Effects of dietary fatty acid composition on basal and hormone-stimulated hepatic lipogenesis and on circulating lipids in the rat

BY ANTONIOS ZAMPELAS, LINDA M. MORGAN, NIGEL FURLONGER AND CHRISTINE M. WILLIAMS

The Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford GU2 5XH

(*Received 23 June 1994 – Revised 5 January 1995 – Accepted 16 January 1995*)

Thirty male rats were randomly assigned to one of three dietary groups in which the source of dietary fat was either a mixed oil, maize oil or fish oil. Effects of dietary fatty acid composition on *in vitro* rates of [U-^{14}C]glucose incorporation into hepatic total lipids and into hepatic triacylglycerol were measured under basal, insulin (4 nM)-, gastric inhibitory polypeptide (GIP; 6 nM)- and insulin + GIP (4 nM + 6nM)-stimulated conditions. Effects of the three diets on postprandial plasma triacylglycerol, cholesterol, insulin and GIP concentrations were also measured. The fish-oil diet decreased rates of basal glucose incorporation into hepatic total lipids ($P < 0.05$) and hepatic triacylglycerol ($P < 0.01$) compared with the mixed-oil diet. The presence of insulin + GIP in the incubation medium stimulated glucose incorporation into hepatic total lipids in the maize-oil ($P < 0.01$) and fish-oil groups ($P < 0.05$), as well as into hepatic triacylglycerol in the maize-oil group ($P < 0.005$). In addition, the fish-oil diet decreased postprandial plasma triacylglycerol levels compared with both other dietary groups ($P < 0.05$ both cases), and the mixed-oil diet markedly increased postprandial plasma insulin levels compared with the other dietary groups ($P < 0.001$).

Saturated fatty acids: Polyunsaturated fatty acids: Lipids: Hormones: Rats.

Numerous studies support the view that the most pronounced and consistent effect of *n*-3 polyunsaturated fatty acids (PUFA) is a decrease in plasma triacylglycerol (TAG) concentrations whereas effects on cholesterol-rich lipoproteins are less consistent (Sanders & Rochanai, 1983; Harris *et al.* 1988; Deck & Radack, 1989). On the other hand, *n*-6 PUFA have been shown to produce consistent reductions in fasting plasma total cholesterol (T-C) and low-density-lipoprotein-cholesterol (LDL-C) concentrations, although when these fatty acids are substituted isoenergetically for saturated fatty acids (SFA), effects on TAG, very-low-density lipoprotein cholesterol (VLDL-C) and high-density-lipoprotein cholesterol (HDL-C) are not found to be consistent (Ahrens *et al.* 1957; Shepherd *et al.* 1980).

Since the metabolic effects of *n*-3 PUFA appear to be quite distinct from those of *n*-6 PUFA, further research needs to be carried out to elucidate the physiological differences in the metabolism of these two classes of PUFA.

Reported inhibitory effects of *n*-3 PUFA on rates of hepatic lipogenesis measured *in vivo* (Triscari *et al.* 1978) may be due to: (1) direct inhibitory actions of *n*-3 PUFA on key enzymes of lipogenesis and/or diversion of substrate into other metabolic pools, (2) inhibitory effects of *n*-3 PUFA on secretion of lipogenic hormones (e.g. insulin, gastric inhibitory polypeptide), or (3) inhibition of the action of these hormones at target tissue level. Further studies are needed to clarify the major locus for the effects of these fatty acids on hepatic TAG metabolism and this requires studies to be carried out *in vitro ex vivo* as

well as in the *in vivo* state. The dietary conditions under which experiments such as these are conducted also require further consideration. Most of the existing studies, which have largely investigated effects of dietary fatty acids on *in vivo* rates of hepatic lipogenesis, have not placed sufficient emphasis on the need to ensure comparable rates of feed intake in the different dietary groups. A further problem is that most studies of effects of dietary fatty acid composition on hepatic lipogenesis have been conducted on fasted animals whereas studies investigating responses of hepatic lipogenesis in the postprandial state are particularly important for interpreting the data for effects of fish oils on plasma postprandial TAG levels in human subjects.

There is also a need to study effects of lipogenic hormones on stimulation of hepatic lipogenesis under different dietary conditions, since this area of research has received little attention. Insulin is the main regulatory hormone controlling hepatic VLDL production (Gibbons, 1990), and in the adipocyte, changes in insulin binding have been reported in animals fed on high-fat diets (Begum *et al.* 1982).

Gastric inhibitory polypeptide (GIP) is stimulated by fat ingestion (Falko *et al.* 1975), inhibits glucagon-induced glycogenolysis in liver and potentiates the effects of insulin on incorporation of fatty acids into TAG in adipose tissue (Beck, 1989). Hartmann *et al.* (1986) also suggest that GIP exerts direct effects on hepatic glycogen metabolism predominantly in a situation where insulin is simultaneously present. However, there have been no studies which have investigated effects of GIP, or GIP together with insulin, on hepatic lipid metabolism, despite the proposed central role of these hormones in the regulation of lipid homeostasis.

As a result, the present study was carried out to compare the effects of three different oils (maize oil, fish oil and a mixture of oils replicating the UK dietary fatty acid intake) on hepatic lipogenesis in pair-fed rats, with tissue removed from animals in the non-fasted state, to investigate effects of these oils on plasma lipid (T-C, TAG) and hormone (insulin, GIP) levels, and to determine effects of insulin and GIP, separately and in combination, on hepatic lipogenesis under the dietary and nutritional conditions described above.

MATERIALS AND METHODS

Animals

Thirty male Wistar Albino rats were randomly allocated into three groups of ten, kept in stainless steel cages, and allowed free access to water. Three animals were randomly allocated to each dietary group daily over a period of 10 consecutive days, until all thirty animals were on one of the three diets. Animals were maintained on the diets for 2 weeks with slaughter of one animal from each dietary group on a daily basis over a period of 10 d.

Diets

Each of the dietary groups consumed a 50 g fat/kg semi-purified diet, providing 10% of the energy from fat. The diets differed in their fatty acid composition, containing either a mixture of oils consisting of palm oil, coconut oil, olive oil and maize oil (42% SFA, 43% monounsaturated fatty acids (MUFA) and 16% PUFA, to mimic the UK current dietary fatty acid composition), or maize oil (rich in *n*-6 fatty acids), or a deodorized fish-oil concentrate (British Cod Liver Oils, Hull, Humberside) which was rich in *n*-3 fatty acids. The nutrient composition of the diets was (g/kg): maize starch 600, casein 200, Solka floc 48, and vitamin–mineral mixture 50, all supplied by Special Diet Services, Witham, Essex. Sucrose (50 g/kg), the appropriate oil (50 g/kg) and methionine (2 g/kg; Sigma Chemical Co., Poole, Dorset) were added to the diets. The compositions of the diets are tabulated in Table 1 and the detailed fatty acid compositions in Table 2. The animals were maintained on the experimental diets for 2 weeks. Diets were prepared weekly and stored at 4°. The

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

Dietary component	Mixed-oil diet	Maize-oil diet	Fish-oil diet
Starch	600	600	600
Sucrose	50	50	50
Casein	200	200	200
Solka floc	48	48	48
Coconut oil	11	—	—
Olive oil	11	—	—
Maize oil	6	50	—
Palm oil	22	—	—
Fish oil	—	—	50
Methionine	2	2	2
Vitamin–mineral mixture*	50	50	50

* SDS Complete Vitamin/Mineral Premix for Maintenance (Special Diets Services (SDS), Witham, Essex).

Table 2. *Fatty acid composition of the diets* (g/100 g total fatty acids)*

Fatty acid	Mixed-oil diet	Maize-oil diet	Fish-oil diet
12:0	11·9	—	—
14:0	5·6	0·8	7·1
16:0	26·7	14·0	17·5
18:0	3·2	2·3	4·2
20:0	0·5	0·3	—
22:0	—	—	—
24:0	—	—	—
14:1	—	—	0·3
16:1	0·6	0·4	9·9
18:1	39·0	30·0	12·9
20:1	—	0·3	2·5
22:1	—	0·3	1·1
18:2	12·0	50·0	4·2
20:2	—	—	4·4
18:3	0·5	1·6	—
20:4	—	—	1·6
22:4	—	—	1·2
20:5	—	—	18·2
22:5	—	—	2·1
22:6	—	—	12·8

* Calculated from food tables (Paul & Southgate, 1978).

Table 3. *Daily food intake, body-weight gain, and liver weights after slaughter, of rats fed on diets containing a mixed oil, maize oil or fish oil**

(Mean values and standard deviations for ten rats)

	Mixed-oil group		Maize-oil group		Fish-oil group	
Variable	Mean	SD	Mean	SD	Mean	SD
Food intake (g/rat per d)	20·7	1·5	20·8	1·5	20·1	1·4
Body-wt gain (g/rat per d)	3·4	0·8	3·4	1·2	3·9	1·4
Liver weight (g)	15·0	1·8	13·9	1·8	14·2	1·8

For details of diets and procedures, see Table 1 and pp. 382–384.

feed pots were changed daily, and daily feed intakes and body weights, as well as liver weights after slaughter were recorded for all the animals (Table 3). The pair-feeding technique was used to ensure that the animals in each dietary group consumed the same amount of feed. This involved providing feed for consumption on an *ad lib.* basis on the first day and recording the 24 h dietary intake by weighing the feed pots full and weighing the pots including any uneaten food 24 h later. The dietary group which ate the least was allowed to eat *ad lib.* the following day and animals in the other groups were given the average amount of feed consumed by the first group over the previous 24 h. This procedure continued throughout the study.

Procedure

Apart from the pair-feeding, which was carried out continuously throughout the experiment, the animals were meal-fed on the evening before slaughter to ensure that animals were in the fed state at the time of removal of the liver the following morning (09.00 hours). On the morning of the day before the slaughter of individual animals the normal procedure of replenishing empty feed pots was not followed. Instead, a weighed portion of feed (equivalent to the total amount eaten for the 24 h period of the preceding day) was given at 17.00 hours for consumption during the night before slaughter (15 h feeding time). Animals consumed all feed presented to them. On the morning of the day of slaughter, one animal from each of the dietary groups was anaesthetized under diethyl ether, and blood was taken by cardiac puncture. Plasma was separated and stored at $-20°$ for further analysis of cholesterol, TAG, insulin and GIP. Values for nine animals are presented since on one study day the blood samples collected from the three animals killed that morning were lost during centrifugation. After blood collection, rats were killed and the liver was put directly into incubation medium (Medium 199 enriched with 20 g/l fatty-acid-free bovine serum albumin both supplied by Sigma Chemical Co.) which was kept at 37°. Rates of basal, insulin-, GIP-, and insulin + GIP-stimulated [U-^{14}C]glucose incorporation into liver total lipids and TAG were measured.

Analyses

Hepatic lipogenesis. Liver explants (approximate weight 200 mg) were incubated in 2 ml Medium 199 with addition of 55·5 kBq [U-^{14}C]glucose and in the absence or presence of hormones for 3 h, at 37° in a shaking water-bath, under an atmosphere of O_2–CO_2 (95:5, v/v). The concentration of the unlabelled glucose in the Medium 199 was 5·6 mmol/l. The reaction was terminated by the addition of 1 ml perchloric acid (600 ml/l). The liver explants were then transferred to a filter paper (Whatman no. 1), incubation buffer and perchloric acid were removed under vacuum, and the liver was washed with 20 ml saline (9 g NaCl/l) solution (addition of 5 ml aliquots to a total volume of 20 ml). Saline was also removed under vacuum.

The Bligh & Dyer (1959) lipid extraction method was used to separate the lipids from the liver explants, using a slightly modified procedure. Liver explants were transferred in a Soveril tube and 200 μl chloroform and 400 μl methanol were added. A glass rod was used to homogenize the liver explants in the organic solvents. The contents were vortex-mixed for 1 min. A further 200 μl chloroform was then added and the contents were mixed for 30 s. Distilled water (200 μl) was also added, the contents were vortex-mixed for 30 s and the homogenate was centrifuged at 1500 *g* for 5 min to separate the aqueous and the chloroform phases. The aqueous phase was discarded, 150 μl of the chloroform phase was transferred to a glass tube and a 50 μl portion was transferred into a scintillation vial where 4 ml scintillant was also added.

Radioactivity was measured in a β-radiation counter (Wallac 1410; Wallac Oy, Turku 10, Finland). A 60 s counting time was used and chemical, colour and chemiluminescence

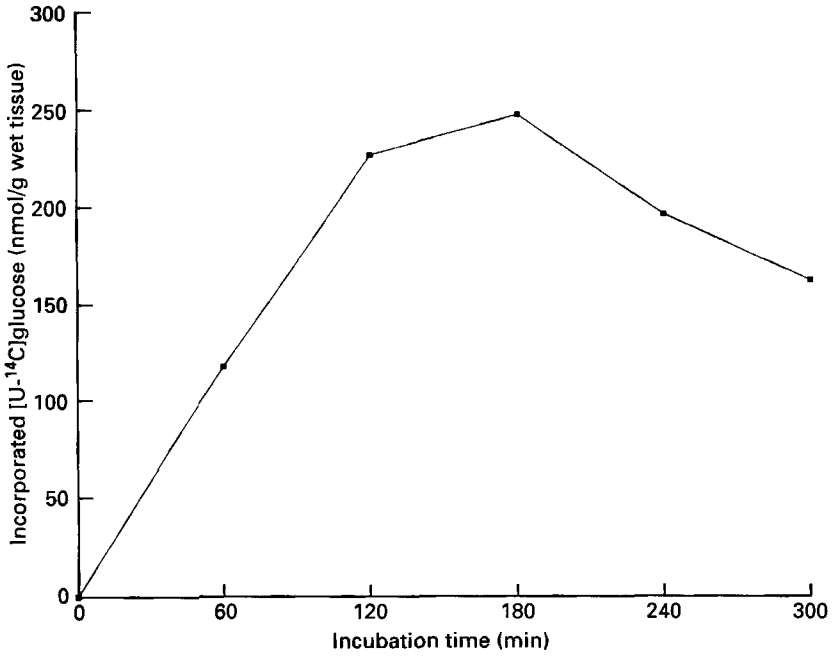

Fig. 1. Incorporation of [U-^{14}C]glucose into hepatic total lipids: time-course validation experiment.

correction were carried out automatically, using an external-standards channel-ratio method. The coefficient of variation (CV) for hepatic lipogenesis in total lipids was found to be 8·4%.

The remainder of the chloroform phase, obtained after the lipid extraction procedure (100 μl), was dried under N_2 and stored at 4° for later separation of the TAG subfraction. Thin layer chromatography (TLC) was used to separate TAG using silica gel plates (Merck, Darmstadt, Germany) and a mobile phase of hexane–diethyl ether–acetic acid (80:20:1, by vol.). The CV was found to be 19·1%.

A time-course validation experiment and incubations with different physiological hormone concentrations in the medium were carried out to assess maximum incorporation of the label. Basal lipogenesis was measured following 1, 2, 3, 4, and 5 h incubation in the medium. Hormone-stimulated lipogenesis was measured after incubating explants in media containing either 1, 2, 4, and 8 nmol insulin/l, or 1, 3, 6, and 10 nmol GIP/l, or 2+3, 4+6 and 8+10 nmol/l insulin and GIP together.

Automatic enzymic colorimetric tests were used to determine plasma TAG (TAG Uni-kit II; Roche Diagnostics Ltd., Welwyn Garden City, Herts), and cholesterol (cholesterol esterase Uni-kit II; Roche Diagnostics Ltd.) in a Cobas-bio centrifugal analyser.

Plasma immunoreactive insulin was measured by a double-antibody radioimmunoassay technique using antiserum supplied by Guildhay Antisera, Guildford, Surrey. Plasma immunoreactive GIP was measured by a double-antibody radioimmunoassay technique using antiserum which was raised against natural porcine GIP (Morgan *et al.* 1978).

Statistical analysis

Tabulated results are presented as mean values and standard deviations. Data in figures are given as mean values with their standard errors to facilitate clarity of presentation. Comparisons of rates of lipogenesis and of lipid and metabolite concentrations between the three dietary groups and differences between groups were identified using one-way

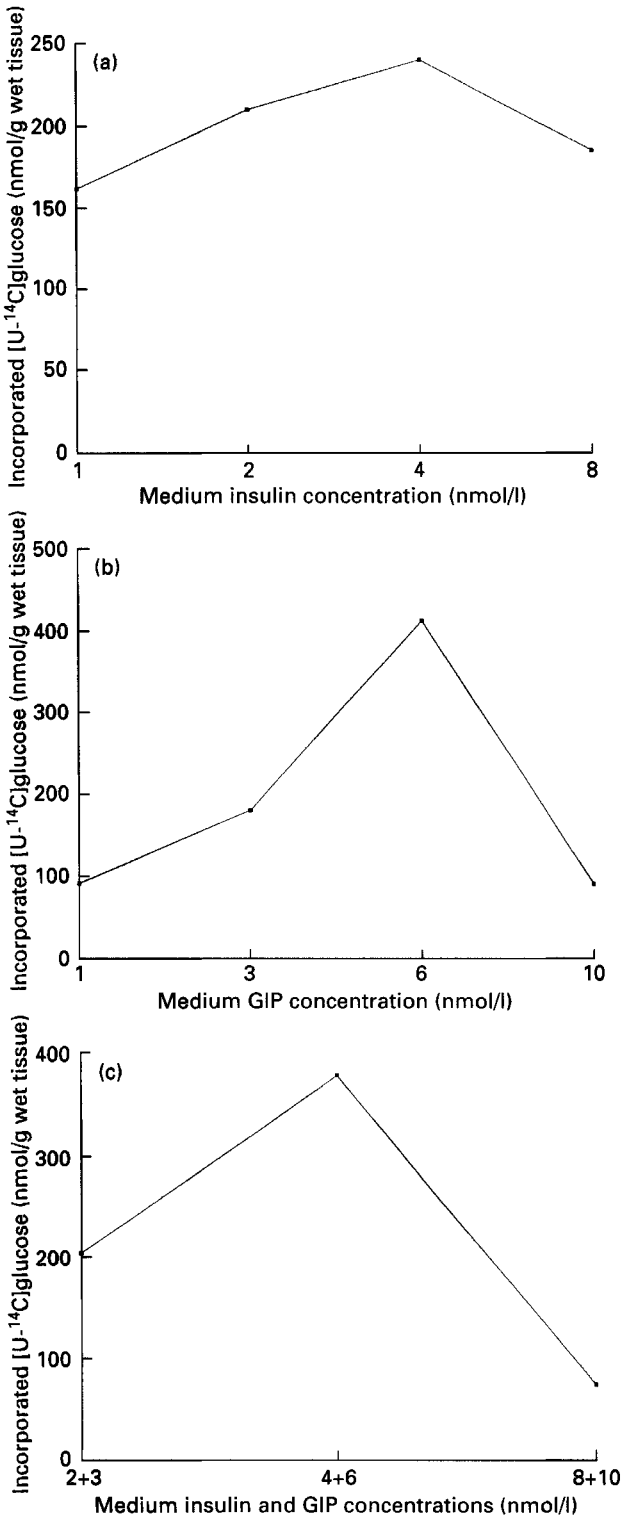

Fig. 2. Effect of (a) different insulin concentrations, (b) different gastric inhibitory polypeptide (GIP) concentrations and (c) different concentrations of both insulin and GIP on incorporation of [U-^{14}C]glucose into hepatic total lipids *in vitro*. For details of procedures, see pp. 382–385.

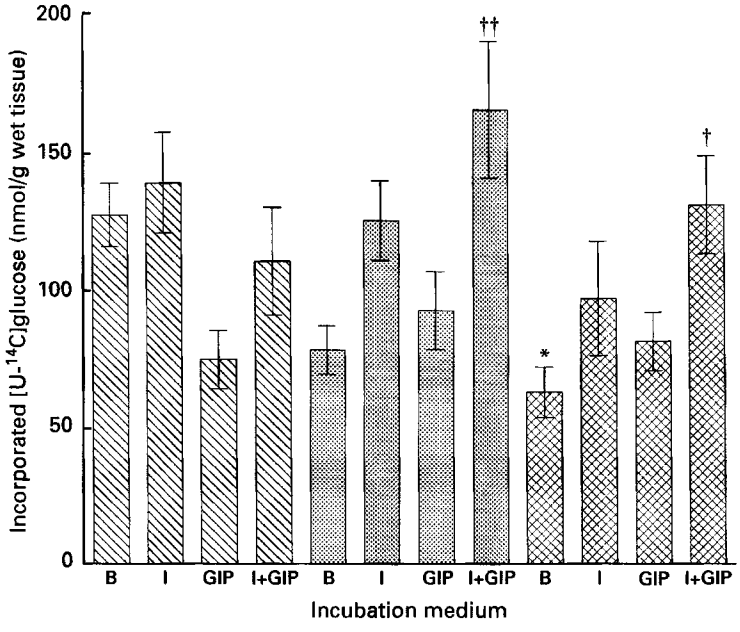

Fig. 3. Effect of dietary fatty acid composition on lipid synthesis by the livers of rats fed on diets containing a mixed oil (▨), maize oil (▩), or fish oil (▩), incubated *in vitro* under different conditions. B, basal incorporation of glucose into hepatic total lipids; I, incubation in the presence of insulin; GIP, incubation in the presence of gastric inhibitory polypeptide; I + GIP, incubation in the presence of insulin and GIP. Values are means for nine rats, with their standard errors indicated by vertical bars. * Mean value was significantly different from B for the mixed-oil group ($P < 0.05$). Mean values were significantly different from B within the same dietary group: † $P < 0.05$, †† $P < 0.01$. For details of diets and procedures, see Table 1 and pp. 382–387.

ANOVA, with Duncan's range test used to locate and assess the level of significance of any differences found. In the case of lipogenesis measurements, logarithmic transformation was carried out to normalize data distribution because of high heteroscadacity. A P value of less than 0·05 was taken as the lowest level of statistical significance.

RESULTS

Average feed intake, body-weight gain, and liver weights

The mean daily feed intakes, weight gain and liver weights after slaughter of rats are given in Table 3 which shows that these values were similar in each of the dietary groups.

Time-response curves and incubations with different hormone concentrations

Incorporation of the label into hepatic total lipids was measured at different incubation times and the maximum incorporation was found in the 3 h incubation (Fig. 1). Maximum incorporation was also observed when liver explants were incubated for 3 h in the presence of 4 nM-insulin (Fig. 2(a)), or 6 nM-GIP (Fig. 2(b)), or in the presence of 4 nM-insulin and 6 nM-GIP in the medium (Fig. 2(c)). These were the concentrations used in the study to investigate hormone-stimulated lipogenesis in the liver.

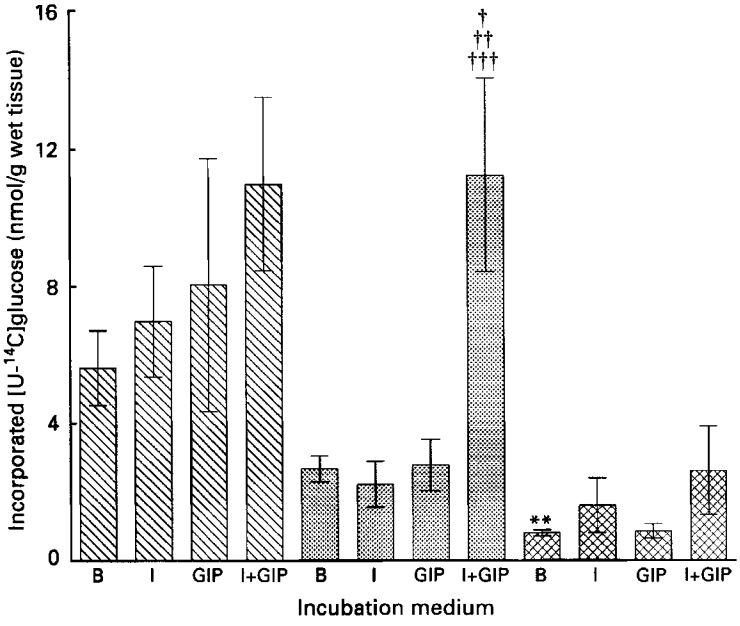

Fig. 4. Effect of dietary fatty acid composition on triacylglycerol (TAG) synthesis by the livers of rats fed on diets containing a mixed oil (▨), maize oil (▩), or fish oil (▩), incubated *in vitro* under different conditions. B, basal incorporation of glucose into hepatic TAG; I, incubation in the presence of insulin; GIP, incubation in the presence of gastric inhibitory polypeptide; I+GIP, incubation in the presence of insulin and GIP. Values are means for seven rats, with their standard errors indicated by vertical bars. ** Mean value was significantly different from B for the mixed-oil group ($P < 0.01$). † Mean value was significantly different from B within the same dietary group ($P < 0.05$). †† Mean value was significantly different from GIP within the same dietary group ($P < 0.05$). ††† Mean value was significantly different from I within the same dietary group ($P < 0.01$). For details of diets and procedures, see Table 1 and pp. 382–387.

Incorporation of [U-^{14}C]glucose into hepatic total lipids

The fish-oil diet significantly reduced the basal levels of incorporation of glucose into hepatic lipids compared with the mixed-oil diet ($P < 0.05$) (Fig. 3). There was also a tendency for basal levels of incorporation to be lower in the maize-oil group than the mixed-oil group, but this did not reach a level of statistical significance.

Insulin alone did not stimulate hepatic lipogenesis in any of the dietary groups, although there was a tendency for levels of lipogenesis to be higher in insulin-treated compared with basal tissue in the maize- and fish-oil groups, which did not reach levels of statistical significance. However, the presence of both hormones together in the incubation medium enhanced the incorporation of glucose in the maize-oil ($P < 0.01$) and fish-oil ($P < 0.05$) dietary groups (Fig. 3). It is notable that GIP alone inhibited incorporation of glucose into total hepatic lipids in the mixed-oil group ($P < 0.01$).

Incorporation of [U-^{14}C]glucose into hepatic triacylglycerol

The fish-oil diet significantly reduced the basal incorporation of glucose into hepatic TAG compared with the mixed-oil diet ($P < 0.01$) (Fig. 4). A similar tendency was observed in the maize-oil group, which did not reach a level of statistical significance.

Insulin and GIP alone did not stimulate incorporation of glucose into hepatic TAG in any dietary group (Fig. 4). However, the presence of both hormones increased the incorporation of the label into hepatic TAG in the maize-oil group ($P < 0.005$) (Fig. 4). In the same dietary group, levels of incorporation in the insulin + GIP-treated tissue were also

Table 4. *Plasma lipid and hormone levels in rats following 2 weeks pair-feeding of diets containing a mixed oil, maize oil or fish oil**

(Mean values and standard deviations for nine rats)

Plasma variable	Mixed-oil diet		Maize-oil diet		Fish-oil diet	
	Mean	SD	Mean	SD	Mean	SD
Cholesterol (mmol/l)	1·18	0·36	1·20	0·23	1·11	0·20
Triacylglycerol (mmol/l)	1·31[a]	0·74	0·89[a]	0·36	0·58[b]	0·19
Insulin (ng/ml)	12·10[c]	8·30	2·60[d]	1·90	1·90[d]	0·90
GIP (pmol/l)	126·30	36·40	130·30	33·00	103·50	21·40

GIP, gastric inhibitory polypeptide.

Mean values within a row not sharing a common superscript letter were significant different: [a,b] $P < 0·05$, [c,d] $P < 0·01$.

* For details of diets and procedures, see Table 1 and pp. 382–387.

significantly higher than in the presence of either insulin ($P < 0·01$) or GIP ($P < 0·05$) alone. Although there was a tendency for higher levels of lipogenesis for insulin+GIP-treated tissue for both mixed- and fish-oil groups, these differences did not reach levels of statistical significance.

Plasma triacylglycerol and cholesterol levels

The fish-oil diet significantly decreased plasma TAG levels compared with both other diets ($P < 0·05$) (Table 4), but plasma total cholesterol levels were similar among the three diets (Table 4).

Plasma insulin and GIP levels

Plasma insulin concentrations were significantly higher in the mixed oil-dietary group than in the other two dietary groups ($P < 0·001$) (Table 4). However, the three diets did not significantly alter plasma GIP concentrations even though there was a tendency for the plasma GIP levels to be lower in the fish-oil group than in the other two dietary groups (Table 4), although this did not reach a level of statistical significance.

DISCUSSION

The main findings of the present study were lower rates of incorporation of glucose into hepatic total lipids and hepatic TAG in the fish-oil dietary group compared with the mixed-oil dietary group. Responses of hepatic lipogenesis to the addition of hormones also differed between the three dietary groups with increased rates of incorporation of glucose into hepatic total lipids in the presence of insulin and GIP (together) observed in both the fish-oil and maize-oil groups, but not in the mixed-oil group. There were also increased rates of incorporation into hepatic TAG compared with basal in the presence of both hormones in the maize-oil group and similar non-significant trends in the mixed- and fish-oil groups. Furthermore, significantly lower plasma TAG levels were observed in the fish-oil group compared with both other groups. A notable observation was also the markedly higher plasma insulin concentrations seen in the mixed-oil group compared with both other groups.

From the beginning of the pair-feeding periods the average daily feed intakes and weight gains were similar in all dietary groups and, as a result, differences observed in the glucose

incorporation into hepatic lipids, as well as differences in plasma TAG and insulin levels, between the dietary groups cannot be credited to different feed intakes or to different growth rates in the three dietary groups.

The results obtained in the present study suggest that the decrease in plasma TAG, seen in the fish-oil-fed animals, can in part be attributed to a decrease in hepatic total lipid synthesis. Indeed, the effects of dietary fatty acids on rates of hepatic total lipid and TAG synthesis were in agreement with their effects on circulating TAG concentrations with mixed oil > maize oil > fish oil. This conclusion is in general agreement with other workers' findings (Haug & Hostmark, 1987) and suggests that a reduction in hepatic synthesis is a mechanism by which fish oils produce their circulating-TAG-lowering effect. In contrast with our findings, Lakshman *et al.* (1988) observed a decrease in plasma TAG but no effect on liver TAG contents in rats given fish-oil diets. In the present study the incorporation of the label into the TAG fraction was unexpectedly much lower than into total lipids for each of the dietary groups. This suggests that label was incorporated into other lipid classes such as phospholipids, cholesterol ester and free fatty acids. However, measurement of incorporation into these lipid fractions was not included as part of this study, so that no definite conclusions regarding these surprising results can be drawn.

The suppression of hepatic lipogenesis observed in the present study could be a result of direct influence of *n*-3 PUFA on lipogenic enzyme activities in the liver, mainly acetyl CoA carboxylase (*EC* 6.4.1.2), diacylglycerol acyltransferase (*EC* 2.3.1.20) and phosphatidic acid phosphohydrolase (*EC* 3.1.3.4). These enzymes are involved not only in fatty acid synthesis (acetyl CoA carboxylase) but also in the synthesis of TAG in the small intestine (diacylglycerol acyltransferase), and in the liver (diacylglycerol acyltransferase, phosphatidic acid phosphohydrolase). In addition to effects of *n*-3 fatty acids on enzyme activities, increases in membrane fluidity by changes in its fatty acid composition due to the *n*-3 PUFA-enriched diet have also been observed (Iritani *et al.* 1980; Marsh *et al.* 1987; Rustan *et al.* 1988; Muriana *et al.* 1992). The present study confirms previous findings that *n*-3 fatty acids decrease hepatic TAG synthesis, but more work should be carried out to investigate the effects of these fatty acids on both the glycerol and fatty acid moieties of TAG.

However, *n*-3 PUFA may also decrease circulating TAG levels through effects on lipoprotein lipase (LPL; *EC* 3.1.1.34) activity and thereby enhance the removal of both chylomicron- and VLDL-TAG. This mechanism may be particularly pertinent within the context of the present study which was carried out with animals in the fed state, unlike many previous studies which have investigated animals fasted for periods of up to 24 h. Adipose tissue LPL activity and LPL mRNA levels were measured in some of the animals of each dietary group of the present study and these results have been previously reported (Murphy *et al.* 1993). Although there was a tendency for post-heparin adipose tissue LPL activity to be higher in fish-oil-fed animals, this difference was not statistically significant. However, LPL mRNA was elevated in fish-oil-fed animals suggesting increased synthesis of the enzyme. Therefore, these results together suggest that *n*-3 PUFA produce modulatory effects on both hepatic and peripheral tissue lipid metabolism and lower plasma TAG levels in fish-oil-fed animals reflecting both decreased production and increased clearance of hepatic and/or intestinally derived lipoproteins.

The present study found insulin concentrations to be lower in both PUFA-fed groups and there was also a tendency for GIP levels to be lower in the fish-oil group. Although caution must be applied in interpreting the significance of single time-point measurements of these hormones, the possibility cannot be excluded that lower circulating concentrations of these hormones before slaughter in PUFA-fed animals contributed to the lower rates of lipogenesis measured *in vitro*.

With respect to effects of the lipogenic hormones, the findings of the present study are important, since they show, for the first time, stimulatory effects of insulin and GIP together, on lipogenesis, and suggest that the secretion of GIP in response to dietary fat may have important physiological effects on the subsequent processing of endogenous and exogenous lipid substrates in the liver. However, further work is needed to confirm and extend the findings reported here. The results are of particular interest because of other observations which show that whilst GIP has little effect on LPL activity in the absence of insulin, it potentiates insulin action when added to incubation media at the same concentrations as used in the present study (Knapper *et al.* 1993). Therefore, this potentiating effect of GIP on insulin, previously observed in adipose tissue, may apply to hepatic actions of the hormone as well. The mechanism for the enhanced lipogenic responses in the presence of insulin and GIP require further study. It is speculated that dietary-induced alteration of membrane phospholipid composition, in combination with the GIP in the incubation medium, may produce altered effects of insulin on hepatic lipogenesis.

Another potentially important finding of the present study was the significantly higher levels of insulin found in mixed-oil-fed animals compared with both groups of animals fed on PUFA-rich diets. Further, more detailed, measurements of postprandial insulin concentrations in animals fed on diets of varying fatty acid compositions are required to confirm these findings. However, a possible interpretation of these results is that mixed-oil-fed animals were developing a degree of insulin resistance and this may relate to effects of dietary fatty acids on membrane phospholipid fatty acid composition (Ginsberg *et al.* 1982; Tepperman & Tepperman, 1985).

In conclusion, a fish-oil diet decreased hepatic lipogenesis and postprandial plasma TAG levels compared with a diet rich in SFA, and insulin and GIP together had a stimulatory effect on hepatic lipogenesis. In addition, the diet rich in SFA markedly increased the postprandial plasma insulin levels.

REFERENCES

Ahrens, E. H., Hirsch, J., Insull, W., Tsaltas, T. T., Blomstrand, R. & Peterson, M. L. (1957). The influence of dietary fats on serum-lipid levels in man. *Lancet* **1**, 943–953.

Beck, B. (1989). Gastric inhibitory polypeptide: a gut hormone with anabolic functions. *Journal of Molecular Endocrinology* **2**, 169–174.

Begum, N., Tepperman, H. M. & Tepperman, J. (1982). Effect of high fat and high carbohydrate diets on adipose tissue pyruvate dehydrogenase and its activation by a plasma membrane-enriched fraction and insulin. *Endocrinology* **110**, 1914–1921.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total extraction and purification of lipids. *Canadian Journal of Physiology* **37**, 911–917.

Deck, C. & Radack, K. (1989). Effects of modest doses of omega-3 fatty acids on lipids and lipoproteins in hypertriglyceridemic subjects. *Archives of Internal Medicine* **149**, 1857–1862.

Falko, J. M., Crockett, S. E., Cataland, S. & Mazzaferri, E. L. (1975). Gastric inhibitory polypeptide (GIP) stimulated by fat ingestion in man. *Journal of Clinical Endocrinology and Metabolism* **41**, 260–265.

Gibbons, G. F. (1990). Assembly and secretion of hepatic very-low density lipoprotein. *Biochemical Journal* **268**, 1–13.

Ginsberg, B. H., Jabour, J. & Spector, A. A. (1982). Effect of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. *Biochimica et Biophysica Acta* **690**, 157–164.

Harris, W. S., Connor, W. E., Alam, N. & Illingworth, D. R. (1988). Reduction of postprandial triglyceridemia in humans by dietary *n*-3 fatty acids. *Journal of Lipid Research* **29**, 1451–1460.

Hartmann, H., Ebert, R. & Creutzfeldt, W. (1986). Insulin-dependent inhibition of hepatic glycogenolysis by gastric inhibitory polypeptide (GIP) in perfused rat liver. *Diabetologia* **29**, 112–114.

Haug, A. & Hostmark, A. T. (1987). Lipoprotein lipases, lipoproteins and tissue lipids in rats fed fish oils or coconut oil. *Journal of Nutrition* **117**, 1011–1017.

Iritani, N., Fukuda, E., Inoguchi, K., Tsubosaka, M. & Tashiro, S. (1980). Reduction of lipogenic enzymes by shellfish triglycerides in rat liver. *Journal of Nutrition* **110**, 1664–1670.

Knapper, J. M. E., Puddicombe, S. M., Morgan, L. M., Fletcher, J. M. & Marks, V. (1993). Glucose dependent insulinotropic polypeptide and glucagon-like peptide-1(7-36) amide: effects on lipoprotein lipase activity. *Biochemical Society Transactions* **21**, 135S.

Lakshman, M. R., Chirtel, S. J. & Chambers, L. L. (1988). Roles of ω-3 fatty acids and chronic ethanol in the regulation of plasma and liver lipids and plasma apoproteins A_1 and E in rats. *Journal of Nutrition* **118**, 1299–1303.

Marsh, J. B., Topping, D. L. & Nestel, P. J. (1987). Comparative effects of dietary fish oil and carbohydrate on plasma lipids and hepatic activities of phosphatidate phosphohydrolase, diacylglycerol acyltransferase and neutral lipase activities in the rat. *Biochimica et Biophysica Acta* **922**, 239–243.

Morgan, L. M., Morris, B. A. & Marks, V. (1978). Radioimmunoassay of gastric inhibitory polypeptide. *Annals of Clinical Biochemistry* **15**, 172–177.

Muriana, F. J. G., Ruiz-Gutierrez, V. & Vazquez, C. M. (1992). Influence of dietary cholesterol on polyunsaturated fatty acid composition, fluidity and membrane-bound enzymes in liver microsomes of rats fed olive and fish oil. *Biochimie* **74**, 551–556.

Murphy, M. C., Zampelas, A., Puddicombe, S. M., Furlonger, N. P., Morgan, L. M. & Williams, C. M. (1993). Pretranslational regulation of the expression of the lipoprotein lipase (*EC* 3.1.1.34) gene by dietary fatty acids in the rat. *British Journal of Nutrition* **70**, 727–736.

Paul, A. A. & Southgate, D. A. T. (1978). *McCance and Widdowson's The Composition of Foods*, 4th ed. London: H.M. Stationery Office.

Rustan, A. C., Nossen, J. O., Christiansen, E. N. & Drevon, C. A. (1988). Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A: 1,2-diacylglycerol acyltransferase. *Journal of Lipid Research* **29**, 1417–1426.

Sanders, T. A. B. & Roshanai, F. (1983). The influence of different types of omega-3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. *Clinical Science* **64**, 91–99.

Shepherd, J., Packard, C. J., Grundy, S. M., Yeshurun, D., Gotto, A. M. & Taunton, O. D. (1980). Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. *Journal of Lipid Research* **21**, 91–99.

Tepperman, H. M. & Tepperman, J. (1985). Membranes and the response to insulin. *Proceedings of the Nutrition Society* **44**, 211–220.

Triscari, J., Hamilton, J. G. & Sullivan, A. C. (1978). Comparative effects of saturated and unsaturated lipids on hepatic lipogenesis and cholesterogenesis *in vivo* in the meal-fed rat. *Journal of Nutrition* **108**, 815–825.

Printed in Great Britain