Quantification *in vivo* of the effects of different types of dietary fat on the loci of control involved in hepatic triacylglycerol secretion

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Polyunsaturated fatty acids (PUFA) have been suggested to exert their hypotriglyceridaemic effect through several possible mechanisms that would be expected to decrease the rate of hepatic very-low-density-lipoprotein-triacylglycerol secretion. We have quantified the role played *in vivo* by changes in the pattern of partitioning of (i) acyl-CoA between oxidation and esterification, (ii) diacylglycerol between synthesis of triacylglycerol and of the major phospholipids, and (iii) triacylglycerol between secretion and storage within the liver, in response to two dietary levels of $n-6$ and $n-3$ PUFA. In order to achieve this we used the technique of selective labelling of hepatic fatty acids *in vivo*. Compared with a predominantly saturated fatty acid diet, both $n-6$ and $n-3$ PUFA intake resulted in a decrease in the proportion of acyl moieties that were secreted by the liver, through an increased diversion of acyl-CoA towards oxidation and a lower fractional rate of secretion of newly synthesized triacylglycerol. In addition, a diet rich in $n-3$ fatty acids resulted not only in a greater magnitude of these effects but also in a doubling of the partitioning of diacylglycerol towards phospholipid labelling. It is shown that the overall 50% reduction achieved by fish oil feeding in the proportion of acyl groups that were secreted by the liver was distributed over all three branch points. The contribution of each of these adaptations was quantified. The application of such an approach, i.e. the localization and *in vivo* quantification of the importance of loci of control, in studies on dietary and pharmacological agents that affect lipaemia, is discussed.

INTRODUCTION

The ability of dietary polyunsaturated fatty acids (PUFA), particularly of the $n-3$ series, to decrease plasma triacylglycerol (TAG) and cholesterol concentrations for a given level of fat intake is well established [1–3]. Multiple mechanisms have been proposed to account for this effect, including the decreased secretion of very-low-density lipoprotein (VLDL)-TAG by the liver [4,5], increased lipoprotein lipase activity in peripheral tissues [6] and an increased rate of clearance of apolipoprotein B (apoB)-containing lipoproteins from the circulation [7]. Of these possibilities, the one that has attracted most attention has been the mediation by fish oil diets rich in $n-3$ fatty acids, and particularly in eicosapentanoic acid (EPA), of a decrease in VLDL-TAG secretion by the liver. This effect of $n-3$ fatty acids has been suggested to occur in the livers of many species and is thought to be involved in the lipid-lowering effects of fish-oil-based diets in humans [8].

Any decrease in the rate of secretion of VLDL-TAG by the liver would be expected to reflect the overall result of a decrease in the supply of fatty acids and/or the diversion of acyl moieties away from TAG secretion through the sequential set of partitionings of flux at several hepatic metabolic branch points. The major ones are: (i) the partitioning of cytosolic acyl-CoA between esterification to the glycerol moiety and oxidation; (ii) the partitioning of diacylglycerol (DAG) between the formation of the major phospholipids and of triacylglycerol; and (iii) the partitioning of TAG between the secretory pathway and the cytosolic pool.

In numerous studies, all of these three major branch points have been variously suggested to be affected by PUFA-rich diets. The increased diversion of hepatic fatty acid metabolism into peroxisomal and/or mitochondrial oxidation [9–11], together with induction of carnitine palmitoyltransferase I (CPT I) activity [12,13], have been described. The activities of key enzymes of DAG synthesis have been found to be either decreased (e.g. phosphatidate phosphohydrolase [12]) or increased (e.g. glycerol 3-phosphate acyltransferase; see [14]) by PUFA-rich diets. The diminished ability of liver homogenates to hydrolyse phosphatidate has been claimed to account for the lowering of plasma TAG levels in fish-oil-fed rats [15,16]. Several studies have described the preferential incorporation of PUFA themselves into phospholipids rather than into TAGs and, perhaps more importantly, to enhance the diversion of overall DAG metabolism towards phospholipid synthesis [17,18]. A direct effect of $n-3$ fatty acids on the partitioning of TAG between intrahepatic storage and secretion has been suggested through the observation of an increased rate of degradation of apoB in hepatocytes isolated from fish-oil- or EPA-fed rats [19,20].

Because these various observations (only a small representative sample of which are cited above) were obtained almost entirely through the use of *in vitro* preparations, ranging from crude liver homogenates [21] to isolated mitochondria [11], isolated cells [17] and perfused liver preparations [5], it has not been possible to obtain a quantitative assessment of the relative importance, if any, of each of the individual possible mechanisms in the physiological setting *in vivo*. Such *in vitro* preparations, although useful in the identification of candidate control points, are necessarily studied under highly artificial conditions (see [22,23]). Moreover, individual studies have often focused on only selected aspects of fatty acid partitioning.

In view of the obvious need to assess quantitatively the relative degree of control exerted at each of these branch points *in vivo*, we have applied the technique of selective labelling of hepatic

Abbreviations used: PUFA, polyunsaturated fatty acids; TAG, triacylglycerol; VLDL, very-low-density lipoprotein; apoB, apolipoprotein B; EPA, eicosapentanoic acid; DAG, diacylglycerol; CPT I, carnitine palmitoyltransferase I; NEFA, non-esterified fatty acids.

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fatty acids *in vivo* [22,23] to rats maintained on diets of different fatty acid compositions. This recently developed method enables the monitoring of the partitioning of hepatic fatty acid metabolism (and in particular that of [^{14}C]oleate) in awake, unrestrained rats, i.e. under entirely physiological conditions. In the present study, it has enabled us to assess quantitatively how each of the partitioning steps is affected in relation to the lipid-lowering effects of the different diets.

MATERIALS AND METHODS

Animals

Female Wistar rats (initial body wt. 120–130 g) were housed in wire-bottomed cages and maintained under a 12 h light/12 h dark cycle. They were randomly assigned to one of four diet groups. Each diet was based on a fat-free (21% casein, 53% sucrose, 16% corn starch, 5% alphacel) powdered, mineral- and vitamin-supplemented diet (fat-free diet AIN-76A; ICN Biochemicals) to which was added (10% by wt.) one of the four different fats studied: lard, corn oil, safflower oil or menhadden oil. The lard diet contained 0.25% corn oil to prevent essential fatty acid deficiency. The diets were prepared fresh every 2 days. The animals were maintained on the respective diets for 25 days. Food intake (average 14.7 g per rat per day) was very similar for all four groups of animals, as was the weight gain (average 2.8 g per day). At the time of the experiment, the rats weighed 190–200 g. When intravenous injections were to be performed [see (i) and (iii) below], the animals were anaesthetized and fitted with a jugular cannula 4 days before being used, by which time their food intake had returned to normal.

Three series of experiments were performed on separate sets of animals: (i) to monitor the partitioning of hepatic fatty acid metabolism, (ii) to measure plasma and liver concentrations of cholesterol, TAG and non-esterified fatty acids (NEFA), and (iii) to measure the rate of hepatic secretion of TAG. Animals for (i) and (ii) were used in the fed, absorptive state, 2–8 h into the light phase of the light/dark cycle; those in series (ii) were anaesthetized with pentobarbitone (60 mg per kg body wt.) before tissue samples were obtained. The rates of appearance of TAG (series iii) were measured in post-absorptive rats (i.e. 6–8 h into the light phase) so as to minimize the contribution of intestinal secretion of chylomicron-TAG.

Monitoring of hepatic fatty acid partitioning

This was performed as described in [22]. Briefly, a pulse of [1-^{14}C]oleate was generated in the livers of the experimental animals by the intravenous injection (through the jugular cannula) of VLDL and chylomicron remnant lipoproteins labelled with cholesteroyl [1-^{14}C]oleate. The fate of the [^{14}C]oleate generated intrahepatically through the hydrolysis of the ester was monitored over the first 60 min by collection of $^{14}CO_2$ in the exhaled air and by measuring the incorporation of ^{14}C into hepatic and plasma glycerolipids, after injection of Triton WR-1339 (at 15 min) into the animals. The preparation of apoC-poor VLDL and chylomicron remnants, and their labelling with cholesteryl [1-^{14}C]oleate and [^{3}H]cholesteroyl ether, were performed as described previously [22] except that the lipoproteins were separated from the labelling and KBr-containing media by gel filtration on a column (1 cm × 20 cm) of Sephadex G-25 equilibrated with 0.15 M NaCl.

Rate of TAG secretion

A parallel set of experiments was performed on post-absorptive rats (see ii, above). Food was removed from the animals 6–8 h before the determinations were performed. An initial blood sample (0.2 ml) was taken to measure TAG and NEFA concentrations and 1 ml of a 10% solution of Triton WR-1339 in saline was injected into the animals through the jugular cannula. Successive samples of blood (0.1 ml) were withdrawn through the cannula at 30, 60 and 90 min for measurement of TAG accumulated in the plasma. Rates of appearance of TAG were linear during this period.

Analyses

Separation and analysis of lipids extracted from the liver and plasma were performed as described previously [22]. Lipid extracts of the diets were performed by the same standard procedures and analysis of the fatty acid composition of the TAGs was performed by gas-liquid chromatography after hydrolysis and formation of the methyl esters. Commercially available kits were used to measure TAG (Sigma, Poole, Dorset, U.K.), NEFA (NEFAC, Wako Chemicals, Neuos, Germany) and cholesterol (Boehringer Mannheim, Lewes, E. Sussex, U.K.). Phospholipid phosphorus was measured as in [24].

Statistical analysis

Unpaired Student's *t* test was used throughout.

RESULTS AND DISCUSSION

The aim of the present study was not to describe the effects of the various diets on liver and plasma lipid parameters (which are very well documented; see the Introduction). Rather, it was to quantify the extent to which control is exerted at each of the possible sites that have been individually suggested to be involved in determining the flux of TAG towards secretion by the liver in rats fed diets enriched in different types of fatty acids (see Table 1 for composition). However, several observations of interest emerged from our plasma and liver analyses. Thus it is evident (Table 2) that rats maintained on the fish oil diet showed the

Table 1 Fatty acid compositions of corn oil, safflower oil, menhadden oil and lard

The various fats were added (10%, w/w) to a fat-free powdered diet. The analyses were performed as described in the Materials and methods section.

	Content (% of total fatty acids)			
Fatty acid	Lard	Safflower oil	Menhadden oil	Corn oil
$C_{14:0}$	1.7	0.1	9.7	< 0.1
$C_{16:0}$	24.3	7.1	21.4	9.6
$C_{16:1}$	3.9	–	15.2	–
$C_{18:0}$	14.1	2.3	5.3	1.9
$C_{18:1,n-9}$	43.2	14.6	15.9	29.0
$C_{18:2,n-6}$	10.7	74.4	3.2	56.4
$C_{18:3,n-3}$	1.8	1.2	3.5	2.7
$C_{20:3}$	< 0.1	0.2	0.5	0.1
$C_{20:4}$	0.2	< 0.1	2.6	–
$C_{20:5,n-3}$	< 0.1	0.2	14.1	0.4
$C_{22:5,n-3}$	< 0.1	–	1.8	–
$C_{22:6,n-3}$	< 0.1	–	6.9	–
Saturated	40.1	9.5	36.4	11.5
Monosaturated	47.1	14.6	31.0	29.0
Polyunsaturated	12.9	76.0	32.6	60.0
n−3	1.9	1.4	26.3	3.1
n−6	10.7	74.4	3.2	56.4

Table 2 Plasma and liver lipid concentrations in animals fed diets rich in different fatty acids

Samples were obtained either from absorptive rats (A) 2–3 h into the light period or from post-absorptive rats (PA) 6–8 h into the light period. Values are means ± S.E.M. for 3–6 determinations. Values that are significantly different ($P < 0.01$) from those observed in animals fed on lard are indicated by an asterisk.

		Plasma			Liver		
Diet		TAG (mM)	NEFA (mEq/l)	Cholesterol (mM)	TAG (mg/g)	Cholesterol (mg/g)	Phospholipid (μmol/g)
Lard	A	1.28 ± 0.14	0.18 ± 0.04	0.94 ± 0.07	5.8 ± 0.6	1.2 ± 0.1	19.4 ± 0.6
	PA	0.58 ± 0.10	0.35 ± 0.04				
Corn oil	A	1.35 ± 0.20	0.19 ± 0.03	0.86 ± 0.06	4.7 ± 0.4	1.1 ± 2.5	19.6 ± 1.7
	PA	0.69 ± 0.11	0.43 ± 0.03				
Safflower oil	A	1.01 ± 0.20	0.25 ± 0.05	0.82 ± 0.13	6.5 ± 0.9	1.2 ± 0.1	16.2 ± 0.7
	PA	0.37 ± 0.06	0.40 ± 0.06				
Menhadden oil	A	0.27 ± 0.05*	0.10 ± 0.03*	0.54 ± 0.01*	8.1 ± 1.1	1.1 ± 0.1	19.3 ± 0.7
	PA	0.43 ± 0.03	0.39 ± 0.03	–			

largest depression of plasma TAG in the absorptive phase (against all other diets) rather than in the post-absorptive phase (versus lard and corn oil diets). As expected, the plasma cholesterol concentration was also depressed in rats maintained on a fish oil diet (Table 2). There were no differences between the different groups in plasma NEFA concentrations in post-absorptive rats, but in absorptive rats (in which the concentrations were generally lower) NEFA concentrations were significantly depressed in the menhadden-fed group (cf. [25]). There were no significant differences in hepatic TAG, cholesterol or phospholipid content between rats maintained on any of the diets, although animals fed on menhadden oil tended to have higher hepatic TAG levels (Table 2). Other studies have found variable effects of dietary fish oil on liver TAG content (see [18,26,27]).

Partitioning of hepatic fatty acid metabolism *in vivo*

The use of selective labelling of hepatic fatty acids *in vivo* enabled the quantification of their partitioning in terms of (i) the oxidation-to-esterification ratio, (ii) the phospholipid-to-TAG ratio, and (iii) the fractional rate of secretion of TAG labelled during the effective pulse of [^{14}C]oleate given to the liver. Direct measurement of intrahepatic and secreted [^{14}C]glycerolipids (which accumulated in the plasma after Triton WR-1339 injection) were obtained for the same animals (see [22,23]).

Oxidation and esterification

As expected from the fact that all the animals were studied in the early post-absorptive phase (2–3 h into the light phase, i.e. in the 'fed' state), the proportion of the cholesteryl ester metabolized in the liver that was recovered in esterification products (plasma and liver) was greater than that which was oxidized (cf [28]). However, there was a large difference between the proportion that was metabolized to glycerolipids in lard-fed animals (76.9%) and that observed for fish-oil-fed animals (57.2%), corresponding to esterification/oxidation ratios of 3.1 and 1.1 respectively. The safflower oil and corn oil diets gave intermediate values. This observation implies that partitioning of acyl-CoA towards glycerolipid formation is inhibited by 10%, 14% and 25% in livers of animals maintained on corn, safflower and menhadden oils respectively compared with lard-fed rats. The decrease in the proportion of acyl-CoA committed to esterification reflects the increased oxidative capacity of the liver (see the Introduction). In particular, several studies have shown that fish-oil-based diets result in a 25–50% increase in the activity of CPT I [12,13,29]. Although such an increase was not observed by Wong et al. [30], these authors described a marked decrease in the sensitivity of CPT I to malonyl-CoA, which, together with a lower hepatic malonyl-CoA concentration in the liver [31], would favour the use of acyl-CoA for oxidation. It might appear surprising that, in spite of these various adaptations of the oxidative pathway (including an increase in peroxisomal oxidation; see the Introduction), the overall result is only a modest decrease (25%) in the partitioning of acyl-CoA towards esterification by menhadden oil feeding. This may be due to the other changes that occur concomitantly in the livers of fish-oil-fed rats that would be expected to counteract the effects of an increased capacity for fatty acid oxidation. Thus the activities of both microsomal and mitochondrial glycerol-3-phosphate acyltransferases, which compete for cytosolic acyl-CoA, are increased [9,30,31], although phosphatidic acid phosphatase activity is generally decreased by dietary PUFA [32]. Our *in vivo* data suggest that these opposing changes in the activities of the enzymes involved in DAG synthesis may limit the contribution made by the acyl-CoA partitioning step towards the overall hypotriglyceridaemic effect of PUFA intake.

TAG and phospholipid labelling

The ^{14}C label incorporated into phospholipid as a proportion of total acylglyceride labelling was very similar (also compare with values for chow diet [28,33]) in the livers of rats maintained on all the diets except menhadden oil. Thus rats fed the fish oil diet had a phospholipid/total glycerolipid labelling ratio that was more than double that observed for the other diets. Because of the fact that incorporation of label into phospholipid from the preformed (exogenous) hepatic fatty acid pool is normally very low [22,28,33] (see also work on hepatocytes [34]), this doubling of the diversion of labelled DAG towards phospholipid had only a modest effect on the fraction of the total glycerolipid-associated label that was found in TAG (0.77 for menhadden oil diet versus 0.90 for lard diet). Therefore, compared with the lard diet, the menhadden oil diet resulted in only a 15% decrease in the proportion of [^{14}C]DAG that entered the TAG pools. However, the effect is exerted cumulatively on top of the 25% inhibition in the partitioning of acyl-CoA towards esterification. Consequently, the overall result is amplified. It is noteworthy, however, that contrary to the case at the acyl-CoA partitioning step

Table 3 Effect of dietary fat on glycerolipid partitioning in rat liver

Rats were used 2–3 h into the light phase. They were injected (through an indwelling jugular catheter) with cholesteryl [1-^{14}C]oleate-labelled remnant lipoproteins (see the Materials and methods section). After 15 min Triton WR-1339 was injected, and after a further 45 min samples of liver and plasma were obtained. Values are means (±S.E.M.) for four animals in each group. Values that are statistically significantly different from those obtained for lard-fed rats are indicated by an asterisk ($P < 0.01$).

Diet	Total glycerolipids (% of cholesterol [^{14}C]oleate metabolized in liver)	Phospholipid (% of total glycerolipid labelled)	TAG secreted (% of total TAG labelled)
Lard	76.9±4.0	9.6±1.0	66.6±3.5
Corn oil	69.2±4.1	9.7±0.4	65.6±3.5
Safflower oil	66.6±6.7	11.4±1.0	59.3±3.1
Menhadden oil	57.2±4.8*	23.1±1.8*	52.9±4.3*

Table 4 Comparison of the proportion of ^{14}C label secreted as TAG after selective labelling of hepatic fatty acids and the absolute rate of TAG appearance in post-absorptive rats

Values are means (±S.E.M.) for four determinations in each series of experiments. Those that are statistically significantly different from values for lard-fed rats are indicated by $*P < 0.05$ or $**P < 0.01$.

Diet	[^{14}C]TAG secreted (% of cholesterol [^{14}C]oleate metabolized in liver)	TAG appearance rate (μmol/h)
Lard	46.5±3.8	45.2±3.6
Corn oil	41.1±4.0	45.9±2.1
Safflower oil	34.9±3.2*	34.7±2.3*
Menhadden oil	23.6±3.4**	30.6±1.6**

(above), only the fish oil diet had any effect on the partitioning of DAG. This is in keeping with the exclusive property of EPA to inhibit DAG acyltransferase activity through a direct inhibitory effect of eicosapentanoyl-CoA [2,35], although the expression of DAG acyltransferase (measured with other acyl-CoA substrates) is either unchanged or increased [9,29,36].

The opposing effects of increased DAG acyltransferase expression and the direct effect of EPA-CoA on enzyme activity appear to result in a much more modest net effect on the partitioning of DAG *in vivo* than has been predicted from *in vitro* enzymological studies (see above). In previous studies, conducted on *in vitro* liver preparations, much larger effects of pure EPA on phospholipid-to-TAG partitioning were observed, presumably because of the high concentrations of EPA used as the sole fatty acid source (see e.g. [17]). At these concentrations, both the inhibition of DAG acyltransferase by EPA-CoA and the mass action effect of the preferential incorporation of PUFA into the *sn*-2 position of phospholipids (see [37]) would be expected to be operative. In this context it is to be emphasized that in the present study the ^{14}C label was associated with oleate and, therefore, such direct effects will not have been contributory factors. The concentrations of EPA experienced by the liver *in vivo* would be expected to be much lower than those used for cell incubations *in vitro*.

Fractional rate of TAG secretion

The last metabolic branch point at which TAG secretion by the liver can be inhibited is through a greater degree of incorporation of newly synthesized TAG into the cytosolic pool at the expense of secretion (see [34]). The data in Table 3 indicate that the livers of lard-fed rats secreted the highest proportion (66.6%) of the newly labelled TAG. The fractional rate of secretion was not significantly different from this value in the livers of corn-oil- and safflower-oil-fed rats, but the menhadden oil diet decreased it significantly (Table 3), such that the fractional rate of secretion of the newly labelled TAG was inhibited by 21% by this diet when compared with lard-fed rats.

Correlation between TAG secretion rates and partitioning of ^{14}C label into secreted TAG

Studies performed on isolated perfused rat liver and isolated hepatocytes have shown that the rates of uptake of PUFA and other fatty acids (e.g. oleate) by these preparations are very similar when equivalent concentrations of the respective NEFA are supplied exogenously [5,38]. It would be anticipated, therefore, that when the rate of delivery of NEFA to the liver *in vivo* is similar in different experimental groups of animals, the partitioning of the label observed in our experiments should provide reasonably quantitative estimates of the relative overall hepatic fluxes of fatty acids into the various pathways. The data in Table 2 indicate that plasma NEFA concentrations are very similar in rats fed on different fat diets when they are sampled in the post-absorptive state. Consequently, during this period, the rate of TAG secretion by the livers of the different groups of animals should be determined primarily by the pattern of partitioning of acyl-CoA at the three metabolic branch points studied, as the availability of NEFA is very similar. In particular, for any given level of plasma NEFA, the fraction of the label metabolized in the liver that appears as secreted [^{14}C]TAG should be quantitatively related to the absolute rate of hepatic TAG secretion. This is borne out by the data in Table 4, in which the experimentally determined rates of TAG secretion in the post-absorptive state are presented alongside the proportion of the [1-^{14}C]oleate label metabolized that was secreted into the plasma when hepatic fatty acids were selectively labelled *in vivo*. This correlation occurred even though the two sets of experiments were performed at different phases of the prandial/absorptive cycle. Thus measurement of hepatic TAG secretion rates had to be performed in the post-absorptive phase to minimize interference from intestinal TAG secretion, whereas *in vivo* labelling of hepatic fatty acids was performed on 'fed' rats, i.e. in the absorptive phase. Menhadden-oil-fed rats showed the lowest rate of TAG secretion by the liver in the post-prandial period and were also the ones that channelled the lowest proportion of label into secreted TAG (Table 4). It is to be stressed that the latter adaptation would be expected to result in an even bigger difference between fish-oil-fed and lard-fed rats in the absolute rate of hepatic TAG secretion during the absorptive state because, in that state, the plasma NEFA concentration of the former is also significantly lower (Table 2), such that a smaller proportion of a lower rate of NEFA delivery to the liver would be secreted. Such combined effects could contribute towards the achievement of the much lower plasma concentrations of TAG observed in these animals in the absorptive phase (Table 2).

The effect of dietary fish oil, and especially of its $n-3$ fatty acid content, on the diversion of TAG away from the secretory pathway is presumed to be due to the ability of PUFA to increase the rate of degradation of apoB, as observed in experiments using cultured rat hepatocytes [39]. (The levels of apoB mRNA

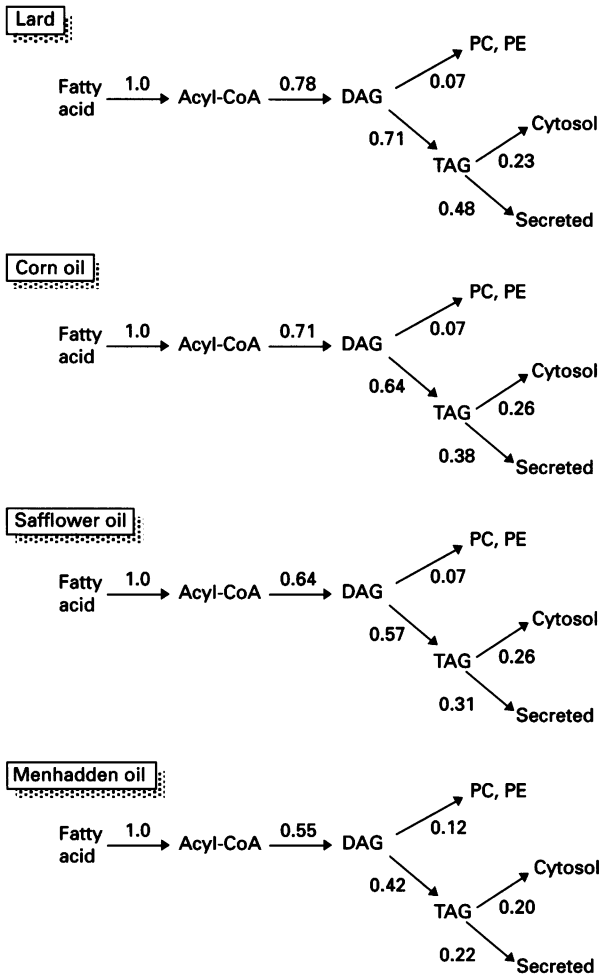

Figure 1 Quantification of the partitioning of flux of acyl moieties at the three major branch points involved in fatty acid utilization (for oxidation or glycerolipid synthesis) in rat liver

The overall utilization of fatty acid (generated from exogenous NEFA) is set at 1.0 for each condition. The numbers against each branch of metabolism relate to the proportion of this total flux that would be expected to enter each pathway, as assessed from the partitioning data obtained *in vivo*. The contribution of each successive partition to the overall proportion of the labelled TAG that is secreted by the liver in animals fed the different diets is demonstrated. PC, phosphatidylcholine; PE, phosphatidylethanolamine.

[19] and the rate of synthesis of the protein [39] remain unaltered.) In HepG2 cells, low concentrations of EPA affect overall apoB expression to a much greater degree than apoB (and associated TAG) secretion [20], suggesting that the primary effect of EPA is to enhance intracellular degradation of apoB.

Overall effect of diets

The contributions of the adaptations at each of the metabolic branch points towards the overall effects of the different diets are quantified in Figure 1. It can be seen that dietary menhadden oil achieves a 58% reduction in the proportion of label that is secreted as [^{14}C]TAG from the liver, compared with lard, with intermediate values for the corn oil and safflower oil diets. It can be computed that for the fish oil diet the overall effect is achieved by the multiplicative effects of decreases (compared with the lard diet) of 29.5%, 15.6% and 25.8% in the degree of partitioning of acyl-CoA, DAG and TAG respectively towards that proportion of the acyl moiety that follows the pathway leading to TAG secretion. Therefore the increased diversion of acyl-CoA towards oxidation and the increased intracellular retention of TAG at the expense of secretion make an approximately equal contribution, whereas the effect of increased diversion of DAG towards phospholipid synthesis contributes about a fifth of the overall effect.

The value of the type of experimental approach adopted here is that it quantifies the distribution of control exerted by a physiological variable such as diet and demonstrates how the large and apparently dominant effects suggested from experiments conducted *in vitro* (see, e.g. [15,16]) are tempered *in vivo* to provide a much wider spread of metabolic control (see [8]). It should be possible to apply the same principles to identify the *in vivo* mode(s) of action (and the time span over which they act) of specific fatty acids and hypolipidaemic drugs.

In the present study we have used commercially available cholesteryl [1-^{14}C]oleate to deliver label specifically to the liver. It should be possible to study the fate of other fatty acids by using the appropriately labelled cholesteryl esters. In this manner it should be possible to study the effects of diets enriched in a particular fatty acid not only on its own metabolism but also on the metabolism of a second fatty acid of different chain length and/or degree and type of unsaturation.

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REFERENCES

1 Sanders, T. A., Vickers, M. and Haines, A. P. (1981) Clin. Sci. **61**, 317–324
2 Rustan, A. C., Christiansen, E. N. and Drevon, C. A. (1992) Biochem. J. **283**, 333–339
3 Clarke, S. D. and Jump, A. (1994) Annu. Rev. Nutr. **14**, 83–98
4 Lottenberg, A., Oliveira, H., Nakanadakare, E. R. and Quintas, E. (1992) Lipids **27**, 326–330
5 Zhang, Z. J., Wilcox, H. G., Castellani, L. W., Fungwe, T. V., Elam, M. B. and Heimberg, M. (1993) Lipids **28**, 419–425
6 Levy, E., Roy, C. C., Goldstein, R., Bar-on, H. and Zir, E. (1991) J. Am. Coll. Nutr. **10**, 69–78
7 Ventura, M. A., Woollet, L. A. and Spady, D. K. (1989) J. Clin. Invest. **84**, 528–537
8 Zammit, V. A. (1994) Diabetes Rev. **2**, 132–155
9 Willumsen, N., Skore, J., Hexeberg, S., Rustan, A. C. and Berge, R. K. (1993) Lipids **28**, 683–690
10 Yamazaki, R. K., Shen, T. and Schade, G. B. (1987) Biochim. Biophys. Acta **920**, 62–67
11 Bergseth, S., Christiansen, E. N. and Bremer, J. (1986) Lipids **21**, 508–514
12 Surette, M. E., Whelan, J., Bronghton, K. S. and Kinsella, J. E. (1992) Biochim. Biophys. Acta **1126**, 199–205
13 Aarsland, A., Lundquist, M., Borretsen, B. and Berge, R. K. (1990) Lipids **25**, 546–548
14 Wong, S. and Marsh, J. B. (1988) Metabolism **37**, 1177–1181
15 Halminski, M. A., Marsh, J. B. and Harrison, E. H. (1991) J. Nutr. **121**, 1554–1561
16 Coniglio, J. G. (1992) Nutr. Rev. **50**, 195–197
17 Rustan, A. C., Hassen, J. O., Christiansen, E. N. and Drevon, C. A. (1988) J. Lipid Res. **29**, 1417–1426
18 Yeo, Y. K. and Holub, B. J. (1990) Lipids **25**, 811–814
19 Ribeiro, A., Mangeney, M., Cardot, P., Loriette, C., Rayssiguier, Y., Chamboz, J. and Bereziat, G. (1991) Eur. J. Biochem. **196**, 499–507
20 Byrne, C. D., Wang, T. W. and Hales, C. N. (1992) Biochem. J. **288**, 101–107
21 Berge, R. K., Nilsson, A. and Husoy, A. M. (1988) Biochim. Biophys. Acta **960**, 417–426
22 Moir, A. M. B. and Zammit, V. A. (1992) Biochem. J. **285**, 655–660
23 Zammit, V. A. and Moir, A. M. B. (1994) Trends Biochem. Sci. **19**, 313–317
24 Bartlett, G. R. (1959) J. Biol. Chem. **234**, 469–471
25 Baltzell, J. K., Jekkins, R. L., Wooten, J. T. and Otto, D. A. (1993) Biochim. Biophys. Acta **1167**, 197–203
26 Otto, D. A., Baltzell, J. K. and Wooten, J. T. (1992) Lipids **27**, 1013–1017

27 Otto, D. A., Tsai, C. E., Baltzell, J. K. and Wooten, J. T. (1991) Biochim. Biophys. Acta **1082**, 37–40
28 Moir, A. M. B. and Zammit, V. A. (1993) Biochem. J. **289**, 49–55
29 Willumsen, N., Hexeberg, S., Skorre, J., Lundquist, M. and Berge, R. K. (1993) J. Lipid Res. **34**, 13–22
30 Wong, S.-H., Nestel, P. J., Trimble, R. P., Storer, G. P., Illman, R. J. and Topping, D. L. (1984) Biochim. Biophys. Acta **792**, 103–109
31 Wilson, M. D., Blake, W. L., Salati, L. M. and Clarke, S. D. (1990) J. Nutr. **120**, 544–552
32 Lawson, N., Jennings, R. J., Pollard, A. D., Sturton, R. G., Ralph, S. H., Marsden, C. A., Fears, R. and Brindley, D. N. (1981) Biochem. J. **200**, 265–273
33 Moir, A. M. B. and Zammit, V. A. (1993) Biochem. J. **291**, 241–246
34 Duerden, J. M., Marsh, B., Burnham, F. J. and Gibbons, G. F. (1990) Biochem. J. **271**, 761–766
35 Coleman, R. and Bell, R. M. (1976) J. Biol. Chem. **251**, 4537–4543
36 Marsh, J. B., Topping, D. L. and Nestel, P. J. (1987) Biochim. Biophys. Acta **922**, 239–243
37 Tijburg, L. B. M., Geelen, M. J. H. and Van Golde, L. M. G. (1989) Biochim. Biophys. Acta **1004**, 1–19
38 Zhang, Z. J., Wilcox, H. G., Elam, M. B., Castellani, L. W. and Heimberg, G. M. (1991) Lipids **26**, 504–511
39 Wang, H., Chen, X. and Fisher, E. A. (1993) J. Clin. Invest. **91**, 1380–1404

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