# Effect of dietary fish oil on the sensitivity of hepatic lipid metabolism to regulation by insulin

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**Abstract** **The contribution of dietary fat content and type to changes in the sensitivity of hepatic lipid metabolism to insulin was studied in primary hepatocyte cultures from donor rats maintained on a low-fat diet (LF), or on diets enriched in olive oil (OO) or fish oil (FO). The higher rate of fatty acid oxidation in hepatocytes from the FO-fed group was resistant to the inhibitory effects of insulin observed in hepatocytes from the other groups. Insulin stimulation of fatty acid incorporation into triglyceride (TG) was also less pronounced in hepatocytes from the FO-fed group than in those from the OO-fed group but there was no difference in the stimulatory effect of insulin on fatty acid incorporation into phospholipid (PL) in these two groups. In the case of fatty acid incorporation into both PL and TG, hepatocytes from the LF group were refractory to stimulation by insulin. At each concentration of insulin, hepatocytes from the FO-fed group secreted less very low density lipoprotein (VLDL) TG than those from the other groups. However, the absolute suppression of VLDL TG secretion by insulin was similar irrespective of the diet of the donor animals. We conclude that chronic consumption of a particuar type of dietary fat does not affect the insulin sensitivity of the major pathways of hepatic lipid metabolism in a consistent manner.**—Baker, P. W., and G. F. Gibbons. **Effect of dietary fish oil on the sensitivity of hepatic lipid metabolism to regulation by insulin.** ***J. Lipid Res.*** **2000.** 41: **719–726.**

**Supplementary key words** fish oil • olive oil • insulin • very low density lipoprotein • hepatocyte culture • fatty acid metabolism • triglyceride • phospholipid • oxidation

Insulin plays a major role in the regulation of carbohydrate and lipid metabolism in muscle, adipose tissue, and the liver. A diminished effectiveness of insulin action, commonly referred to as insulin resistance, leads to perturbations in carbohydrate and lipid metabolism which are commonly associated with chronic hyperlipidemia and hyperinsulinemia, and an increased risk of coronary artery disease. An important contribution to the hyperlipidemia in insulin-resistant states, such as obesity and non-insulin-dependent diabetes mellitus, is an increased secretion of very low density lipoprotein (VLDL) triglyceride (TG) from the liver (1, 2). It is now generally accepted that in insulin-sensitive subjects acute hyperinsulinemia suppresses the hepatic secretion of VLDL TG (3, 4). It is unclear, however, whether in insulin-resistant subjects the increased secretion of VLDL TG is the result of an inability of insulin to regulate VLDL TG secretion subsequent to the chronic hyperinsulinemia, and so this remains a controversial area (4).

The inhibitory effect of insulin on VLDL TG secretion in vivo is due partly to an acute suppression of non-esterified fatty acid (NEFA) flux from adipose tissue to the liver, thereby reducing the availability of substrate for TG production (3, 4). Importantly, however, insulin has been shown to have a more direct inhibitory effect on VLDL TG and apoB secretion, independent of any reduction in the levels of TG precursor reaching the liver (1, 3). This direct effect has also been shown in vitro using isolated hepatocytes (for reviews see refs. 5, 6).

Many other aspects of hepatic lipid metabolism including fatty acid oxidation, lipogenesis, and glycerolipid synthesis are also subject to regulation by insulin (for reviews see refs. 7–9). High dietary intakes of certain fatty acids, such as those found in olive oil, result in severe insulin resistance (10, 11). Isocaloric substitution of these fatty acids with fish oil, however, protect against insulin resistance (10, 12) and also reduce VLDL secretion (for review see ref. 13). This latter effect has been shown to be due, at least in part, to the direct suppression of VLDL apoB by eicosapentaenoic acid (EPA) (14). Little is known, however, of whether, and if so how, such dietary-induced changes in insulin sensitivity affect the insulin-dependent regulation of those aspects of hepatic lipid metabolism described above. Primary cultures of hepatocytes retain for some time the metabolic characteristics of the donor animals (15). For this reason, we have used hepatocytes from rats fed diets rich in olive oil (OO) or fish oil (FO) to

Abbreviations: VLDL, very low density lipoprotein; apo, apolipoprotein; TG, triglyceride; PL, phospholipid; NEFA, non-esterified fatty acids; DGAT, diacylglycerol acyltransferase; CoA, coenzyme A; CPT-I, carnitine palmitoyltransferase I; ACC, acetyl-CoA carboxylase; DG, diacylglycerol; EPA, eicosapentaenoic acid.

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study the effects of these dietary manipulations on the hepatic response of *i*) fatty acid esterification into TG and phospholipid (PL), *ii*) fatty acid oxidation, *iii*) TG storage, and *iv*) VLDL TG secretion, to a direct insulin challenge in vitro. We have used hepatocytes from animals fed a low-fat (LF) diet as a benchmark for insulin sensitivity.

## METHODS

### Materials

All reagents were of analytical grade. Waymouth's medium and Dulbecco's phosphate-buffered saline (PBS) were obtained from Gibco Ltd. (Paisley, Scotland, U.K.), [9,10(n)-$^3$H]oleate from Amersham International (Aylesbury, Buckinghamshire, U.K.), and dexamethasone, bovine insulin, and sodium oleate were from Sigma (Poole, Dorset, U.K.). MaxEPA was kindly supplied by Seven Seas Ltd. (Kingston-upon-Hull, U.K.). Filippo Berio Extra Virgin Olive Oil was used for this study.

### Maintenance of animals and dietary regime

Male rats of the Wistar strain (140–170 g) were housed individually in wire-bottomed cages in a room lit artificially between 08:00 h and 18:00 h. The room temperature was kept constant at 20 ± 2°C. Rats were randomly allocated and fed either a low-fat diet or diets enriched with 18% (w/w) olive oil (76% C18:1, n–9) or 18% (w/w) fish oil (MaxEPA; 18% C20:5 and 12% C22:6, both n–3). These diets were designated LF, OO, and FO, respectively, and were fed ad libitum for 14 days before preparation of hepatocytes. Food was replaced every 24 h and any remaining pellets were discarded. Food intake and body weight were monitored daily. The LF diet was Rat and Mouse No.3 Breeding Diet from Special Diet Service (Witham, Essex, U.K.). This diet contained 4.3% fat, 51.2% carbohydrate (mainly starch), 22.3% protein, 4.5% fiber, and 7.7% ash (w/w). The OO and FO diets were prepared by mixing 180 g of the respective oil with 800 g of powdered LF diet and 400 ml of deionized water. After thorough mixing, the diets were re-pelleted and allowed to dry in a well-ventilated area with subdued light. All diets contained the following anti-oxidants; α-tocopherol (110 mg/kg), vitamin E (120 mg/kg), vitamin C (10 mg/kg), and β-carotene (200 μg/kg). After preparation of the diets, Folch extraction (16) followed by *trans*-methylation of the fatty acids and quantitative GC analysis indicated that these anti-oxidants were sufficient to prevent oxidation of the n–3 fatty acids during preparation. The OO and FO diets were kept frozen at −20°C until use. The LF diet contained 3.66 cal/g while the OO and FO diets contained 4.69 cal/g.

### Hepatocyte isolation and culture procedures

Hepatocytes were prepared at 10:00 h under sterile conditions and were plated out onto 60-mm dishes coated with rat tail collagen (17) as a suspension ($0.65 \times 10^6$ cells/ml, 3 ml/dish) in Waymouth's medium MB752/1 supplemented with 10% fetal calf serum, penicillin (100000 U/l), streptomycin (100 mg/l), and added amino acids (3.60 mm glutamine, 0.36 mm alanine, and 0.45 mm serine). Initial cell viability as assessed by Trypan Blue exclusion was 87 to 92%. There were no differences in viability between hepatocytes isolated from animals fed the LF, OO, or FO diets. The initial cell plating densities were 1.67 ± 0.13, 1.81 ± 0.22, and 1.81 ± 0.20 $\times 10^6$ cells/dish with corresponding protein and DNA levels, after the 4-h attachment period, of 0.75 ± 0.03, 0.92 ± 0.07, and 1.18 ± 0.06 mg/dish and 19.6 ± 2.9, 19.6 ± 2.2, and 20.7 ± 1.7 μg/dish for hepatocytes isolated from the LF-, OO-, and FO-fed groups, respectively. Hence, there were no significant differences in plating efficiency between the groups. Due to the significant increase in the amount of protein per cell in hepatocytes isolated from the OO- and FO-fed groups, all data are expressed per dish (DNA was not measured for every data point and so it could not be used to normalize data). After 4 h, the serum-containing medium was removed and the cell-monolayer was washed twice with PBS. Serum-free Waymouth's medium (3 ml) containing the above amino acid and antibiotic supplements, dexamethasone (1 μm), pyruvate (1 mm), and lactate (10 mm), was added and the cells were cultured either in the absence or presence of oleate (750 μm or 2250 nmol; 830 dpm/nmol of [9,10(n)-$^3$H]oleate) bound to fatty acid-free albumin (75 μm). Albumin alone (75 μm) was added to cells that were cultured in the absence of oleate. Insulin was tested at initial concentrations of 0.5, 7.8, 78, and 780 nm.

### Measurement of cellular and VLDL lipids

At the end of the 24-h culture, the medium was removed, the cells were harvested, and the secreted VLDL was isolated from the medium by ultracentrifugation ($d < 1.006$ g/ml) as previously described (18). Cellular and VLDL lipids were extracted by Folch extraction (16), dried under nitrogen, and reconstituted in ethanol (500 μl). To account for losses during Folch extraction [$^{14}$C]oleate was added to each sample, prior to extraction, as an internal standard. From the lipid extracts triglyceride (TG) mass was determined using the GPO-PAP kit from Boehringer Mannheim (Lewes, U.K.). Cellular and VLDL TG and PL were separated, after Folch extraction, by thin-layer chromatography on silica plates with n-hexane–diethyl ether–glacial acetic acid 70:30:1.6 (v/v/v) (18). After chromatographic separation, TG and PL bands were visualized using Rhodamine 6G, scraped into scintillation pots, and incorporation of $^3$H was determined to enable calculation of the rate of incorporation of exogenous [$^3$H]oleate into newly synthesized TG and PL.

### Other methods

Cellular protein was determined colorimetrically by the method of Lowry et al. (19). DNA was determined colorimetrically by the method of Burton (20). Folch extraction (16) of the VLDL infranate followed by scintillation counting of an acidified aliquot of the aqueous phase was used to determine the extent of oxidation of [9,10(n)-$^3$H]oleate. This phase contains the water-soluble lipid oxidation products such as water, acetate, β-hydroxybutyrate, acetoacetate, and other small molecular weight products. The VLDL infranate is the fraction of medium ($d >$ 1.006 g/ml) obtained after flotation of VLDL ($d < 1.006$) by centrifugation. Similarly, scintillation counting of an aliquot of the organic phase of the VLDL infranate was used to determine the amount of [9,10(n)-$^3$H]oleate remaining, and, thus, by subtraction, the extent of cellular oleate uptake. There was little, if any, esterified lipid in this fraction. All plasma measurements were made on plasma obtained by cardiac puncture. NEFA was measured using the Wako NEFA C ACS-ACOD kit supplied by Alpha Laboratories (Eastleigh, Hampshire, U.K.). Glucose was determined enzymatically using hexokinase and glucose-6-phosphate dehydrogenase as described by Kunst, Draeger, and Riegenhorn (21). Ketone bodies (i.e., acetoacetate and β-hydroxybutyrate) were determined enzymatically as described by Williamson, Mellanby, and Krebs (22). Insulin was determined radiometrically using a kit supplied by Pharmacia and Upjohn (Knowlhill, Milton Keynes, Buckinghamshire, U.K.). Catalase activity was measured in freshly isolated hepatocytes as described by Baudhuin (23). Adipose tissue was collected from the intraperitoneal and subcutaneous depots. VLDL apoB was determined by ELISA as described previously (24), this method measures both apoB-100 and apoB-48.

### Statistical analysis

All statistical tests (two sample *t*-tests: assuming equal variance and ANOVA: two-factor with repeated measures where indicated) were performed using the data analysis package in Microsoft Excel for Windows 95, Version 7.0. Significance was determined at the 95% confidence interval, i.e., $P < 0.05$; *a* and *b* refer to a significant change from the LF- or OO-fed groups, respectively (ANOVA) and *c* refers to a significant change from initial cellular TG levels (*t*-test). ANOVA was performed by treating all points within a data set as a group; points were not treated individually.

## RESULTS

### Effects on body mass, adiposity, and plasma biochemistry

Animals were given unrestricted access to food and water. The daily energy intake in the FO- and OO-fed groups (117 ± 3 and 111 ± 3 cal/day, respectively) was higher than that in the LF-fed group (95 ± 2 cal/day), despite similar food intakes (25 g/day). This was due to the increased fat content of the FO and OO diets. Body mass gain in the FO-fed group, however, was lower than that in the OO-fed group (**Table 1**). Similarly, at the end of the 14-day feeding period, the total mass of subcutaneous and intraperitoneal adipose tissue in the FO-fed group was lower than that in the OO-fed group and comparable to that in the LF-fed group (Table 1). These observations suggest that FO-feeding shifts the balance of whole body metabolism towards a more catabolic state. The hepatic activity of catalase was higher in the FO-fed group than in the LF- or OO-fed groups (Table 1). A significant hyperketonemia was observed in the FO-fed group while no changes in plasma glucose were observed in either of the groups (Table 1). Significant relationships were observed between plasma insulin and TG ($P < 0.02$; $r^2 = 0.43$) and plasma insulin and NEFA ($P < 0.001$; $r^2 = 0.67$) with the FO-fed group being intermediate between the LF- and OO-fed groups (Table 1).

### Effects on VLDL TG secretion

The basal rates of VLDL TG secretion and both the absolute and fractional suppression of these rates at various concentrations of insulin are shown in **Fig. 1**. In the absence of insulin, hepatocytes from the FO-fed group secreted less TG than those from the LF- or OO-fed groups (Fig. 1, upper panel). In absolute terms, the maximal suppression of VLDL TG secretion by insulin in all groups was similar (approximately 22 ± 1 nmol/dish/24 h), although the fractional suppression was greater in hepatocytes from the FO-fed group compared to the OO-fed group, with the effect being intermediate in hepatocytes from the LF-fed group (52 ± 7, 26 ± 7, and 36 ± 9%, respectively) (Fig. 1, lower panel). The increased fractional suppression by insulin in the FO-fed group, however, may be more a function of the lower initial rate of VLDL TG secretion than perhaps an actual increase in the sensitivity of this process to inhibition by insulin. Nevertheless, at each concentration of insulin, hepatocytes from the FO-fed group secreted significantly less VLDL TG (Fig. 1, upper panel). In contrast to the marked effects of insulin on VLDL TG secretion, insulin reduced the fractional secretion of VLDL apoB to a lesser extent (19 ± 8% on average, data not shown). It is of interest however, that in the absence of insulin hepatocytes from the FO- and OO-fed groups secreted less VLDL apoB than those from the LF-fed group (3.77 ± 0.77 and 4.01 ± 0.36 vs. 5.12 ± 0.33 μg/dish, respectively).

### Effects on exogenous oleate esterification into TG and PL

The basal rates of incorporation of exogenous [$^3$H]oleate into TG and PL and the effect of insulin on these rates are shown in **Fig. 2** and **Fig. 3**. These parameters were investigated by adding exogenous [$^3$H]oleate to the culture media as described in the Methods. The hepatic uptake of [$^3$H]oleate was the same for all groups: 90 ± 1% of the [$^3$H]oleate added to the media or 2025 nmol of fatty acid.

The basal rate of oleate incorporation into TG was 40 to 50% lower in hepatocytes from the FO-fed group than from the OO-fed group (Fig. 2, upper panel). The ability of insulin to stimulate this rate in the FO-fed group was identical to that observed in the LF-fed group and considerably lower than that observed in the OO-fed group (Fig. 2, lower panel). In contrast, the basal rate of oleate incorporation into PL was 20 to 30% higher in the FO-fed

TABLE 1. Effect of dietary fat composition on body mass, adiposity, and plasma biochemistry

| Parameter | LF-Fed | OO-Fed | FO-Fed |
|---|---|---|---|
| Body mass gain (g/day) | 6.04 ± 0.26 | 6.93 ± 0.18[a] | 6.63 ± 0.22 |
| Adipose tissue (% of body mass) | 4.88 ± 0.22 | 6.42 ± 0.34[a] | 5.13 ± 0.11[b] |
| Hepatic catalase (nmol/min/μg protein) | 124 ± 9 | 141 ± 20 | 190 ± 13[a,b] |
| Plasma TG (mg/dl) | 37.9 ± 1.2 | 88.5 ± 12.9[a] | 40.9 ± 7.4[b] |
| Plasma insulin (μU/ml) | 12.7 ± 0.7 | 21.32 ± 2.0[a] | 16.1 ± 2.4[a] |
| Plasma NEFA (mm) | 0.68 ± 0.07 | 1.04 ± 0.17[a] | 0.86 ± 0.09 |
| Plasma ketone bodies (μm) | 255 ± 2 | 251 ± 32 | 558 ± 116[a,b] |
| Plasma glucose (mm) | 12.3 ± 0.5 | 11.2 ± 0.7 | 11.6 ± 0.8 |

A low-fat diet (LF) or diets enriched in fish oil (FO) or olive oil (OO) were fed for 14 days. At the end of this period blood was obtained by cardiac puncture and tissues were removed. Plasma was isolated by centrifugation at 3000 rpm for 10 min. Body mass was recorded daily. Adipose tissue refers to the mass of the sum of the subcutaneous and interperitoneal adipose depots. Plasma ketone bodies refers to the sum of acetoacetate and β-hydroxybutyrate. Results are expressed as mean ± SEM (n = 4).

[a,b] Significant difference from the LF- or OO-fed groups, respectively.

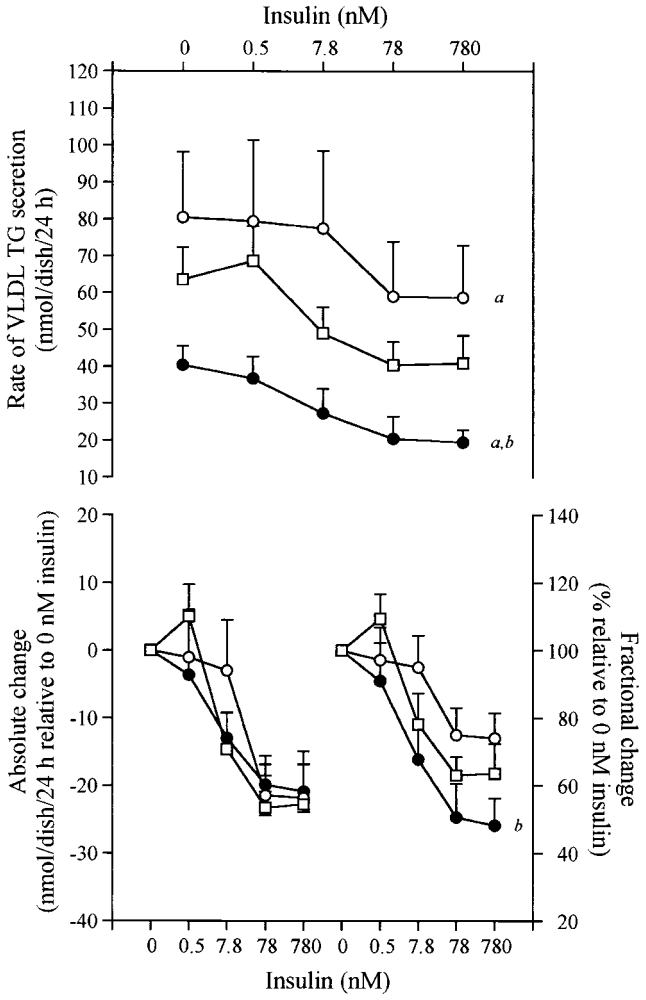


**Fig. 1.** Effect of dietary fat type and content on the rate of hepatic VLDL TG secretion (upper panel) and its sensitivity to the inhibitory effects of insulin (lower panels). A low-fat diet (LF) or diets enriched in fish oil (FO) or olive oil (OO) were fed for 14 days before preparation of hepatocytes. Hepatocytes ($0.65 \times 10^6$ cells/ml, 3 ml/dish) were cultured for 24 h. Insulin was present initially at the concentrations indicated. At the end of the culture period, the medium was removed, the secreted VLDL were isolated by density centrifugation, and VLDL TG was determined enzymatically after Folch extraction as described in Materials and Methods. Results are expressed as mean ± SEM (n = 6 for LF and n = 5 for OO and FO); LF (□), OO (○), and FO (●). *a* and *b* refer to a significant difference from the LF- or OO-fed groups, respectively (ANOVA).

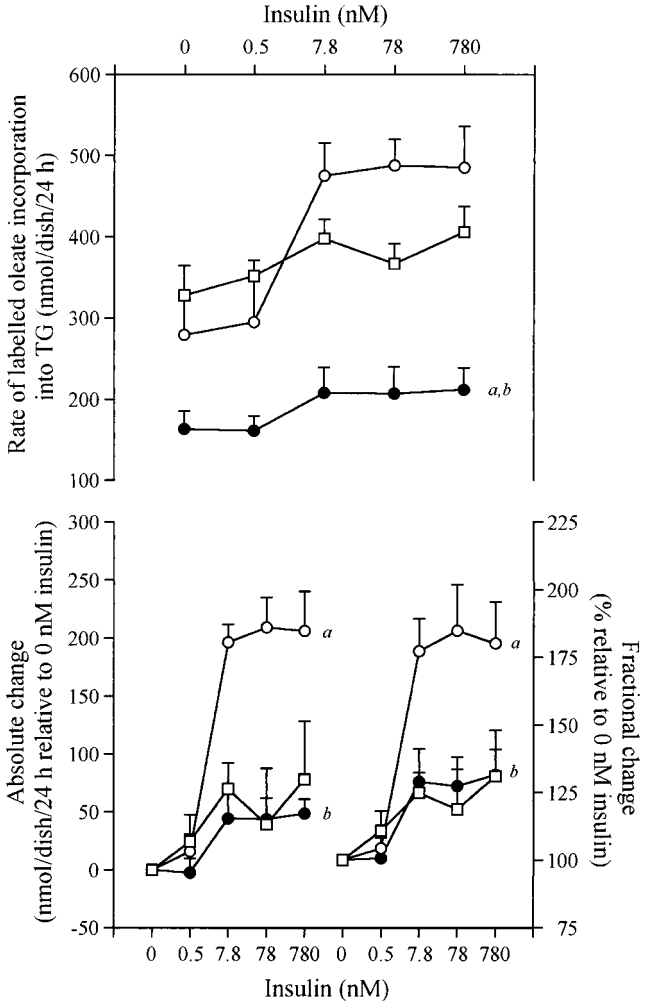


**Fig. 2.** Effect of dietary fat type and content on the rate of incorporation of exogenous [$^3$H]oleate into newly synthesized TG (upper panel) and the sensitivity of this rate to the stimulatory effects of insulin (lower panels). Hepatocytes ($0.65 \times 10^6$ cells/ml, 3 ml/dish) were cultured for 24 h in the presence of exogenous oleate (0.75 mm or 2250 nmol, 830 dpm/nmol of [9,10(n)-[$^3$H]oleate) bound to fatty acid-free albumin (75 μm). Insulin was present initially at the concentrations indicated. At the end of the culture period, the medium was removed, the cells were harvested, and the secreted VLDL were isolated by density centrifugation. The incorporation of exogenous [$^3$H]oleate into newly synthesized TG, both cellular and VLDL, was determined by scintillation counting after Folch extraction and thin-layer chromatography on silica plates as described in Materials and Methods. Results are expressed as mean ± SEM (n = 6 for LF and n = 5 for OO and FO); LF (□), OO (○), and FO (●). *a* and *b* refer to a significant difference from the LF- or OO-fed groups, respectively (ANOVA).

group than the OO-fed group (Fig. 3, upper panel) and the effect of insulin on this rate in the FO-fed group mirrored that observed in the OO-fed group, which was higher than that observed in the LF-fed group (Fig. 3, lower panel). It is important to recognize at this point that the incorporation of oleate into PL represents only a small proportion (10 to 20%) of its total incorporation into both TG and PL. In combination, therefore, the basal rate of incorporation of oleate into both TG and PL was considerably lower in the FO-fed group than in the OO- and LF-fed groups (209 ± 23, 314 ± 40, and 363 ± 35 nmol/dish/24 h, respectively). Despite this, however, the ability of insulin to stimulate this rate was similar in the FO- and LF-fed groups, and considerably lower than that observed in the OO-fed group (data not shown but can be deduced from Figs. 2 and 3). Thus, FO-feeding maintained a relatively normal sensitivity to stimulation by insulin of the incorporation of exogenous oleate into glycerolipids.

### Effects on exogenous oleate oxidation

Recovery of water-soluble products of [$^3$H]oleate oxidation after Folch extraction of culture media enabled a de-

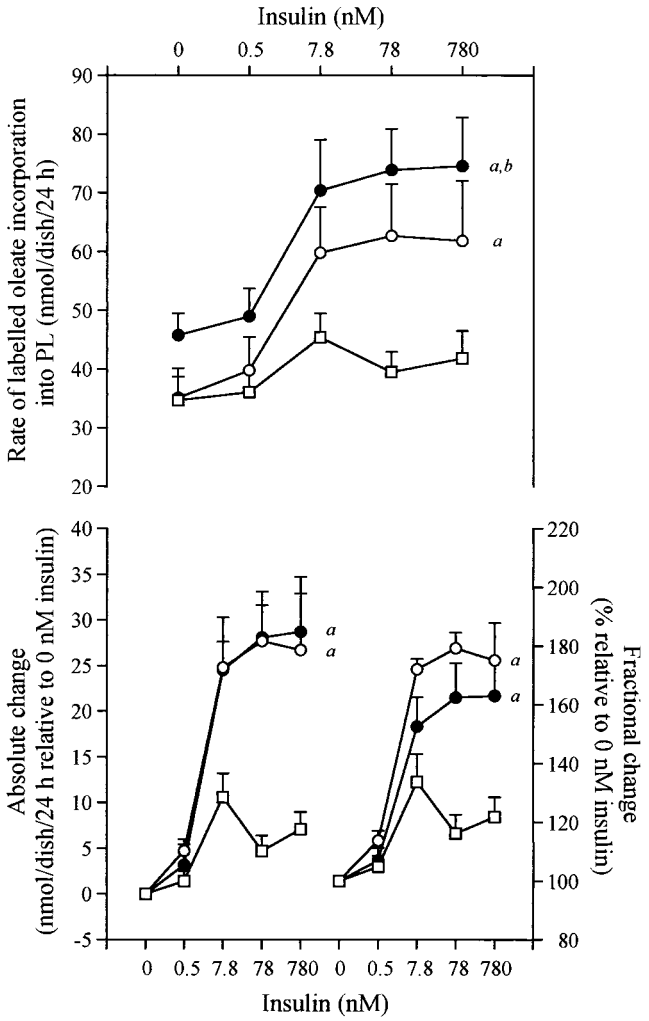


**Fig. 3.** Effect of dietary fat type and content on the rate of incorporation of exogenous [$^3$H]oleate into newly synthesized PL (upper panel) and the sensitivity of this rate to the stimulatory effects of insulin (lower panels). Incubation conditions, harvesting of cells, isolation of VLDL, and determination of incorporation of exogenous [$^3$H]oleate into newly synthesized PL, both cellular and VLDL, are described in the legend to Fig. 2. Results are expressed as mean ± SEM (n = 6 for LF and n = 5 for OO and FO); LF (□), OO (○), and FO (●). *a* and *b* refer to a significant difference from the LF- or OO-fed groups, respectively (ANOVA).

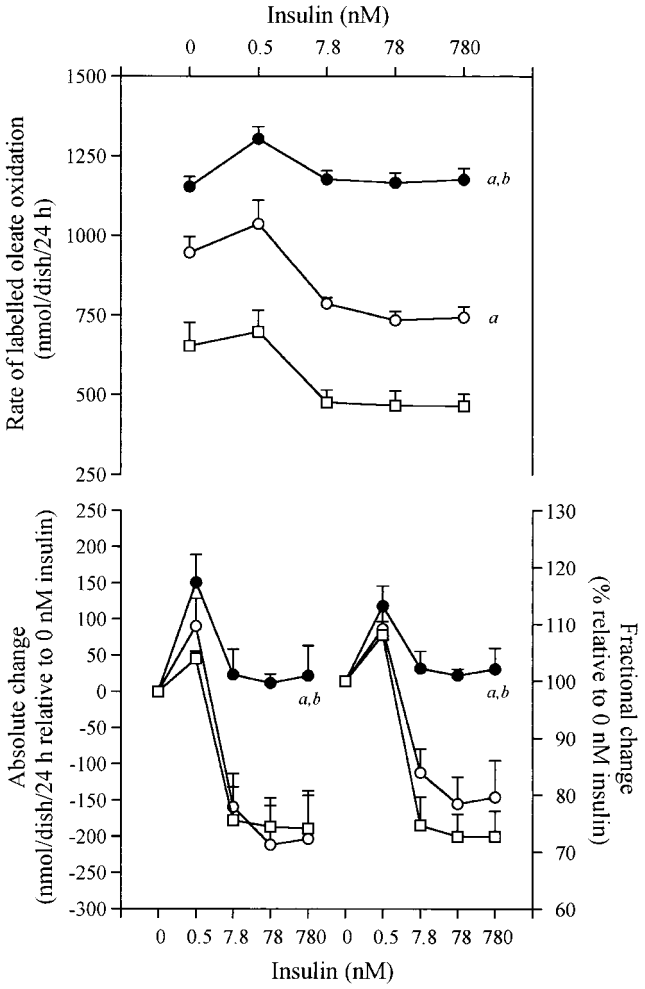


**Fig. 4.** Effect of dietary fat type and content on the rate of oxidation of exogenous [$^3$H]oleate (upper panel) and the sensitivity of this rate to the inhibitory effects of insulin (lower panel). Incubation conditions are described in the legend to Fig. 2. Folch extraction of the VLDL infranate followed by scintillation counting of an acidified aliquot of the aqueous phase was used to determine the extent of [$^3$H]oleate oxidation as described in Materials and Methods. Results are expressed as mean ± SEM (n = 6 for LF and n = 5 for OO and FO); LF (□), OO (○), and FO (●). *a* and *b* refer to a significant difference from the LF- or OO-fed groups, respectively (ANOVA).

termination of the overall rate of oxidation of exogenous [$^3$H]oleate as described in the Methods. The rates of conversion of exogenous [$^3$H]oleate to water-soluble $^3$H oxidation products and the effects of insulin on these rates are shown in **Fig. 4**. The basal rate of oxidation of oleate in hepatocytes from the FO-fed group was higher than in the OO- or LF-fed groups (1153 ± 31 vs. 947 ± 49, and 653 ± 74 nmol/dish/24 h, respectively) (Fig. 4, upper panel). Thus, of the oleate taken up by the cells, a higher percentage was oxidized in the FO-fed group than in the LF- or OO-fed groups (57 vs. 47 and 30%, respectively). More importantly, however, insulin was unable to suppress oleate oxidation in hepatocytes from the FO-fed group (Fig. 4, lower panel).

### Effects on cellular TG storage

The levels of cellular TG at the beginning and end of the culture period and the effects of insulin on these levels are shown in **Fig. 5**. At the beginning of culture, cellular TG levels in hepatocytes from the LF-, OO-, and FO-fed groups were 63 ± 5, 191 ± 28, and 96 ± 10 nmol/dish, respectively (Fig. 5, left upper panel). Culture in the absence of oleate resulted in a 57% reduction in cellular TG in all groups, equating to a mobilization of 36 ± 5, 115 ± 34, and 55 ± 11 nmol/dish/24 h of cellular TG in the LF-, OO-, and FO-fed groups, respectively (Fig. 5, right upper panel). Thus, hepatocytes from the FO-fed group do not appear to have an inability to mobilize cellular TG. In the presence of insulin,

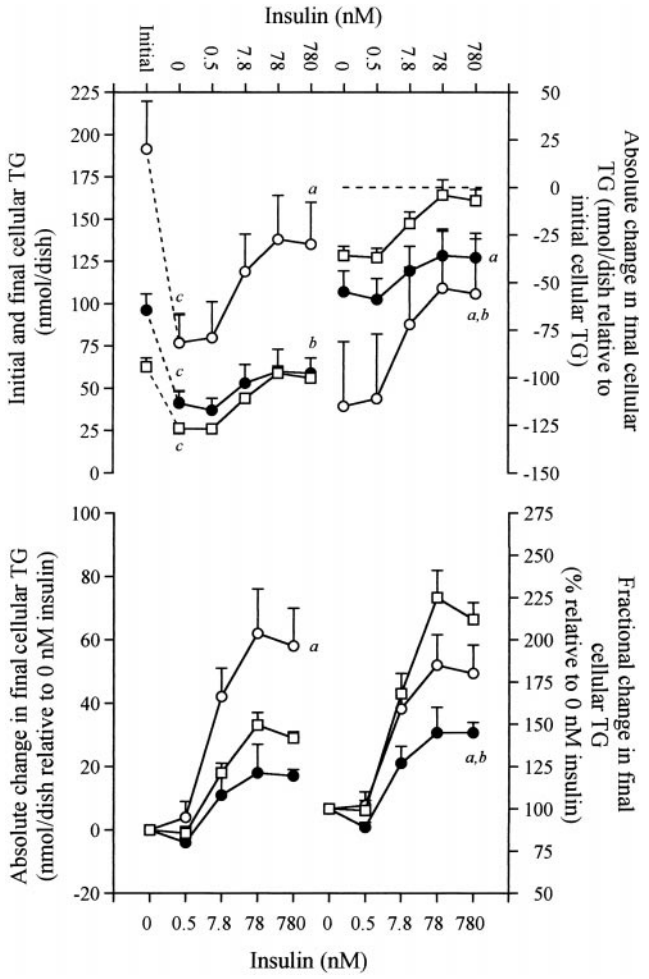


**Fig. 5.** Effect of dietary fat type and content on initial (far left, left upper panel only) cellular TG levels and the sensitivity of final cellular TG levels to the stimulatory effects of insulin. Incubation conditions are described in the legend for Fig. 1. Initial cellular TG levels were determined at the end of the 4-h attachment period while final cellular TG levels were determined after the 24-h culture period. Cellular TG was determined enzymatically after Folch extraction as described in Materials and Methods. Results are expressed as mean ± SEM (n = 6 for LF and n = 5 for OO and FO); LF (□), OO (○), and FO (●). *a* and *b* refer to a significant difference from the LF- or OO-fed group, respectively (ANOVA) while *c* refers to a significant difference from the initial cellular TG levels (*t*-test).

however, the FO-fed group were less able to store cellular TG as evidenced by the smaller increases in the levels of cellular TG remaining at the end of culture (Fig. 5, lower panel).

## DISCUSSION

Hepatocytes cultured in vitro retain, for some time, the metabolic characteristics of the liver of the donor animals (15, 25). The present study utilized hepatocytes from rats fed chronically with different types of dietary fat to determine the effects of these fats on the regulation by insulin of metabolic processes which might be expected to influence the secretion of VLDL. The results of this study confirm previous observations (26–28) that dietary fish oil supplementation decreases the incorporation of fatty acid into TG (Fig. 2) and the secretion of VLDL TG (Fig. 1), while increasing fatty acid oxidation (Fig. 4).

The major novel finding is that FO-feeding dramatically altered the sensitivity to insulin of some, but not all, aspects of hepatic fatty acid and glycerolipid metabolism in vitro. For instance, FO-feeding abolished the inhibitory effect of insulin on the oxidation of exogenous oleate which occurred in hepatocytes from animals fed either the LF- or OO-containing diets (Fig. 4). However, compared to that observed in hepatocytes from the OO- or LF-fed animals, FO-feeding either attenuated or had no effect on the ability of insulin to stimulate the incorporation of oleate into TG (Fig. 2). Neither did dietary FO supplementation give rise to any marked change in the sensitivity of VLDL TG secretion to inhibition by insulin, especially when compared with that observed in hepatocytes from the LF-fed group (Fig. 1). A marginal ($P < 0.05$) increase in the fractional inhibition caused by insulin was observed in comparison with that which occurred after feeding OO. However, with all diets, the maximum absolute decrease in VLDL TG output resulting from insulin was similar (~20 nmol/dish/24 h) (Fig. 1).

It has been shown previously that, in vitro, insulin promotes the storage of hepatic TG by preventing its secretion in VLDL (5, 29). It can be seen from Fig. 5 that, in the absence of insulin, culture of cells from animals in each of the dietary groups led to a loss of cellular TG and that, in each case, this loss was attenuated by insulin. However, the effect of insulin in protecting against the loss of cellular TG occurred to a different extent in each of the dietary groups with the largest effect, in absolute terms, in the OO-fed group and the lowest in the FO-fed group. Although studies have shown that in livers from rats fed a LF diet, fatty acids released from endogenous cellular TG appear to be poor substrates for oxidation (30, 31), this may not be the case in animals fed diets rich in FO. If so, then the inability of insulin to suppress oleate oxidation (Fig. 4) following FO-feeding may underlie its inability to prevent the loss of cellular TG during culture (Fig. 5).

It seems apparent from the above results that chronic dietary supplementation with FO may affect the insulin-sensitivity of metabolic pathways in the liver to different extents. Thus, the sensitivity of some is enhanced, some may be diminished, and that of others is unaffected. Perhaps the most pronounced effect of the FO-diet in this regard was the almost complete abolition of the insulin-dependent suppression of exogenous oleate oxidation (Fig. 4). The β-oxidation of fatty acids is regulated primarily by their rate of entry into the mitochondrial matrix, mediated by carnitine palmitoyltransferase I (CPT-I). Elevated levels of cellular malonyl-CoA, the product of acetyl-CoA carboxylase (ACC), suppress CPT-I (for a review, see ref. 32) and, as ACC is stimulated by insulin (33, 34), this effect is responsible for the inhibition of fatty acid oxidation in response to an elevated concentration of insulin. The sensitivity of CPT-I to inhibition by malonyl-CoA is

also thought to be an important factor in the physiological regulation of fatty acid oxidation (32, 35). Chronic (36) but not acute (37) consumption of EPA, a major component of fish oil, decreases the activity of ACC, increases the activity of CPT-I (38–41), and decreases (4-fold) the sensitivity of CPT-1 to inhibition by malonyl-CoA (26, 39, 42). Although speculative, in the present work these changes may contribute to the increased oxidation of oleate and, more importantly, the inability of insulin to suppress oleate oxidation in the hepatocytes from the FO-fed rats. This observation is clearly of considerable interest and requires further study.

It might be argued that insulin's inability to divert oleate away from the oxidation pathway in the FO-fed group (Fig. 4) decreased the intracellular availability of oleate for incorporation into TG and attenuated its sensitivity to stimulation by insulin when compared to the OO-fed group (Fig. 2). Several observations, however, suggest that other factors may also be involved. First, insulin inhibited oleate oxidation to the same extent in the LF- and OO-fed groups (Fig. 4) and, thus, similarly spared oleate for incorporation into TG. Yet the response to insulin of oleate incorporation into TG was similar in the LF- and FO-fed groups. Second, although phospholipids (PL) and TG share a common pool of fatty acid precursors and a common pathway to the point of diacylglycerol (DG), the ability of insulin to stimulate oleate incorporation into PL in the FO-fed group was greater than that in the LF-fed group and similar to that in the OO-fed group. This is a novel finding and argues against a simple intracellular lack of fatty acids as the sole cause of insulin's inability to stimulate oleate incorporation into TG in hepatocytes from the FO-fed group. It would appear, therefore, that FO-feeding not only leads to a relative decrease in the conversion of fatty acids into TG compared to PL (Figs. 2 and 3), a finding which confirms that of others (27, 28), but that this difference is potentiated in the presence of insulin. An explanation for the finding that FO-feeding reduces oleate incorporation into TG whilst increasing its incorporation into PL may be that the activity of diacylglycerol acyltransferase (DGAT), the final and committed step of TG synthesis, is reduced (43, 44), thus diverting DG to PL synthesis. However, this is not a universal finding (45, 46). Another possibility is that DGAT has a lower affinity for DG than the final enzymes in the conversion of DG to PL (9). During conditions of reduced DG formation, therefore, such as occurs with an increased rate of fatty acid oxidation (28, 47), partitioning of DG to PL synthesis is favored. Whatever the cause, the effect is accentuated by insulin. This differential stimulation of oleate incorporation into PL relative to TG was unique to hepatocytes from the FO-fed rats. The reason(s) for this effect is unclear and merits further study. In this respect, it is of interest that a similar relative increase in PL compared to TG synthesis occurs in rats in vivo during the starved to refed transition (9).

In the present study, FO-feeding prevented the increases in plasma TG that was observed after OO-feeding (Table 1). In addition, there was a clear trend towards lower plasma insulin and NEFA levels after FO-feeding compared to OO-feeding, although this trend did not reach significance. Such effects after FO- and OO-feeding have been observed by others with concomitant increases and decreases in in vivo insulin sensitivity, respectively (10, 48). These changes were quantified by measuring differences in whole body glucose disposal (euglycemic hyperinsulinemic clamps) and insulin secretion in response to a glucose load (oral glucose tolerance test). Thus, it may be reasonable to speculate that in our model insulin resistance in vivo, as determined by parameters relating to carbohydrate metabolism, would rank in the order OO $>$ FO $>$ LF. It cannot be assumed, however, that changes in the sensitivity of certain aspects of carbohydrate metabolism to insulin reflect similar changes in the sensitivity of all other aspects of both carbohydrate and lipid metabolism to insulin. This point is adequately demonstrated by the differential changes in the sensitivity to insulin of various aspects of hepatic lipid metabolism observed in this study.

We would like to thank Reginald Hems for his expert assistance in the preparation of hepatocytes. Fish oil (MaxEPA) was a generous gift from Dr. F. W. Vas-Dias, Seven Seas Ltd., Marfleet, Hull, HU9 8NJ. One of us (P.W.B.) thanks the National Heart Foundation of Australia for a travel grant. This work was funded by a Programme Grant from the U.K. Medical Research Council (M.R.C.) and we would like to thank the Council for its continued support. G.F.G. is a member of the External Scientific Staff of the M.R.C. and Research Lecturer at the University of Oxford.

*Manuscript received 13 May 1999, in revised form 11 November 1999, and in re-revised form 25 January 2000.*

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