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Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats

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Dietary supplementation with oligofructose (OFS; 100 g/kg), a non-digestible oligomer of β-D-fructose, decreases serum triacylglycerols in serum and VLDL of rats. In order to investigate the role of hepatic metabolism in the hypolipidaemic effect of OFS, male Wistar rats were fed on a standard diet with or without 100 g Raftilose® P_{95}/kg as OFS source for 30 d. OFS feeding (1) significantly decreased triacylglycerol and phospholipid concentrations in both blood and liver, (2) increased the glycerol-3-phosphate liver content but decreased the hepatic activity of glycerol-3-phosphate acyltransferase (*EC* 2.3.1.15), suggesting a decrease in acylglycerol synthesis, (3) did not affect the blood non-esterified fatty acid concentrations, but (4) reduced by 54% the capacity of isolated hepatocytes to synthesize and secrete triacylglycerols from labelled acetate; the activity of fatty acid synthase, a key lipogenic enzyme was also significantly decreased. These findings suggest that OFS decreases serum triacylglycerols by reducing *de novo* fatty acid synthesis in the liver; the lower insulin level in the serum of OFS-fed rats could explain, at least partly, the metabolic effect induced by such non-digestible carbohydrates.

Fructans: Liver: Lipogenesis: Very-low-density lipoproteins

Many attempts have been made to control serum triacylglycerol (TAG) concentration by modifying dietary habits. Thus, the hypotriacylglycerolaemic effect of non-digestible but fermentable carbohydrates, including resistant starch or fructo-oligosaccharides, has been described both in human subjects (Glore *et al.* 1994) and in animals (Tokunaga *et al.* 1986; Vigne *et al.* 1987; Overton *et al.* 1994; de Deckere *et al.* 1995). The mechanism of their lowering effect on serum lipids still remains to be fully elucidated.

Feeding rats on a diet supplemented with oligofructose (OFS; 100 g/kg), a non-digestible but fermentable oligomer of β-D-fructose obtained by enzymic hydrolysis of chicory (*Cichorium intybus*) inulin, significantly lowers serum TAG and phospholipid (PL) concentrations (Delzenne *et al.* 1993). This is exclusively due to a decrease in the concentration of plasma VLDL (Fiordaliso *et al.* 1995). The hepatic synthesis of VLDL involves the biosynthesis of both lipids and apoproteins, their assembly into nascent VLDL particles, and the secretion of mature VLDL into the circulation (Gibbons, 1990).

Since newly-synthesized fatty acids are preferentially channelled into VLDL, the lipogenic activity of the liver is a key factor in hepatic VLDL-TAG output (Gibbons, 1990; Ribiero *et al.* 1991; Arbeeny *et al.* 1992; Park *et al.* 1992). Among the key enzymes that control lipogenesis, fatty acid synthase (FAS) is the most sensitive to nutrients and hormones (Boll *et al.* 1994).

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Fatty acid esterification is regulated at the reactions catalysed by glycerol-3-phosphate acyltransferase (*EC* 2.3.1.15; GPAT; Stals *et al.* 1994) and phosphatidate phosphohydrolase (*EC* 3.1.3.4; PAP; Sturton *et al.* 1978), but it also depends on the supply of the substrates, i.e. glycerol-3-phosphate (Declercq *et al.* 1982) and fatty acids; the availability of the latter depends on the balance between lipogenesis and β-oxidation, as well as on the serum concentration of non-esterified fatty acids (NEFA; Stals *et al.* 1994).

Hepatocytes isolated from OFS-fed rats have a lower capacity to esterify [^{14}C]palmitate into TAG (Fiordaliso *et al.* 1995); this suggests that OFS lowers the hepatic output of VLDL by reducing the availability of esterified fatty acids. In order to test this hypothesis the enzyme activities involved in the control of fatty acid synthesis and esterification, as well as the concentration of rate-limiting substrates, were measured in OFS-fed rats.

In addition, insulin and glucose have been shown to be important effectors regulating fatty acid and TAG synthesis, both *in vivo* (Kaiser *et al.* 1983; Katsurada *et al.* 1990) and *in vitro* (Topping & Mayes, 1972; Beynen *et al.* 1980; Spence & Pitot, 1982; Giffhorn-Katz & Katz, 1986). Non-digestible carbohydrates are known to modify the kinetics of absorption of carbohydrates, thus lowering glycaemia and insulinaemia (Stanley & Newsholme, 1985; Ulrich, 1987; Leclère *et al.* 1994). Consequently, both variables were also measured in OFS-fed rats to test the hypothesis of a regulatory role for these factors in the modulation of hepatic lipid metabolism induced by oligofructose.

MATERIALS AND METHODS

Chemicals

Raftilose® P_{95} (Raffinerie Tirlemontoise, Tienen, Belgium), a mixture of glucosyl-$(fructosyl)_n$-fructose (64%) and $(fructosyl)_m$-fructose (36%) with an average degree of polymerization of 4·8, was used as OFS source.

[1-^{14}C]acetic acid (specific activity 60 mCi/mmol) was obtained from Amersham International Plc (Amersham, Bucks.). NA Beckman scintillation solution was obtained from Beckman (Geneva, Switzerland). Enzymes and coenzymes were purchased from Boehringer Mannheim (Mannheim, Germany). Fatty acid-free bovine serum albumin (fraction V; BSA) and all other chemicals were of the purest analytical grade available from Sigma Chemical Co. (St Louis, MO, USA).

Maintenance of animals

Thirty-two male Wistar rats ICOPS-WY IOPS from Iffa Credo (Les Oncins, France) weighing initially about 120 g were housed individually and maintained on a 12 h dark–12 h light alternating schedule (random design). Rats were randomly assigned to one of two groups. Control animals were fed *ad libitum* on a powdered basal diet obtained from a commercial source (UAR A04; UAR, Villemoisson-sur-Orge, France). OFS-fed rats received the same diet containing 100 g Raftilose® P_{95}/kg.

On day 30, at 09.00 hours, corresponding to the postprandial period, twenty animals (ten control and ten OFS-fed rats) were anaesthetized with pentobarbital (60 mg/kg body weight); blood was collected from the descending vena cava and the liver was excised and clamped immediately in liquid N_2 for hepatic enzyme activity and metabolite concentration measurements.

Analytical procedures

Serum TAG, PL, total cholesterol, NEFA and glucose were measured using enzymic kits (Sopar-Biochem, Brussels, Belgium). Intra-assay CV were 2·0, 1·7, 0·9, 2·2 and 1·2 respectively. The same kits were used for liver lipid analysis, after chloroform–methanol

extraction according to Folch *et al.* (1957). Intra-assay CV were 5·2, 6·9 and 11·2% for liver TAG, PL and total cholesterol respectively.

Insulin concentrations were determined using a radioimmunoassay kit from Novo Nordisk (Bagsvaerd, Denmark). The intra-assay CV was 6·2.

The esterification pathway was assessed by measuring glycerol-3-phosphate hepatic content (Hohorst, 1965) and GPAT as well as Mg^{2+}-dependent PAP activities (Bates & Saggerson, 1977; Cheng & Saggerson, 1978). The intra-assay CV was 12·4% for GPAT activity and 11·9% for PAP activity.

The availability of fatty acyl-S-CoA was assessed by measuring carnitine palmitoyltransferase (*EC* 2.3.1.21; CPT I; Schäfer *et al.* 1993) and fatty acid synthase (*EC* 2.3.1.38, *EC* 2.3.1.39, *EC* 2.3.1.41, *EC* 1.1.1.100, *EC* 4.2.1.58, *EC* 4.2.1.59, *EC* 1.3.1.10 and *EC* 3.1.2.2; FAS) activities (Linn, 1981). Intra-assay CV were 6·9 and 6% for CPT I and FAS activities respectively.

Protein was assayed by the method of Lowry *et al.* (1951), using BSA, as the standard. All enzyme activities increased linearly with respect to protein concentration.

At the end of the 30 day feeding period, seven control and five OFS-fed rats were used for hepatocytes isolation. Liver perfusion was carried out under pentobarbital (60 mg/kg) anaesthesia at 10.00 hours. Hepatocytes were isolated according to the standard procedure described by Krack *et al.* (1980). The isolated cells were kept separately for each rat and incubated in one batch at a concentration of 1×10^6 cells/ml with Krebs-bicarbonate buffer (pH 7·4) containing 10 mM-glucose, 1 mM-L-carnitine, fatty acid-free BSA (10 g/l) and 2 mM-[1-^{14}C]acetate (0·2 mCi/mmol). Incubations were carried out at 37° under an atmosphere of O_2–CO_2 (95:5, v/v) in a shaking water-bath.

At 30 min intervals, samples were removed from the suspension and cells were sedimented by centrifugation. TAG synthesis was measured by quantifying the incorporation of [1-^{14}C]acetate into intra- and extracellular ^{14}C-labelled TAG, as previously described by Deboyser *et al.* (1989). Total ^{14}C-labelled TAG synthesis from labelled acetate was calculated as the sum of intra- and extracellular ^{14}C-labelled TAG at 180 min of incubation.

Statistical analysis

Data were analysed (Fleiss, 1986) using *BMDP New System Professional Edition* statistical software (Statistical Solutions Inc., 1995). Serum and hepatic fatty acid metabolism data are reported as arithmetic means with their standard errors of the arithmetic mean. Variates were checked for outliers and for skewness in the control group and in the OFS-fed group of rats. One outlier was observed in the OFS-fed group for the GPAT enzyme and this single outlier has been removed for computing robust significance levels. There was no skewed distribution. Therefore, variates were compared for variability between control and OFS-fed rats using the Levene F test with $P < 0{\cdot}01$ level for rejecting the equal variance assumption. The effect of OFS feeding on those variates was assessed at the two-sided $P < 0{\cdot}05$ level using either Student's t test for equal variances or Student's t test for unequal variances with the conservative choice of the minimum sample size minus one as degree of freedom. Labelled TAG data were analysed on a logarithmic scale because of a proportional relationship between means and standard deviations, but data are reported as geometric mean with the antilog of standard error of the logarithmic mean. ANOVA was performed for a three-factor design: (1) a between-groups factor with two levels for control and OFS-fed rats (the diet factor), (2) a within-groups factor with two levels for cellular and extracellular content (the localization factor), and (3) a repeated-measures factor with four unequal-spaced levels for 30, 60, 120 and 180 min time-point measurements (the time factor). A Greenhouse-Geisser (GG) adjustment to the degree of freedom for tests of the

repeated-measures factor was chosen because of significant sphericity tests ($P = 0.035$ for time error and $P < 0.001$ for time × localization error) and non-decreasing correlation pattern as the time intervals increased. Despite significant linear ($P < 0.001$) and quadratic ($P < 0.001$) components in time-trend analysis, no significant interaction with diet was observed (GG $P = 0.54$) and there was a significant diet effect ($P = 0.031$). The second-order interaction was borderline (GG $P = 0.07$). Therefore, the effect of OFS feeding on labelled TAG data was assessed by submitting only the 180 min time-point measurements to a two-factor analysis of variance (the diet and the localization factor). The diet × localization interaction P value was used to assess the effect of OFS feeding on the secretion of newly-synthesized TAG.

RESULTS AND DISCUSSION

The average daily feed intake was not significantly different between control rats (23·5 (SE 0·9) g/d) and OFS-fed rats (24·1 (SE 0·6) g/d); the average body weight at the end of the feeding period was 297 (SE 8) and 302 (SE 9) g for OFS-fed and control rats respectively.

Analysis of serum lipids (Table 1) confirmed that oral administration of OFS to rats significantly lowers serum TAG and PL concentrations by 40 and 18% respectively, without affecting total cholesterol level. These lipid-lowering effects are greater than those observed with other fermentable compounds such as guar gum which, at the same dose, decrease TAG levels by 15–20% (Overton *et al.* 1994). TAG and PL contents are also lower in the liver of OFS-fed rats (Table 2). This latter observation supports the hypothesis that OFS decreases the liver capacity for fatty acid esterification. Three questions have been addressed in order to formulate new hypotheses with regard to the mechanism of action of OFS on hepatic lipid metabolism.

Which are the effects of feeding oligofructose on key factors controlling acylglycerol synthesis in the liver?

As OFS feeding not only lowers TAG but also PL, it could act on fatty acid esterification by modifying the activity of one of the enzymes that drive the esterification pathway, namely GPAT, which catalyses the first reaction of the pathway, or PAP, which converts phosphatidate to diacylglycerol, the common intermediate for PL and TAG biosynthesis (Bates & Saggerson, 1977; Sturton *et al.* 1978; Stals *et al.* 1994). Results in Table 2 show that PAP activity was not affected by OFS feeding while GPAT activity was significantly lower in the liver of four OFS-fed rats compared with all control rats (n 5). One OFS-fed rat had abnormally high GPAT activity and, therefore, was excluded from the statistical analysis. This suggests that OFS feeding could decrease fatty acid esterification, at least partly, through modulation of GPAT activity.

The extent of fatty acid esterification also depends on the availability of both substrates, glycerol-3-phosphate and fatty acyl-CoA. Declercq *et al.* (1982) have suggested that glycerol-3-phosphate is a limiting factor for fatty acid esterification *in vivo* for contents below 0·3 and 0·5 μmol/g in livers of fed and starved rats respectively. Results in Table 2 show that the hepatic glycerol-3-phosphate concentration exceeded that threshold in OFS-fed rats and was even significantly higher than that in control rats. This relative increase in glycerol-3-phosphate liver content might be due to a decrease in its utilization for fatty acid esterification, as shown by the lower GPAT activity. These results are in line with our previous work in which we had shown that daily administration of OFS slightly but significantly reduced the hepatocyte capacity to esterify [^{14}C]palmitate into TAG (Fiordaliso *et al.* 1995).

Fatty acid availability is considered as the rate-limiting factor for TAG synthesis under

Table 1. *Effect of feeding oligofructose (OFS) for 30 d on serum variables of rats*†

(Mean values with their standard errors for ten rats per dietary group)

Dietary group...	Control		OFS-fed	
	Mean	SEM	Mean	SEM
Triacylglycerols (g/l)	1·70	0·11	1·04**	0·09
Phospholipids (g/l)	1·62	0·06	1·35**	0·05
Total cholesterol (g/l)	0·68	0·04	0·65	0·04
Non-esterified fatty acids (g/l)	0·036	0·004	0·032	0·005
Glucose (mmol/l)	10·13	0·38	8·44**	0·35
Insulin (μg/l)	5·44	0·49	3·67*	0·49

Mean values were significantly different from those for the control group (Student's t test): * $P < 0·05$, ** $P < 0·01$.

† For details of diets and procedures, see pp. 882–883.

Table 2. *Effect of feeding oligofructose (OFS) for 30 d on hepatic fatty acid metabolism of rats*†

(Mean values with their standard errors for ten rats per dietary group except where indicated)

Dietary group...	Control		OFS-fed	
	Mean	SEM	Mean	SEM
Hepatic content (mg/g liver)				
Triacylglycerols	8·65	0·47	6·66*	0·55
Phospholipids	16·38	0·26	14·77**	0·29
Total cholesterol	2·29	0·04	2·15	0·08
Glycerol-3-phosphate (μmol/g liver)	0·24	0·01	0·38**	0·04
Enzymes activities (mU/mg protein)				
GPAT‡	3·35	0·07	2·98*	0·06
PAP	5·5	0·4	4·9	0·53
CPT I	24·3	1·8	22·4	1·4
FAS	63·7	4·8	37·3**	3·4

GPAT, glycerol-3-phosphate acyltransferase (*EC* 2.3.1.15); PAP, phosphatidate phosphohydrolase (*EC* 3.1.3.4); CPT I, carnitine palmitoyltransferase I (*EC* 2.3.1.21); FAS, fatty acid synthase.

Mean values were significantly different from those for the control group (Student's t test): * $P < 0·05$, ** $P < 0·01$.

† All variables except GPAT were measured in freeze-clamped liver of ten control and ten OFS-fed rats. For details of diets and procedures see pp. 882–883.

‡ GPAT activity was measured in liver microsomes prepared from only five control and four OFS-fed rats.

physiological conditions (Stals *et al.* 1994); therefore, OFS could affect esterification either by increasing fatty acid catabolism, or by decreasing the hepatic fatty acid input.

The hypothesis of an increased fatty acid catabolism to explain a lower availability is unlikely: the activity of CPT I, the rate-limiting enzyme in the hepatic mitochondrial β-oxidation (MacGarry & Foster, 1980), was not increased but even slightly decreased in the liver of OFS fed rats (Table 2). Moreover, we have previously shown that mitochondria-enriched fractions prepared from liver of OFS-fed rats had the same capacity to oxidize various fatty acids as those prepared from untreated rats (Kok *et al.* 1993).

Under our experimental conditions (Table 1) the plasma concentration of NEFA was not modified by OFS feeding. This result is in line with those of Sparks & Sparks (1994) who

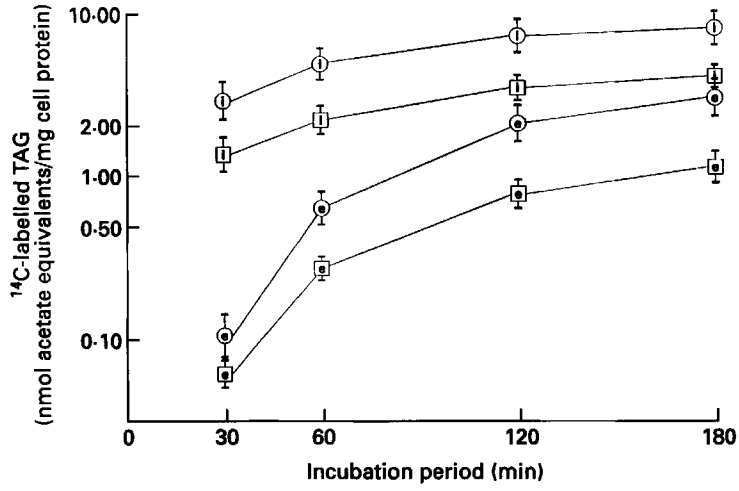

Fig. 1. Effect of feeding oligofructose (OFS) on triacylglycerol (TAG) synthesis from [^{14}C]acetate. Hepatocytes from seven control (○) and five OFS-fed rats (□) were incubated separately for 180 min in the presence of 2 mM-[1-^{14}C]acetate (0·2 mCi/mmol) and analysed. For details of procedures, see p. 883. i, e, intracellular and extracellular (secreted) ^{14}C-labelled TAG respectively. These data were analysed on a logarithmic scale because of a proportional relationship between means and standard deviations, but data are reported as geometric mean and antilog of standard error of the logarithmic mean, represented by vertical bars. A three-factor ANOVA, performed as described on pp. 882–883, showed a significant effect for OFS ($P = 0·031$). For details of diets, see p. 882.

suggested that the capacity for hepatic VLDL-TAG secretion relates to changes in lipogenic enzymes rather than to the availability of extracellular fatty acids.

Recent experimental data support a strong relationship between *de novo* fatty acid synthesis and VLDL secretion: *in vivo*, chronic treatments with modified starch or 5-(tetradecyloxy)-2-furancarboxylic acid decrease acetyl-CoA carboxylase (*EC* 6.4.1.2) and FAS activities in rats and hamsters respectively; this was correlated with a lower VLDL-TAG secretion and a decrease in plasma TAG concentrations (Arbeeny *et al.* 1992; de Deckere *et al.* 1995).

Fatty acid synthesis is controlled by several key enzymes which are coordinately regulated when responding to physiological stimuli (Gibson *et al.* 1972); FAS activity is 40% lower in the liver of OFS-fed rats compared with controls (Table 2), thus suggesting that OFS feeding could decrease lipogenic flux and, thus, the liver VLDL-TAG secretion capacity (Gibbons, 1990; Arbeeny *et al.* 1992). Indeed, using an inhibitor of acetyl-CoA carboxylase, Arbeeny *et al.* (1992) demonstrated with primary cultures of hamster hepatocytes that when fatty acid synthesis from [^{14}C]acetate is almost completely inhibited, VLDL-TAG secretion is decreased by 90%.

Therefore, our results indicate that the lower TAG and PL formation in the liver of OFS-fed rats could be due mostly to a decrease in *de novo* fatty acid synthesis. This hypothesis has been tested by raising the following question.

Is a reduced de novo *lipogenic capacity of the liver involved in the triacylglycerol-lowering effect of oligofructose*?

The time-course of ^{14}C-labelled TAG synthesis and secretion from [^{14}C]acetate was measured in hepatocytes isolated from control and OFS-fed rats (Fig. 1). The use of acetate as a lipogenic substrate is interesting because being activated by acetyl-CoA synthetase it bypasses not only the glycolytic pathway but also both the pyruvate dehydrogenase

Table 3. *Effect of feeding oligofructose (OFS) for 30 d on triacylglycerol synthesis from [^{14}C]acetate in isolated rat hepatocytes**

(Mean values with their standard errors)

	TAG (nmol equiv. acetate/mg protein)					
	Intracellular		Extracellular		Intracellular:extracellular	
Dietary group†	Mean	SEM	Mean	SEM	Mean	SEM
Control rats (*n* 7)	7·72	1·27	2·89	1·29	2·67	1·25
OFS-fed rats (*n* 5)	3·91	1·18	1·10	1·24	3·57	1·22
Statistical significance of difference‡: $P =$	0·041		0·022		0·041	

* Hepatocytes were isolated from seven control and five OFS-fed rats, incubated separately for 180 min in the presence of 2 mM-[1-^{14}C]acetate (0·2 mCi/mmol) and analysed. For details of procedures, see p. 883.

† For details of diets, see p. 882.

‡ Two-factor ANOVA (the diet and the localization factors) was used to assess the effect of OFS feeding on the secretion of newly synthesized triacylglycerols after 180 min.

(*EC* 1.2.4.1)-catalysed reaction and the transport from mitochondria into cytosol (Nishina & Freeland, 1990).

OFS-feeding significantly reduces (by more than 2-fold) TAG synthesis and secretion from acetate in isolated hepatocytes (Fig. 1). After 180 min of incubation, total ^{14}C-labelled TAG synthesis reached 10·65 (SE 1·27) and 5·02 (SE 1·19) nmol acetate equivalents/mg cell protein in the control and the OFS-fed group respectively. The parallelism between the extracellular and intracellular curves suggests that OFS feeding decreased the hepatocyte capacity for fatty synthesis and esterification through modulation of enzyme activity, as confirmed by the decreased activity of GPAT and FAS.

In the control group the newly-synthesized TAG secreted (extracellular) represented only 27·4% of the total synthesis, whereas 72·6% remained inside the hepatocyte (Table 3). These relative amounts were slightly but significantly modified in the OFS-fed group, in favour of cellular TAG, thus indicating that OFS feeding could also affect the VLDL-TAG secretion pathway.

These data thus support the hypothesis that a decreased *de novo* lipogenesis in the liver, through modulation of FAS activity, combined with a decrease in fatty acid esterification, causes a reduction in VLDL-TAG secretion in OFS-fed rats. A similar hypothesis has been proposed by Clark & Jump (1994) who suggested that at least part of the hypotriacylglycerolaemic action of dietary polyunsaturated fats is due to the inhibition of hepatic *de novo* fatty acid synthesis.

This observation of a modulation of the hepatic *de novo* fatty acid and TAG synthesis in OFS-fed rats leaves open the question of its possible mechanism. The third question was raised in order to test one possible hypothesis.

Are postprandial insulinaemia and glycaemia affected by oligofructose feeding?

This question is probably relevant as dietary modulation of FAS activity is often linked to modifications of glucose and/or insulin serum levels: indeed, resistant starch decreases serum TAG concentration in rats (de Deckere *et al.* 1995), reduces FAS activity by 50 and 20% in adipose tissue and liver respectively, and concommitantly lowers postprandial insulin response by 30% without affecting glucose response (Takase *et al.* 1994). Similarly,

acarbose, an intestinal glycosidase inhibitor which delays starch digestion, reduces glucose absorption and postprandial glycaemia and insulinaemia and also decreases FAS activity and mRNA concentration in the liver (Maury *et al.* 1993).

Our data show that OFS ingestion reduces postprandial glycaemia and insulinaemia by 17 and 26% respectively (Table 1). This could explain the lower activity of FAS, an enzyme whose transcription is primarily activated by glucose and insulin (Spence & Pitot, 1982; Giffhorn-Katz & Katz, 1986). In the same way, GPAT activity and transcription are also activated by insulin or a high-carbohydrate, fat-free diet (Bates & Saggerson, 1977; Jerkins *et al.* 1995). This further confirms the hypothesis of a decreased level of insulin as a possible mediator of the hepatic effects of OFS.

Nevertheless, although the stimulatory action of insulin on hepatic lipogenesis and TAG synthesis is accepted generally in the literature, the direct effect of this hormone on hepatic VLDL secretion *in vitro* is often a matter for controversy. Early studies using rat liver perfusions or isolated hepatocytes suggested that insulin stimulated VLDL secretion (Topping & Mayes, 1972; Beynen *et al.* 1979, 1980), whereas several more recent studies using human subjects (Lewis *et al.* 1995) or primary cultures of rat hepatocytes for periods up to 24 h demonstrated that this process was in fact inhibited by insulin (Björnsson *et al.* 1992). But this inhibitory effect is no longer present and there is even a stimulation if cells are incubated in the presence of insulin for 48 h (Duerden *et al.* 1989). It is possible that the effect of insulin on VLDL-TAG secretion is influenced by the prevailing hormonal and metabolic conditions within the hepatocyte at the time of preparation. This could explain the positive relationship between plasma insulin and total TAG and VLDL observed in chronic hyperinsulinaemia as well as in insulin-deficient states (Sparks & Sparks, 1994). We could postulate, therefore, that the decrease in insulinaemia and glycaemia may contribute to the reduction in fatty acid and TAG synthesis and be part of the mechanism of the OFS-induced decrease in VLDL secretion by the liver, processes which are, as recently reviewed, strongly stimulated by insulin (Gibbons, 1990; Sparks & Sparks, 1994).

However, other hypotheses cannot be ruled out and need to be tested. The level of TAG in the blood results from the balance between production of TAG-rich lipoproteins (TRL) and their utilization. Therefore, we cannot rule out the possibility that part of the hypotriacylglycerolaemic effect of OFS may be due to increased clearance of TRL. Insulin has a direct stimulatory effect on adipose tissue lipoprotein lipase (*EC* 3.1.1.34) activity (for review, see Braun & Severson, 1992) and as feeding OFS decreases postprandial insulinaemia, we could postulate that the activity of the latter enzyme should be lower in OFS-fed rats. Further studies are needed to clarify the effect of feeding OFS on TRL catabolism and LPL activity.

We postulate that insulin modifications could, at least partly, explain the metabolic effect of OFS but other mediators could also be involved. In particular, OFS is largely fermented in the caeco-colon, leading to the production of short-chain carboxylic acids (Wang & Gibson, 1993) and the portal concentrations of acetate and propionate are increased by more than twofold in OFS-fed rats (Delzenne & Roberfroid, 1993). Since propionate has been reported to inhibit hepatic fatty acid synthesis (Nishina & Freeland, 1990; Wright *et al.* 1990; Lin *et al.* 1995), further work is in progress to test the effect of this putative mediator on lipogenic enzyme activity and the VLDL-TAG secretion capacity of liver cells.

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