Metabolic fate of oleic acid, palmitic acid and stearic acid in cultured hamster hepatocytes

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Unlike other saturated fatty acids, dietary stearic acid does not appear to raise plasma cholesterol. The reason for this remains to be established, although it appears that it must be related to inherent differences in the metabolism of the fatty acid. In the present study, we have looked at the metabolism of palmitic acid and stearic acid, in comparison with oleic acid, by cultured hamster hepatocytes. Stearic acid was taken up more slowly and was poorly incorporated into both cellular and secreted triacylglycerol. Despite this, stearic acid stimulated the synthesis and secretion of triacylglycerol to the same extent as the other fatty acids. Incorporation into cellular phospholipid was lower for oleic acid than for palmitic acid and stearic acid. Desaturation of stearic acid, to monounsaturated fatty acid, was found to be greater than that of palmitic acid. Oleic acid produced from stearic acid was incorporated into both triacylglycerol and phospholipid, representing 13 % and 6 % respectively of the total after a 4 h incubation. Significant proportions of all of the fatty acids were oxidized, primarily to form ketone bodies, but by 8 h more oleic acid had been oxidized compared with palmitic acid and stearic acid.

INTRODUCTION

It is generally accepted that, in contrast with other long-chain saturated fatty acids (SFA), stearic acid does not raise the serum cholesterol concentration [1–5]. The exact mechanism behind this observation remains unclear; however, whereas it was originally thought that it may be a result of poor absorption of the fatty acid [6–8], it now appears more likely that it is due to its metabolism within the body [4,9]. One suggestion is that stearic acid is more rapidly converted into oleic acid than other SFA, such as palmitic acid. Studies in both laboratory animals [10,11] and humans [4] are consistent with this rapid conversion of stearic acid into oleic acid, by desaturation at the Δ9-position. A stable-isotope study in humans by Emken et al. [12] showed that desaturation of 18:0 (stearic acid) to 9-*cis* 18:1 (oleic acid) was significantly greater than that of 16:0 (palmitic acid) to 9-*cis* 16:1 (palmitoleic acid). They hypothesized that stearic acid may be behaving as a non-cholesterol-raising fatty acid because of its high rate of conversion into oleic acid. In this same study, the incorporation of stearic acid into plasma lipids, compared with that of palmitic acid, was 30–40 % lower for triacylglycerol (TAG) and cholesteryl ester (CE) and approx. 40 % higher for phospholipid (PL), suggesting preferential incorporation of stearate into PL. Bonanome et al. [11] also reported that, in mice, stearic acid was more readily utilized for PL synthesis than palmitic acid, suggesting that stearate which was not desaturated was preferentially incorporated into PL rather than into TAG.

An alternative fate to esterification of fatty acids coming into the liver is oxidation, primarily to ketone bodies. While it is commonly assumed that SFA and unsaturated fatty acids (UFA) are taken up by the liver and oxidized at the same rate [13], some workers have found differences. Gavino et al. [14] have shown that SFA were oxidized more slowly than UFA in rat liver mitochondria. In addition, the rate of oxidation may decrease with increasing fatty acid chain length [15–17]. It is possible that varying rates of fatty acid oxidation may provide a further means by which specific fatty acids exert different overall metabolic effects.

We have recently shown that, in male Golden Syrian hamsters, diets rich in stearic acid result in significantly decreased plasma very-low-density lipoprotein (VLDL) TAG concentrations compared with diets rich in myristic acid, oleic acid or palmitic acid [18]. It is possible that this is due to differences in the partitioning of the fatty acids between esterification and oxidation. While some studies have looked at the selective channelling of polyunsaturated fatty acids into hepatic glycerolipids [19], less is known about the selective channelling of SFA. The present study was designed to investigate the metabolic fate of radiolabelled palmitic acid and stearic acid, compared with that of oleic acid, using monolayer cultures of hamster hepatocytes.

EXPERIMENTAL

Materials

All reagents were of the highest purity commercially available, and solvents (all from Fisons Scientific Equipment) were of AnalaR grade. Radiochemicals ([9,10(n)-^{3}H]oleic acid, [1-^{14}C]oleic acid, [9,10(n)-^{3}H]palmitic acid, [1-^{14}C]palmitic acid and [1-^{14}C]stearic acid) were from Amersham International; choline chloride, Folin and Ciocalteu's phenol reagent, potassium sodium tartrate, silica gel 60 glass and plastic TLC plates (Merck) and silver nitrate were from BDH Laboratory Supplies; collagenase A (from *Clostridium histolyticum*; lyophilized) and Peridochrom Triglyceride GPO-PAP enzymic assay kit were from Boehringer Mannheim U.K. (Diagnostics and Biochemicals) Ltd.; scintillants (Emulsifier-safe and Insta-fluor) were from Canberra Packard; 60 mm-diam. Falcon tissue culture and organ culture dishes (with centre well) were from Fahrenheit Lab Supplies; perchloric acid (60 %), KOH, NaOH, Na_2CO_3 and Tris were from Fisons Scientific Equipment; Leibovitz-L15 medium was from Gibco BRL Life Technologies Ltd.; newborn-calf serum was from Imperial Laboratories (Europe) Ltd.; BSA, collagen, cupric sulphate, 2′,7′-dichlorofluorescein, gentamycin, heparin, Hepes, insulin, non-radioactive fatty acids, penicillin-G, streptomycin, Trypan Blue stain and trypsin inhibitor were from Sigma Chemical Co.

Abbreviations used: CE, cholesteryl ester; MUFA, monounsaturated fatty acids; PL, phospholipid; SFA, saturated fatty acids; TAG, triacylglycerol; UFA, unsaturated fatty acids; VLDL, very-low-density lipoprotein.

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Maintenance of animals

Male DSNI Golden Syrian hamsters (120–150 g) were obtained from the Biomedical Services Unit, University of Nottingham, Notts., U.K. Animals were housed individually in a windowless room, artificially lit between 06:00 h and 18:00 h. They were allowed free access to both food and water *ad libitum*. The hamsters were fed on Rat and Mouse Breeding Diet 422 (Pilsbury's; Heygate & Sons, The Mill, Bugbrooke, Northants., U.K.), a commercially available pelleted diet with a gross composition (by weight) of approx. 20 % digestible protein, 39 % carbohydrate and 4 % total fat [containing 0.008 % (w/w) cholesterol].

Preparation and incubation of hepatocyte cultures

Hepatocytes were prepared essentially by the method of Seglen [20] by collagenase digestion, with modifications as described by Cascales et al. [21] and Salter et al. [22]. The gall bladder, absent in rats, was ligated. Collagenase digestion of the liver was normally completed within 10–15 min. Hepatocytes were attached to collagen-coated dishes, either 60 mm-diam. tissue culture dishes (at a density of approx. 2.75×10^6 cells per dish) or organ culture dishes with a centre well (at a density of approx. 1.7×10^6 cells per dish), in a modified Leibovitz-L15 medium [21] containing 5 % (v/v) newborn-calf serum and 100 nM bovine insulin. After incubation for at least 2 h at 37 °C in a humidified atmosphere, the medium was replaced with 3 ml of fresh medium (as above) to remove the unattached and non-viable cells. After approx. 20 h in culture, each hepatocyte monolayer was washed twice with serum-free medium supplemented with 0.2 % (w/v) fatty-acid-poor BSA, but without insulin, and received a further 2 ml of this medium, to which ^{3}H- or ^{14}C-labelled fatty acids were added at a final concentration of 0.3 mM. The dishes were re-incubated at 37 °C and removed at the indicated times.

Determination of fatty acid incorporation into cellular lipids

Four independent experiments were performed to investigate the incorporation of the different fatty acids into cellular lipids. In two of these, all the fatty acids were ^{14}C-labelled, while in the other two, ^{3}H-labelled oleic acid and palmitic acid were used. As the results of all experiments were similar, data have been pooled.

Cellular lipids were extracted in hexane/propan-2-ol (3:2, v/v) as described by Goldstein et al. [23]. Solvents were evaporated to dryness under nitrogen. Dried lipid extracts were dissolved in 200 μl of chloroform; a 100 μl portion of this extract was loaded on to silica gel 60 plastic TLC plates, and CE, TAG, fatty acids and PL were separated with the solvent system light petroleum (b.p. 40–60 °C)/diethyl ether/acetic acid (90:30:1, by vol.), using non-radioactive standards for identification of each lipid band. CE, TAG, fatty acids and PL (at the origin) bands were cut from the plates into scintillation vials, scintillation cocktail (Packard Emulsifier-safe liquid scintillation cocktail for aqueous samples) was added and radioactivity in each fraction was determined by scintillation counting (Packard Tricarb liquid scintillation counter, model 1900 CA).

Determination of fatty acid incorporation into media lipids

Three independent experiments were performed to measure the incorporation of radiolabelled fatty acids into secreted lipids. In two experiments, all the fatty acids were ^{14}C-labelled, while in the third, ^{3}H-labelled oleic acid and palmitic acid were used. As results were similar in all three experiments, data were pooled.

Lipids from an aliquot of medium (usually 0.5 ml) were extracted by the method of Bligh and Dyer [24], followed by acidification and two further light petroleum extractions from the Bligh and Dyer aqueous phase. The combined media lipid extracts were dried down under nitrogen and lipids (CE, TAG, fatty acids and PL) were separated by TLC as described above. Lipid bands were cut from the plates, scintillant was added and radioactivity was determined by scintillation counting.

At each time point, the amount of extracellular fatty acid in the medium was determined by counting the radioactivity in an aliquot (usually 100 μl) of the collected medium and correcting this value for radioactivity associated with secreted TAG, PL and CE. The amount of fatty acid removed from the medium was calculated by subtracting this value for fatty acid in the medium (in nmol) from 600 nmol (the amount of fatty acid originally added to each plate; equivalent to 0.3 mM).

Determination of absolute mass of cellular and secreted TAG

Three independent experiments were performed to measure the absolute mass of TAG, both in the cells and secreted into the medium, i.e. VLDL TAG. Hepatocytes were prepared as described above, and BSA-bound unlabelled fatty acids were added to the incubation medium at a final concentration of 0.3 mM. Each experiment included a control set of dishes, to which only 12 % (w/v) BSA was added. Hepatocytes were incubated for 8 h at 37 °C.

Lipids were extracted from 1.5 ml of medium by the method of Bligh and Dyer [24]. Cellular lipids were extracted in hexane/propan-2-ol (3:2, v/v) as described by Goldstein et al. [23]. Solvents were evaporated to dryness under nitrogen. Dried lipid extracts were dissolved in 100 μl of propan-2-ol and an aliquot was assayed for TAG enzymically with a Boehringer Triglyceride kit, together with a range of TAG standards (0–50 μg) dissolved in propan-2-ol.

Determination of the conversion of palmitic acid and stearic acid into monounsaturated fatty acids (MUFA)

Three independent experiments were performed to measure the amounts of palmitic acid and stearic acid desaturated to MUFA. Hepatocytes were incubated with ^{14}C-labelled palmitic acid or stearic acid, and TAG and PL were separated by TLC as indicated above. The bands were cut from the plates and extracted; TAG twice with chloroform/methanol (2:1, v/v), and PL with chloroform/methanol (1:2, v/v) and then chloroform/methanol (1:5, v/v). Lipid extracts were dried under nitrogen, during which time 0.5 μmol of both non-radioactive oleic acid and stearic acid in diethyl ether were added as carriers, then fatty acids were *trans*-methylated by the method of Lepage and Roy [25]. Fatty acids (saturated and monounsaturated) were separated by means of silver nitrate TLC. Silica gel 60 glass TLC plates were soaked in 4 % silver nitrate solution in aqueous methanol (9:1, v/v) and oven-dried before use. Dried lipid extracts were dissolved in 200 μl of light petroleum; a 150 μl portion of this extract was loaded on to the prepared TLC plates, and SFA and MUFA were separated using the solvent system light petroleum (b.p. 40–60 °C)/diethyl ether (9:1, v/v). Individual bands on the TLC plate were visualized with 2′,7′-dichlorofluorescein under UV light. Fatty acid bands were scraped into scintillation vials, scintillation cocktail was added (Packard Insta-fluor liquid scintillation cocktail for organic samples) and radioactivity was determined by scintillation counting.

Measurement of fatty acid oxidation and ketogenesis

Four independent experiments were performed to determine fatty acid oxidation and ketone body formation. Hepatocytes

were prepared in organ culture dishes with a centre well and were incubated with ^{14}C-radiolabelled fatty acids. Prior to the start of the incubation period, 1 ml of 3 M KOH was pipetted into the centre well and the dishes were sealed with high-vacuum silicone grease. At the end of the incubation period, 0.2 ml of 60% perchloric acid ($HClO_4$) was injected into the medium surrounding the centre well to release the $^{14}CO_2$. The sealed dishes were shaken for 1 h at room temperature to trap all of the $^{14}CO_2$ in the KOH. Radioactivity was determined by scintillation counting.

Analyses of cell lipids, cell protein and media lipids (including acid-soluble products; see below) were performed using a separate set of dishes without addition of acid at the end of the incubation.

Acid-soluble products (consisting primarily of ketone bodies) [26] were measured by adding 0.2 ml of 60% $HClO_4$ to 1 ml of medium. Following incubation on ice for 15 min, the samples were centrifuged at 1500 ***g*** for 10 min to pellet the acid precipitate formed. A 0.8 ml aliquot of the supernatant was scintillation counted for acid-soluble radioactivity.

Other methods

Cellular protein was measured by the method of Lowry et al. [27]. Oleate, palmitate and stearate were bound to BSA (essentially fatty-acid-free) by a modification of the method of Van Harken et al. [28] to give stock solutions of 10 mM. This was added to cells so that the initial concentration of fatty acid was 0.3 mM, while the BSA concentration in the culture medium was 0.2% (w/v). This resulted in a total fatty acid/BSA ratio of 3.6.

Statistical methods

Each determination represents the average of duplicate or triplicate dishes of hepatocytes prepared from the same hamster, and results are given as means ± S.D. The results of each experiment were reproduced in further independent experiments as indicated. Data were analysed, as appropriate, either by repeated-measures analysis of variance (ANOVA) or by Student's paired *t* test using GraphPad Instat (Instant Statistics) software (GraphPad Software, San Diego, CA, U.S.A.). If a significance of $P < 0.05$ was obtained with ANOVA, further comparison was performed between groups using the Tukey–Kramer multiple comparisons test. Linear regression was also completed using GraphPad Instat software.

Table 1 Amounts of fatty acids removed from the medium by cultured hamster hepatocytes

Hamster hepatocytes were cultured as described in the legend to Figure 1, and were incubated with either ^{3}H- or ^{14}C-labelled extracellular oleic acid, palmitic acid or stearic acid (initial concentration 0.3 mM). Hepatocytes were incubated for the indicated periods of time, the culture medium was collected and the amount of fatty acid removed from the medium was calculated as described in the Experimental section. Results represent means ± S.D., from four (at 4 h) or three (at 8 h) independent experiments. *Significantly lower than oleic acid ($P < 0.05$) and palmitic acid ($P < 0.01$); †significantly lower than palmitic acid ($P < 0.05$).

	Fatty acid removed (%)	
Fatty acid	4 h	8 h
Oleic acid	50.1 ± 6.88	70.6 ± 6.21
Palmitic acid	57.0 ± 7.92	80.1 ± 6.24
Stearic acid	39.3 ± 6.80*	57.1 ± 17.08†

RESULTS

Fatty acid esterification

When hepatocytes were cultured with oleate, palmitate or stearate at an initial concentration of 0.3 mM, there was rapid clearance of the extracellular fatty acids from the culture medium. Table 1 summarizes the results from several independent experiments (exact number indicated in table) and shows the percentage of fatty acid removed from the medium by hepatocytes incubated with the labelled fatty acids for 4 h and 8 h. After a 4 h incubation, significantly less stearic acid had been removed from the medium

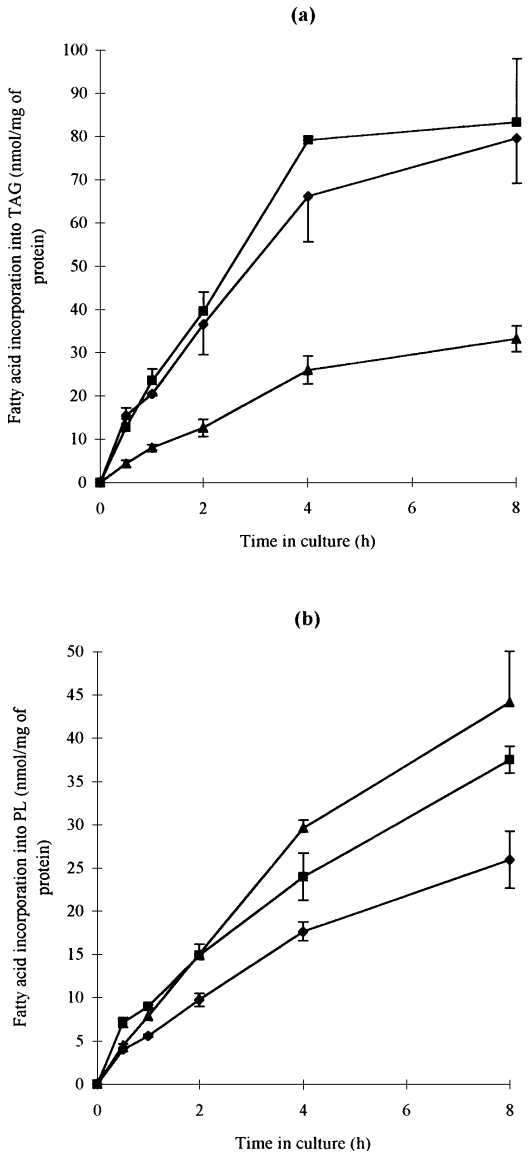

Figure 1 Incorporation of radiolabelled fatty acids into (a) intracellular TAG and (b) intracellular PL

Hamster hepatocytes were incubated for 20 h in medium containing 5% (v/v) newborn-calf serum and then in fresh serum-free medium supplemented with 0.2% (w/v) fatty-acid-free BSA in the presence of either ^{3}H- or ^{14}C-labelled extracellular oleate (◆), palmitate (■) or stearate (▲) (initial concentration 0.3 mM). Hepatocytes were incubated for the indicated periods of time, the culture medium was collected and incorporation of radiolabelled fatty acids into (**a**) cellular TAG and (**b**) cellular PL was determined as described in the Experimental section. Results are means ± S.E.M. of triplicate measurements and are representative of one experiment out of four. Where error bars are not visible, they lie within the symbol.

Table 2 Rate of fatty acid incorporation into intracellular TAG and PL

Hamster hepatocytes were cultured as described in the legend to Figure 1, and were incubated with either ^{3}H- or ^{14}C-labelled extracellular oleic acid, palmitic acid or stearic acid (initial concentration 0.3 mM). Hepatocytes were incubated for 0.5, 1, 2 and 4 h, and the incorporation of fatty acid into intracellular TAG and PL fractions was calculated at each time point, as described in the Experimental section. Results represent the mean rate of incorporation over the 4 h period ± S.D., pooled from four independent experiments. Significance of differences: * significantly lower than palmitic acid ($P < 0.01$) and higher than stearic acid ($P < 0.001$); † significantly lower than palmitic acid and stearic acid ($P < 0.001$); ‡ significantly higher than stearic acid ($P < 0.001$); § significantly lower than oleic acid ($P < 0.05$).

	Fatty acid incorporation (nmol/h per mg of protein)		
Fatty acid	Intracellular TAG	Intracellular PL	TAG/PL
Oleic acid	12.2 ± 1.22*	4.3 ± 0.17†	2.9 ± 1.04
Palmitic acid	16.0 ± 0.75‡	6.1 ± 0.43	2.7 ± 0.75
Stearic acid	6.4 ± 0.36	5.9 ± 0.37	1.2 ± 0.42§

compared with oleic acid and palmitic acid. Again, after 8 h in culture, significantly less stearic acid than palmitic acid had been removed from the medium.

Typical curves of fatty acid incorporation into intracellular TAG and PL are shown in Figures 1(a) and 1(b) respectively. Incorporation of each fatty acid increased linearly up to 4 h, tailing off slightly between 4 h and 8 h. Comparison of regression data from four independent experiments confirmed linear incorporation into both TAG and PL during the first 4 h in culture and showed that the rate of incorporation of stearic acid into intracellular TAG was significantly lower than the rates of incorporation of both oleic acid and palmitic acid (Table 2). The rate of incorporation of oleic acid into cellular TAG was also significantly lower than that of palmitic acid. The rate of incorporation of oleic acid into intracellular PL was significantly lower than the rates of incorporation of the two SFA up to 4 h in culture. The same set of results was used to calculate the ratio of cellular TAG/PL for each fatty acid (Table 2). The ratio was lowest for stearic acid; however, this only attained statistical significance when compared with oleic acid.

The concentration of non-esterified fatty acids in the cells was greatest for stearic acid compared with oleic acid and palmitic acid at the 4 h time point, suggesting that stearate was less readily utilized inside the cell (3.0 ± 0.61 compared with 0.7 ± 0.25 and 0.6 ± 0.17 nmol/mg of protein respectively).

Typical curves of fatty acid incorporation into secreted TAG and PL are shown in Figure 2. During the first 2 h of incubation the output of radioactive fatty acids in secreted TAG and PL remained fairly low for all of the fatty acids; however, after 2 h, the amount of TAG secreted into the medium increased in an essentially linear manner with time. Pooled results from three independent experiments reveal that, after 8 h, hepatocytes incubated with stearate had incorporated significantly less radiolabelled fatty acid into secreted TAG than hepatocytes incubated with oleate or palmitate (11.7 ± 0.73 compared with 28.6 ± 7.76 and 29.0 ± 8.28 nmol/mg of protein respectively). Unlike TAG, radioactive fatty acid output in secreted PL failed to increase after 2 h in culture.

Table 3 shows pooled results from three independent experiments in which the absolute mass of TAG present both in the hepatocyte and in the surrounding medium, i.e. secreted as VLDL, was measured after 8 h in culture with BSA alone (control), oleic acid, palmitic acid or stearic acid. The addition of extracellular fatty acid markedly increased the mass of cellular TAG, significantly so for oleate and stearate, compared with hepatocytes incubated with albumin alone. The mass of secreted

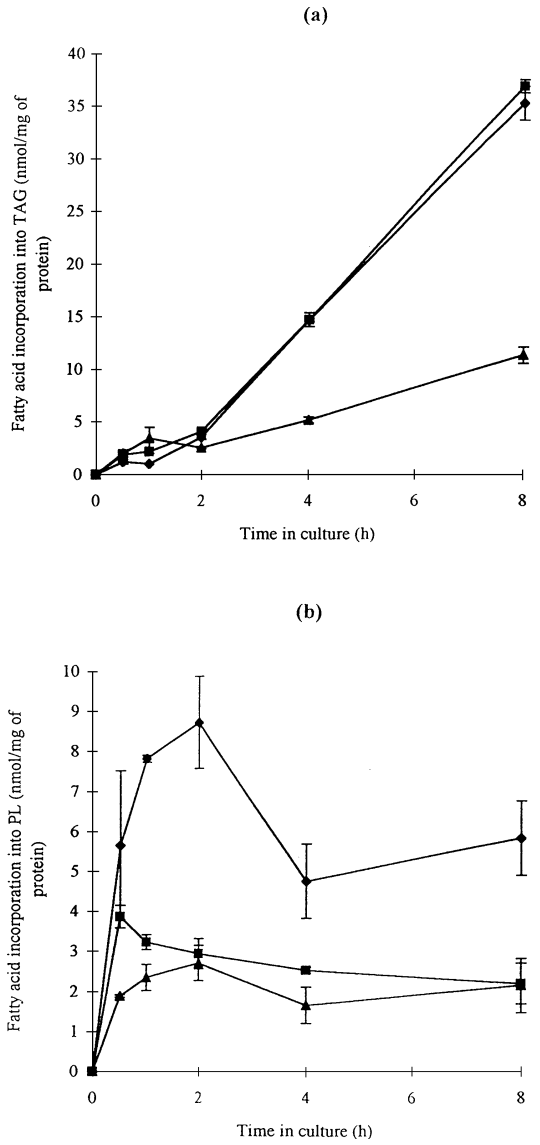

Figure 2 Incorporation of radiolabelled fatty acids into (a) secreted TAG and (b) secreted PL

Hamster hepatocytes were cultured as described in the legend to Figure 1, and were incubated with either ^{3}H- or ^{14}C-labelled extracellular oleate (◆), palmitate (■) or stearate (▲) (initial concentration 0.3 mM). Hepatocytes were incubated for the indicated periods of time, the culture medium was collected and incorporation of radiolabelled fatty acids into (**a**) secreted TAG and (**b**) secreted PL was determined as described in the Experimental section. Results are means ± S.E.M. of triplicate measurements and are representative of one experiment out of three. Where error bars are not visible, they lie within the symbol.

TAG was also significantly stimulated by addition of each of the fatty acids compared with cells incubated with only albumin. However, there were no significant differences between any of the fatty acids in the absolute mass of TAG either in the cell or secreted into the medium.

Fatty acid desaturation

When either palmitic acid or stearic acid was used as substrate, significant amounts of MUFA were incorporated into both TAG and PL (Figure 3). Pooled results from three independent experiments showed that the proportion of MUFA was greater when stearic acid was the substrate [TAG 13.4 ± 2.45 % of total

Table 3 Absolute mass of intracellular and secreted TAG

Hamster hepatocytes were cultured as described in the legend to Figure 1, and were incubated with extracellular BSA, oleic acid, palmitic acid or stearic acid (initial concentration 0.3 mM). Hepatocytes were incubated for 8 h, and the masses of cellular and secreted (VLDL) TAG were determined as described in the Experimental section. Results represent means ± S.D. pooled from three independent experiments. Significance of differences compared with BSA: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

	Mass of TAG (μg/mg of protein)	
Fatty acid	Cellular TAG	VLDL TAG
BSA	19.4 ± 4.70	9.8 ± 0.31
Oleic acid	36.0 ± 12.84*	18.6 ± 1.51***
Palmitic acid	33.8 ± 12.24	18.2 ± 0.70***
Stearic acid	39.8 ± 7.70*	17.3 ± 2.38**

fatty acid incorporated, compared with 3.0 ± 1.20 % ($P < 0.01$); PL 6.2 ± 1.08 % cf. 4.1 ± 0.39 % ($P < 0.05$)].

Fatty acid oxidation

Table 4 shows pooled results from four independent experiments in which fatty acid incorporation into acid-soluble products, i.e. ketone bodies, was determined. Ketogenesis proceeded essentially linearly with time up to 4 h for each fatty acid, with no significant differences between the fatty acids. However, by 8 h, significantly more oleic acid than palmitic acid or stearic acid had been incorporated into ketone bodies.

The amount of fatty acid entirely oxidized to $^{14}CO_2$ increased in a time-dependent manner, relatively slowly at first (up to 2 h), then more rapidly up to 8 h in an essentially linear fashion (results not shown). However, even by 8 h, oxidation to $^{14}CO_2$ did not exceed 10 % of the total fatty acid oxidized ($^{14}CO_2$ plus ketone bodies).

DISCUSSION

Considerable evidence exists to suggest that fatty acids added to the medium of cultured hepatocytes stimulate the synthesis and secretion of VLDL TAG [29,30]. In the present experiment, the mass of TAG synthesized and secreted over an 8 h period was stimulated by approx. 2-fold, independent of whether the added fatty acid was oleic, palmitic or stearic acid. The most obvious conclusion from this observation is that the exogenous fatty acid represents an increased substrate supply for TAG synthesis and is thereby incorporated into the TAG. As the intracellular TAG pool increases, then part of it is mobilized and secreted within VLDL. However, data on the incorporation of radiolabelled fatty acids into the different fractions only partly supports this suggestion. While the increase in TAG synthesis could be accounted for by exogenous oleic or palmitic acid, this is not the case for stearic acid which, on the basis of the incorporation of the radiolabelled fatty acid, is more poorly incorporated into TAG. Thus it appears that stearic acid stimulates the incorporation of endogenous fatty acids into VLDL TAG, perhaps by displacing them from other routes of metabolism, such as PL synthesis. The data do not support the idea that stearic acid suppresses VLDL secretion, only that it is not as readily incorporated itself.

Previous workers have proposed the preferential incorporation of stearic acid into hepatic cellular PL [10–12,17,31], and this is generally associated with preferential assimilation of palmitic acid into cellular TAG. In the present study, we found similar amounts of palmitic acid and stearic acid incorporated into PL

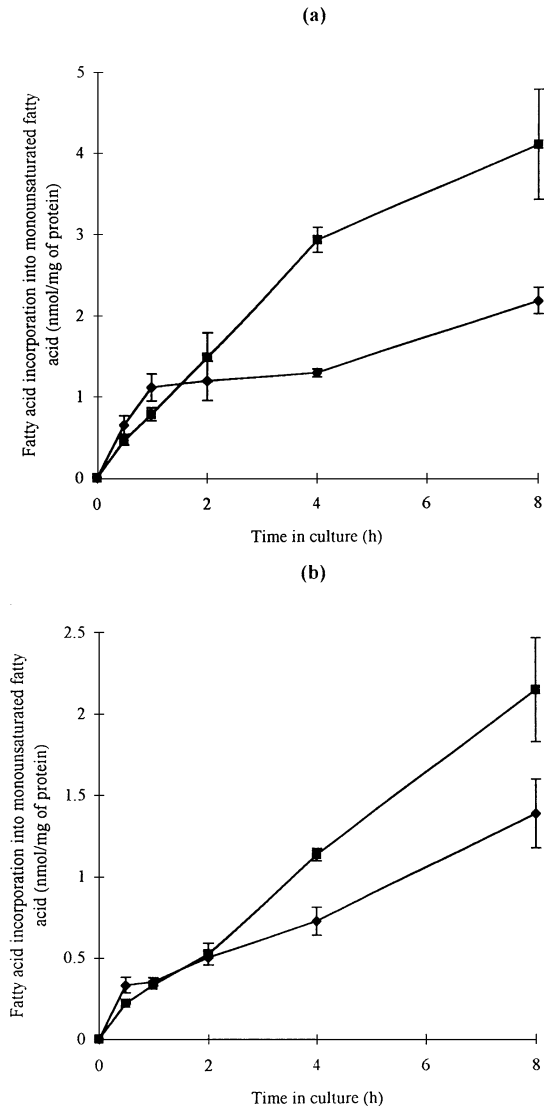

Figure 3 Desaturation of palmitic acid and stearic acid in (a) intracellular TAG and (b) intracellular PL of hamster hepatocytes

Hamster hepatocytes were cultured as described in the legend to Figure 1, and were incubated with extracellular [^{14}C]palmitic acid (◆) or [^{14}C]stearic acid (■) (initial concentration 0.3 mM). At the indicated times, medium was removed, cellular lipids were separated and the amount of either palmitic acid or stearic acid converted into MUFA was determined in (**a**) TAG and (**b**) PL, as described in the Experimental section. Results are means ± S.E.M. of triplicate measurements, and are representative of one experiment out of three. Where error bars are not visible, they lie within the symbol.

but, as detailed above, significantly more palmitic acid accumulated in TAG. Thus, although the results appear to support the idea that, compared with palmitic acid, stearic acid is poorly incorporated into TAG, they do not immediately suggest preferential incorporation into PL. However, it should be taken into account that the rate of stearic acid uptake, and hence the total amount of the fatty acid metabolized at a given time, is reduced compared with the other two fatty acids. If the amount of stearic acid incorporated into PL is expressed as a proportion of the total metabolized, i.e. that incorporated into TAG, PL and ketone bodies, then at 4 h this represents 30 %. This compares with 19 % and 15 % for palmitate and oleate respectively. Hence there may be preferential incorporation of stearate into PL. We also show that significantly less oleic acid than either of the SFA

Table 4 Fatty acid incorporation into ketone bodies

Hamster hepatocytes were cultured as described in the legend to Figure 1, and were incubated with ^{14}C-labelled extracellular oleic acid, palmitic acid or stearic acid (initial concentration 0.3 mM). Hepatocytes were incubated for the indicated lengths of time, and the incorporation of fatty acids into ketone bodies was determined as described in the Experimental section. Results represent means ± S.D. pooled from four independent experiments. * Significantly higher than palmitic acid ($P < 0.05$) and stearic acid ($P < 0.01$).

	Fatty acid incorporation (nmol/mg of protein)	
Fatty acid	4 h	8 h
Oleic acid	51.2 ± 9.32	81.0 ± 25.23*
Palmitic acid	40.7 ± 15.12	53.9 ± 22.01
Stearic acid	29.2 ± 12.71	42.7 ± 16.98

was incorporated into cellular PL. This supports the findings of Wang and Koo [31], who showed that oleic acid, incorporated into chylomicrons, was minimally utilized for PL synthesis in the liver compared with palmitic acid and stearic acid.

Secretion of PL (mainly in VLDL, partly in high-density lipoprotein and potentially in bile) may be expected to show a time-dependent increase similar to secreted TAG, since both lipids are required for lipoprotein secretion. However, Figure 3 shows that incorporation of radiolabelled fatty acids into secreted PL failed to increase after 2 h. One possible explanation for this is that fatty acids synthesized *de novo*, rather than the exogenous labelled fatty acids, are preferentially incorporated into the VLDL PL. This would result in secreted PL containing a greater proportion of unlabelled fatty acid, thus making an increase in PL secretion undetectable. We also cannot rule out the possibility that there is some phospholipase activity associated with the medium.

The difference in the metabolism of stearic acid, compared with the other two fatty acids, may be caused by several factors. It is possible that the conversion of stearate into stearoyl-CoA may be a rate-limiting step, perhaps due to poor metabolism of stearate by the long-chain acyl-CoA synthase [32]. This could explain why stearic acid was always present in the cell at higher concentrations as the non-esterified fatty acid, compared with oleic acid and palmitic acid.

An alternative mechanism by which stearate may be less well utilized for TAG synthesis in the liver is by its rapid conversion into oleate, as observed in humans [4], in laboratory animals [10,11] and in cultured hepatocytes [33]. The results presented here (Figure 3; Table 4) suggest that more stearate than palmitate was converted into oleate, whether in intracellular TAG or PL, but that the extent of desaturation was not sufficient to totally account for the neutral or hypocholesterolaemic effects associated with stearic acid.

Differential oxidation of SFA and UFA has been demonstrated both *in vivo* [16,17] and *in vitro* [34,35]. It has been postulated that different fatty acids are oxidized at a rate relative to their carbon chain length and degree of unsaturation. UFA tend to be oxidized more rapidly than SFA, thus making them less stimulatory to hepatic lipogenesis [16,17,31]. The rate of ketogenesis relies mainly on the direct utilization of extracellular fatty acids, and is therefore dependent on the fatty acid concentration in the medium [36]. In the present study, no significant difference was seen in the amount of oleate oxidized, compared with the two SFA, during the first 4 h of incubation. However, by 8 h significantly more oleate had been oxidized.

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In summary, using hepatocytes *in vitro* to study the metabolic fate of different fatty acids has confirmed distinct utilization and metabolism of stearic acid, compared with oleic acid and palmitic acid. Stearate, bound to albumin, is removed from the medium at a lower rate and, once taken up, is poorly incorporated into TAG and may be preferentially utilized for PL synthesis. Surprisingly, however, these differences do not appear to influence the mass of TAG synthesized and secreted by the cells, suggesting a stimulation of TAG synthesis which is independent of the role of the fatty acids as substrates. More stearic acid than palmitic acid is converted into oleic acid, but this does not appear to be sufficient to entirely explain the neutral or hypolipidaemic effect of the former. It is more likely that this is due to the overall effect of a number of differences in metabolism.

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