in equal proportions. *Lipids* **29**, 491–495.

Jones PJH, Pencharz PB & Clandinin MT (1985*a*) Whole body oxidation of dietary fatty acids: implications for energy utilization. *Am J Clin Nutr* **42**, 769–777.

Jones PJH, Pencharz PB & Clandinin MT (1985*b*) Absorption of ^{13}C-labeled stearic, oleic, and linoleic acids in humans: application to breath tests. *J Lab Clin Med* **105**, 647–652.

Jones PJH & Schoeller DA (1988) Polyunsaturated:saturated ratio of diet fat influences energy substrate utilization in the human. *Metabolism* **37**, 145–151.

Kabir Y & Ide T (1996) Activity of hepatic fatty acid oxidation enzymes in rats fed α-linolenic acid. *Biochim Biophys Acta* **1304**, 105–119.

Kodama AM (1971) In vivo and in vitro determinations of body fat and body water in the hamster. *J Appl Physiol* **31**, 218–222.

Lin DS, Connor WE & Spenler CW (1993) Are dietary saturated, monounsaturated, and polyunsaturated fatty acids deposited to the same extent in adipose tissue of rabbits? *Am J Clin Nutr* **58**, 174–179.

McLean JA & Tobin G (1987) *Animal and Human Calorimetry*. Cambridge, UK: Cambridge University Press.

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.

Metcalfe LD, Schmitz AA & Pelka JR (1966) Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal Chem* **38**, 514–515.

Okuno M, Kajiwara K, Imai S, *et al.* (1997) Perilla oil prevents the excessive growth of visceral adipose tissue in rats by down-regulating adipocyte differentiation. *J Nutr* **127**, 1752–1757.

Pace N & Rathbun EN (1945) Studies on body composition III. The body water and chemically combined nitrogen content in relation to fat content. *J Biol Chem* **158**, 685–691.

Phinney SD, Tang AB, Thurmond DC, Nakamura MT & Stern JS (1993) Abnormal polyunsaturated lipid metabolism in the obese Zucker rat, with partial metabolic correction by γ-linolenic acid administration. *Metabolism* **42**, 1127–1140.

Pinchasov Y & Nir I (1992) Effect of dietary polyunsaturated fatty acid concentration on performance, fat deposition, and carcass fatty acid composition in broiler chickens. *Poultry Sci* **71**, 1504–1512.

Raclot T, Langin D, Lafontan M & Groscolas R (1997) Selective release of human adipocyte fatty acids according to molecular structure. *Biochem J* **324**, 911–915.

Rogers P & Webb GP (1980) Estimation of body fat in normal and obese mice. *Br J Nutr* **43**, 83–86.

Sanz M, Flores A & Lopez-Bote CJ (2000*a*) The metabolic use of energy from dietary fat in broilers is affected by fatty acid saturation. *Br Poultry Sci* **412**, 61–68.

Sanz M, Flores A, Perez de Ayala P & Lopez-Bote CJ (1999) Higher lipid accumulation in broilers fed on saturated fats than in those fed on unsaturated fats. *Br Poultry Sci* **40**, 95–101.

Sanz M, Lopez-Bote CJ, Menoyo D & Bautista JM (2000*b*) Abdominal fat deposition and fatty acid synthesis are lower and β-oxidation is higher in broiler chickens fed diets containing unsaturated rather than saturated fat. *J Nutr* **130**, 3034–3037.

Sheng HP & Huggins RA (1979) A review of body composition studies with emphasis on total body water and fat. *Am J Clin Nutr* **32**, 630–647.

Shillebeer G & Lau DCW (1994) Regulation of new fat cell formation in rats: the role of dietary fat. *J Lipid Res* **35**, 592–600.

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.

Soria A, Chicco A, D'Alessandro ME, Rossi A & Lombardo YB (2002) Dietary fish oil reverses epididymal tissue adiposity, cell hypertrophy and insulin resistance in dyslipemic sucrose fed rat model. *J Nutr Biochem* **13**, 209–218.

St-Onge M-P & Jones PJH (2002) Physiological effects of medium-chain triglycerides: potential agents in the prevention of obesity. *J Nutr* **132**, 329–332.

Su W & Jones PJH (1993) Dietary fatty acid composition influences energy accretion in rats. *J Nutr* **123**, 2109–2114.

Takada R, Saitoh M & Mori T (1994) Dietary γ-linolenic acid-enriched oil reduces body fat content and induces liver enzyme activities relating to fatty acid β-oxidation in rats. *J Nutr* **124**, 469–474.

Takahashi Y & Ide T (2000) Dietary n-3 fatty acids affect mRNA level of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. *Br J Nutr* **84**, 175–184.

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* **127**B, 213–222.

Takeuchi H, Matsuo T, Tokuyama K, Shimomura Y & Suzuki M (1995) Diet-induced thermogenesis is lower in rats fed a lard than in those fed a high oleic acid safflower oil diet, a safflower oil diet or a linseed oil diet. *J Nutr* **125**, 920–925.

Terpstra AHM, Beynen AC, Everts H, Kocsis S, Katan MB & Zock PL (2002) The decrease in body fat in mice fed conjugated linoleic acid is due to increases in energy expenditure and energy loss in the excreta. *J Nutr* **132**, 940–945.

Terpstra AHM, Lapré JA, de Vries HT & Beynen AC (1988) Dietary pectin with high viscosity lowers plasma and liver cholesterol concentrations and plasma cholesteryl ester transfer protein activity in hamsters. *J Nutr* **128**, 1944–1949.

van Marken Lichtenbelt WD, Mensink RP & Westerterp KR (1997) The effect of fat composition of the diet on energy metabolism. *Z Ernaehrungswiss* **36**, 303–305.

West DB, Delany JP, Camet PM, Blohm F, Truett AA & Scimeca J (1998) Effect of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am J Physiol* **275**, R667–R672.

Yeom K-H, van Trierum G, Hovenier R, Schellingerhout AB, Lee K-W & Beynen AC (2002) Fatty acid composition of adipose tissue in goat kids fed milk replacers with different contents of α-linolenic and linoleic acid. *Small Rumin Res* **43**, 15–22.