Octacosanol affects lipid metabolism in rats fed on a high-fat diet

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The effect of dietary octacosanol, a long-chain alcohol, on lipid metabolism was investigated in rats fed on a high-fat diet for 20 d. The addition of octacosanol (10 g/kg diet) to the high-fat diet led to a significant reduction ($P < 0{\cdot}05$) in the perirenal adipose tissue weight without decrease of the cell number, suggesting that octacosanol may suppress lipid accumulation in this tissue, whereas no effect was seen in the epididymal adipose tissue weight and in the lipid content in liver. Octacosanol supplementation decreased the serum triacylglycerol concentration, and enhanced the concentration of serum fatty acids, probably through inhibition of hepatic phosphatidate phosphohydrolase (*EC* 3.1.3.4). Though the activity of hormone-sensitive lipase (*EC* 3.1.1.3) was not influenced by octacosanol, higher activities of lipoprotein lipase (*EC* 3.1.1.34) in the perirenal adipose tissue and the total oxidation rate of fatty acid in muscle were observed. Lipid absorption was not affected by the inclusion of octacosanol. Thus, the present results suggest that the dietary incorporation of octacosanol into a high-fat diet affects some aspects of lipid metabolism.

Octacosanol: Lipid metabolism: Adipose tissue: Serum triacylglycerol: Rat

Octacosanol ($CH_3(CH_2)_{26}CH_2OH$), is an aliphatic primary alcohol. Only small quantities of this compound are available in human diets from plants, mainly as a wax in the superficial layers of fruit, leaves and skin of common plants as well as whole grains.

In previous work (Cureton, 1972) it was shown that extracts from wheat-germ oil had beneficial effects on the physical performance of athletes. Based on these observations, these researchers speculated that one of the several compounds detected in wheat-germ oil, octacosanol, may stimulate catabolism, thereby promoting energy production. More recently, such beneficial effects on physical performance have been suggested in different systems by other groups (Levin, 1963; Passwater, 1982; Saint-John & McNaughton, 1986). Their results also support the hypothesis that octacosanol may stimulate energy mobilization (Cureton, 1972; Passwater, 1982).

In our previous study it was shown that the addition of octacosanol (about 40% purity) to the diets of mice not only caused the enhancement of motor endurance, as estimated by their swimming endurance, but also affected the concentrations of hepatic and serum lipids (Shimura *et al.* 1987). Taken together with previous findings, these results suggest that octacosanol may affect lipid metabolism, thereby causing the increase in energy production needed to improve motor endurance.

However, little information has been available on the effect of octacosanol on lipid metabolism and its mechanism of action on physical performance. Specifically, there are no reports in the literature describing how octacosanol activates energy production needed to enhance motor endurance. Moreover, the influence of dietary long-chain alcohols on metabolism has not been clarified. Such questions remained unanswered because of the difficulty in obtaining sufficient amounts of long-chain alcohols, including octacosanol, for animal experiments.

Recent advances in the technique of supercritical fluid extraction using CO_2 (Hamatani & Takahashi, 1988; Hamatani *et al.* 1991) enabled us to obtain a sufficient amount of octacosanol from sugar cane to study a direct effect on animals, eliminating as far as possible the influence of contaminating compounds. The present study was, therefore, undertaken to test the effect of octacosanol on lipid metabolism in rats.

MATERIALS AND METHODS

Animals and diets

Wistar male rats (7 weeks old, body weight 176 (SE 9·2) g, obtained from Tokyo Laboratory of Animal Science, Tokyo, Japan) were fed on a normal-fat diet (NFC in Table 1) for 1 week to allow them to adapt to their new environment. Rats were individually housed with free access to water and the experimental diets. In all experiments they were kept under light from 07.00 to 19.00 hours with the room temperature maintained at $20 \pm 2°$. The food intake and the body weight were measured daily. Animal care and housing met the guidelines for animal experimentation of Tokyo University of Agriculture.

The compositions of the normal- and high-fat diets are shown in Table 1 (Kawada *et al.* 1986; Kato *et al.* 1992). When octacosanol was added to the diets, an equal amount of starch from the experimental diets was removed. The octacosanol fraction used for the diets was extracted from sugar cane and concentrated using supercritical fluid extraction methods (Hamatani & Takahashi 1988; Hamatani *et al.* 1991). This fraction contained 710 mg octacosanol/g, and 110 mg/g of the remainder was identified as long-chain alcohols and aldehydes at negligible individual amounts estimated by HPLC and gas chromatography using standard chemicals. The rest was considered to be carbohydrate (160 mg/g) and waxes (20 mg/g), as judged by their chemical properties based on gas chromatography and thin-layer chromatography, and each component was present at trace levels. From element analysis this fraction was found to contain only C, H, and O.

Experimental schedule

Expt 1 was designed to study the effect of dietary supplementation with octacosanol on lipid metabolism in rats. Four groups of rats (six rats/group) were pair-fed within the same fat level on the experimental diets with or without 10 g octacosanol of 71% purity/kg diet (29·5 (SE 0·5) g/d per rat for the normal-fat diets NFC and NFO, and 15·8 (SE 0·3) g/d for the high-fat diets HFC and HFO). After 20 d on the experimental diets the rats were deprived of food overnight, and killed by decapitation. Tissue samples were washed in ice-cold saline (9 g NaCl/l), blotted, and weighed and quickly frozen in liquid N_2, then stored at $-80°$ until analysis. Portions of the liver tissues were used directly for enzymic analysis before freezing. For the assay of hepatic enzymes the samples were directly homogenized with a Teflon pestle in the appropriate buffer, as described later (p. 435). The homogenates were centrifuged at 13000 *g* for 45 min. The resulting pellets were used for further enzymic analysis. Deep portions in the middle of the adipose tissues were homogenized with a Teflon pestle in the cognate buffer, as described later (p. 435), and used for further enzymic analysis. All procedures used in preparing the tissue extracts were done in a cold room or

Table 1. *Composition of experimental diets (g/kg)*

Diets... Ingredients	Normal-fat control (NFC)	Normal fat + octacosanol (NFO)	High-fat control (HFC)	High fat + octacosanol (HFO)
Casein	100·0	100·0	100·0	100·0
Starch	691·7	681·7	400·0	390·0
Sucrose	100·0	100·0	100·0	100·0
Lard	50·0	50·0	300·0	300·0
Soya-bean oil	8·3	8·3	50·0	50·0
Mineral mixture*	20·0	20·0	20·0	20·0
Vitamin mixture†	10·0	10·0	10·0	10·0
Cellulose	20·0	20·0	20·0	20·0
Octacosanol	—	10·0	—	10·0

* Supplied (mg/kg diet): KI 2, $CuSO_4.5H_2O$ 6, $ZnCO_3$ 22, $MnSO_4.5H_2O$ 24, Fe-citrate 640, NaCl 930, $MgSO_4$ 1430, NaH_2PO_4 1870, $CaHPO_4.2H_2O$ 2910, KH_2PO_4 5140, Ca-lactate 7020.

† Supplied (mg/kg diet): D-biotin 0·2, pteroylglutamic acid 2, pyridoxine-HCl 8, thiamin-HCl 12, riboflavin 40, D-calcium pantothenate 50, p-aminobenzoic acid 50, menadione 52, nicotinic acid 60, inositol 60, ascorbic acid 300, choline chloride 2000, cyanocobalamin 0·00552, all-rac-α-tocopheryl acetate 50, retinyl acetate 1·72, cholecalciferol 0·0625.

on ice-water. Expts 2 and 3 were performed to examine the influence of octacosanol on the activities of lipases, phosphatidate phosphohydrolase and total fatty acid oxidation rate. Two groups of rats (ten rats/group) were pair-fed on the high-fat diets either with or without octacosanol (16·3 (SE 0·4) g/d per rat for Expt 2 and 16·8 (SE 0·5) g/d per rat for Expt 3) for 20 d, then subjected to analyses.

Analytical procedures

Serum was prepared by centrifugation and analysed enzymically for triacylglycerol, cholesterol, and free fatty acids with commercially available kits (Triglyceride E-test Wako, Cholesterol C-test Wako, NEFA C-test Wako; Wako Chem. Ind., Osaka, Japan). Serum ketone concentration was also assayed enzymically with commercially available kits (Ketone test 'Sanwa'; Sanwa Kagaku Co., L., Nagoya, Japan). Hepatic lipids were first extracted by the method of Folch *et al.* (1957) and the extracts used for further analyses. Hepatic triacylglycerol, cholesterol and free fatty acids were evaluated as described above for serum. DNA content in adipose tissues was measured according to the method of Leyva & Kelley (1974). The activities of lipoprotein lipase (LPL; *EC* 3.1.1.34) and hormone sensitive lipase (HSL; *EC* 3.1.1.3) were assayed by the method of Gasquet & Pequignot (1972), modified slightly according to the methods of Deshaies *et al.* (1988) and Rizack (1961) respectively. The activities of acetyl-CoA carboxylase (ACC; *EC* 6.4.1.2), glucose-6-phosphate dehydrogenase (G6PDH; *EC* 1.1.1.49) and β-hydroxyacyl-CoA dehydrogenase (ACO; *EC* 1.1.1.35) in the liver were estimated by the methods of Tanabe *et al.* (1981), Kornberg & Horecker (1955), and Lazarow (1981) respectively. The activity of phosphatidate phosphohydrolase (PAP; *EC* 3.1.3.4) in hepatic microsomes was measured according to the method of Germershausen *et al.* (1980). Total fatty acid oxidation was measured using labelled palmitic acid as substrate by the method of Reubsaet *et al.* (1990). Protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. The intestinal absorption rate of dietary lipid from faeces was monitored for 3 d, from days 9–11 of Expt 1, and determined by balance experiments (Smyth, 1974). The faecal samples from the same rat were dried, weighed and pulverized.

The faecal lipids were extracted by the method of Folch *et al.* (1957). The absorbed lipids were expressed as fat absorption rate by comparison of lipid contents in the faecal samples with those in the diets.

Statistics

Values are presented as means with their standard errors. After the Bartlett test the results (in Tables 2–4) with statistical differences ($P < 0{\cdot}05$) were analysed using a computerized Kruskal–Wallis test. The other results were compared by two-way analysis of variance (ANOVA) ($P < 0{\cdot}05$). Specific differences were identified by the Dunnett test at a significance level of $P < 0{\cdot}05$, as indicated in the tables. One-way ANOVA was used on the results in Tables 5 and 6. Specific differences were identified by Student's t test at a significance level of $P < 0{\cdot}05$, as described in the tables.

RESULTS

Expt 1

Inclusion of octacosanol in the high-fat diet led to a significant reduction of perirenal adipose tissue weight without significant difference in body-weight gain (Table 2). When the normal-fat diet was supplemented with octacosanol the perirenal adipose tissue also tended to be reduced. In contrast, no significant difference in weight was observed between the other tissues such as the epididymal adipose tissue and the liver of rats fed on the control diets and the octacosanol-supplemented diets (HFC *v.* HFO and NFC *v.* NFO; Table 2). Plasma triacylglycerol concentrations were significantly lowered, and the concentrations of serum fatty acids were enhanced by octacosanol in rats fed on the high-fat diet (Table 3). Serum cholesterol and ketone concentrations were not affected by the dietary supplement of octacosanol. Although hepatic free fatty acids, triacylglycerol and cholesterol were clearly influenced by dietary fat (normal fat *v.* high-fat), there was no clear difference between rats fed on the unsupplemented diets and the octacosanol-supplemented diets (NFC *v.* NFO and HFC *v.* HFO in Table 3).

The results presented in Table 3 show that the faecal excretion of lipids from the diets was not affected by the dietary supplement of octacosanol.

Supplementation of the normal- and high-fat diets with octacosanol did not cause significant changes in the activities of hepatic enzymes such as G6PDH, ACC and ACO, involved in lipid metabolism, although the activities of G6PDH and ACC were affected by diet as expected (Table 4).

Expt 2

The HSL activities in all tested tissues were not affected by dietary octacosanol. In contrast, the significant increase in LPL activity by octacosanol was observed only in the perirenal adipose tissue (Table 5). Analysis of the DNA content in the perirenal adipose tissues suggested that reduction of the adipose tissue may have been due to a decrease of the lipid content within the cells (Table 6).

Expt 3

Total oxidation rate of fatty acid in various tissues and the hepatic activity of PAP were assessed to explore the action of octacosanol on lipid metabolism. Though the total oxidation rates of fatty acid in the liver and heart were not influenced, in the soleus (muscle) it was slightly but significantly enhanced by the inclusion of octacosanol into the high-fat diet (Table 5). The activity of PAP was suppressed by octacosanol supplementation, indicating that the esterification of serum fatty acid into triacylglycerol may have been decreased (Table 5).

Table 2. *Body-weight gain and organ weights in rats fed on normal and high-fat diets with and without octacosanol**

(Mean values with their standard errors for six rats)

Diet...	NFC		NFO		HFC		HFO	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Energy intake (KJ/10 d)	3876	190·6	3872	98·0	3508	105·3	3512	119·4
Body-weight gain (g/10 d)	54·6	3·84	60·4	5·94	40·7	4·93	36·3	3·88
Liver (g)	9·6	0·46	9·1	0·47	9·3	0·53	8·4	0·45
Testis (g)	3·2	0·09	3·1	0·08	3·0	0·11	3·0	0·09
Kidney (g)	2·0	0·07	2·0	0·09	1·8	0·06	1·9	0·06
Brain (g)	2·1	0·03	1·9	0·05	1·9	0·03	1·1	0·04
Heart (g)	1·0	0·04	0·9	0·03	0·8	0·03	0·8	0·04
Pancreas (g)	0·9	0·07	0·9	0·03	0·8	0·05	0·7	0·07
Epididymis (g)	0·9	0·04	0·8	0·03	0·8	0·02	0·7	0·02
Spleen (g)	0·8	0·06	0·6	0·03	0·6	0·03	0·6	0·03
Adrenal gland (g)	0·06	0·004	0·05	0·022	0·06	0·004	0·05	0·002
EAT (g)	6·4	0·06	6·9	0·83	6·4	0·54	6·7	0·86
PAT (g)	7·3^{ab}	0·57	5·5^{bd}	0·42	9·1^{a}	1·13	4·5^{d}	0·62

NFC, normal-fat control; NFO, normal-fat diet with octacosanol; HFC, high-fat control; HFO, high-fat diet with octacosanol; EAT, epididymal adipose tissue; PAT, perirenal adipose tissue.

^{a,b,c,d} *Mean values with unlike superscript letters were significantly different* ($P < 0·05$).

* For details of diets, see Table 1.

Table 3. *Serum, liver and faecal lipids in rats fed on normal and high-fat diets with and without octacosanol**

(Mean values with their standard errors for six rats)

Diet...	NFC		NFO		HFC		HFO	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Serum								
Triacylglycerol (g/l)	0·99[ab]	0·113	0·81[ab]	0·108	1·11[a]	0·076	0·69[b]	0·044
Free fatty acids (mmol/l)	0·83[ab]	0·053	0·88[ab]	0·058	0·80[a]	0·024	0·96[b]	0·057
Cholesterol (g/l)	0·97	0·049	0·82	0·041	0·88	0·061	0·99	0·054
Ketone bodies (g/l)	1·20	0·022	1·38	0·140	1·24	0·301	1·15	0·124
Liver								
Triacylglycerol (mg/g)	2·13[a]	0·440	2·21[a]	0·575	21·8[b]	2·161	20·0[b]	2·219
Free fatty acids (mmol/g)	17·2[a]	0·459	16·3[a]	0·706	23·0[b]	0·791	21·9[b]	0·908
Cholesterol (mg/g)	3·22[a]	0·706	3·05[a]	0·194	4·92[b]	0·420	5·20[b]	0·169
Faecal lipids								
Fat absorption rate (%)	95·8[a]	0·53	95·0[a]	0·58	98·0[b]	0·31	97·2[b]	0·47

NFC, normal-fat control; NFO, normal-fat diet with octacosanol; HFC, high-fat control; HFO, high-fat diet with octacosanol.

[a,b] Mean values with unlike superscript letters were significantly different ($P < 0·05$).

* For details of diets, see Table 1.

Table 4. *Effect of octacosanol supplementation on hepatic enzyme activities in rats**

(Mean values with their standard errors for six rats)

Diet...	NFC		NFO		HFC		HFO	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
G6PDH (*EC* 1.1.1.49; μmol/min per g liver)	33·6[a]	6·85	32·0[a]	9·89	5·06[b]	0·25	4·59[b]	1·34
ACC (*EC* 6.4.1.2; μmol/min per g liver)	504[a]	23·8	536[a]	36·9	408[b]	29·3	387[b]	31·0
ACO (*EC* 1.1.1.35; μmol/min per g liver)	42·3	3·19	43·7	2·53	49·8	6·57	52·3	5·96

NFC, normal-fat control; NFO, normal-fat diet with octacosanol; HFC, high-fat control; HFO, high-fat diet with octacosanol; G6PDH, glucose-6-phosphate dehydrogenase; ACC, acetyl CoA carboxylase; ACO, β-hydroxyacyl CoA dehydrogenase.

[a,b] Mean values with unlike superscript letters were significantly different ($P < 0·05$).

* For details of diets, see Table 1.

DISCUSSION

Previous studies suggested that octacosanol might improve the physical performance and motor endurance of experimental animals (Cureton, 1972; Passwater, 1982; Saint-John & McNaughton 1986). Although it is not certain whether long-chain alcohols, detected mainly as a component of plant waxes, affect lipid metabolism in general, from the previous observations it was speculated that octacosanol may be a factor in the process responsible for active energy release. To clarify such speculation, the present study was conducted to investigate the effect of dietary octacosanol on lipid metabolism. We present evidence here that octacosanol does indeed affect lipid metabolism.

Table 5. *Enzyme activities and total fatty acid oxidation in various tissues of rats fed on high-fat diets with and without octacosanol*†

(Mean values with their standard errors for ten rats)

Diet...	HFC		HFO	
	Mean	SE	Mean	SE
LPL (*EC* 3.1.1.34; μmol/h per mg DNA)				
PAT	155	15·8	268*	18·1
EAT	72·3	4·65	70·2	4·20
Heart	207	19·3	220	16·4
Soleus	66·5	5·93	78·9	3·81
HSL (*EC* 3.1.1.3; μmol/h per mg DNA)				
PAT	3·94	0·586	4·30	0·066
EAT	2·43	0·400	2·36	0·197
PAP (*EC* 3.1.3.4; nmol/min per mg microsomal protein)				
Liver	2·98	0·654	1·70*	0·227
β-Oxidation rate (nmol/min per g tissue)				
Liver	276	3·40	284	3·35
Heart	308	2·27	316	2·81
Muscle	195	2·21	230*	1·36

HFC, high-fat control; HFO, high-fat diet with octacosanol; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; PAT, perirenal adipose tissue; EAT, epididymal adipose tissue; PAP, phosphatidic acid phosphatase.

* Mean values were significantly different from those for HFC, $P < 0·05$.

† For details of diets, see Table 1.

Table 6. *Cell weights and numbers in adipose tissue of rats fed on high-fat diets with and without octacosanol*†

(Mean values with their standard errors for ten rats)

Diet...	HFC		HFO	
	Mean	SE	Mean	SE
Perirenal adipose tissue				
Weight (g)	7·31	0·560	5·03*	0·288
Cell weight (mg/μg DNA)	11·7	0·82	8·17*	0·51
DNA content (μg DNA/pads)	718	76·0	720	49·0
Epididymal adipose tissue				
Weight (g)	4·51	0·154	4·48	0·292
Cell weight (mg/μg DNA)	4·98	0·317	4·81	0·317
DNA content (μg DNA/pads)	895	76·4	915	59·5

HFC, high-fat control; HFO, high-fat diet with octacosanol.

* Mean values were significantly different from those of HFC, $P < 0·05$.

† For details of diets and procedures, see Table 1 and pp. 434–436.

In the present study we used an octacosanol fraction of 71% purity, where small amounts of other long-chain alcohols, aldehydes and unidentified carbohydrates were also included. However, it is unlikely that the other compounds have a measurable effect on lipid metabolism because, even when the proportion of other compounds was increased in the fraction used for the dietary supplement, the effect on lipid metabolism was not enhanced in preliminary experiments in which the serum concentrations of triacylglycerol

and free fatty acids were significantly affected only in the rats fed on the high-fat diet with octacosanol (10 g/kg diet) of 71% purity, and not with octacosanol of 50 and 30% purity. Moreover, significant effects of octacosanol on serum lipids were observed only when 71% purity octacosanol was included at a level of 10 g/kg diet, and not when it was included at 3 or 1 g/kg. Taken together, these observations suggest that octacosanol itself affects lipid metabolism.

We found that supplementation of the high-fat diet with octacosanol led to a significant decrease in the weight of perirenal adipose tissue, while the weights of the epididymal adipose tissue and the other tissues appeared to be unaffected. From the analysis of the DNA content it is likely that the reduction of perirenal adipose tissue weight may be due to a decrease in cell size (Table 6). In this tissue we also observed an unexpected increased activity of LPL with the octacosanol supplement. The reason for this increase, which would normally be associated with an increase in fat storage, remains to be determined.

We investigated the influence of octacosanol supplementation on the hepatic enzymes involved in lipid metabolism. The results presented in Table 4 show that the activities of these enzymes were clearly affected by the fat content of the diets (Dupont, 1965; Numa *et al.* 1965). However, when octacosanol was added to the diet the activity of the rate-limiting enzyme of fatty acid synthesis, ACC, was not affected. Similarly, there was no change in the activities of ACO and G6PDH. In contrast, the activity of PAP, the rate-limiting enzyme of fatty acid esterification into triacylglycerol, was suppressed by octacosanol supplementation (Table 5). Thus, such decreased activity of PAP may lower hepatic synthesis and secretion of triacylglycerol. In fact, reduced concentrations of serum triacylglycerol were found after the addition of octacosanol to the high-fat diet. However, no explanation for the increased concentrations of serum free fatty acids is available so far.

Although octacosanol affected lipid metabolism in rats fed on the high-fat diet, in the rats fed on the normal-fat diet such an effect was not observed. Thus, the action of octacosanol may depend on a dietary condition such as fat content.

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