Decreased Sensitivity to the Inhibitory Effect of Insulin on the Secretion of Very–Low-Density Lipoprotein in Cultured Hepatocytes From Fructose-Fed Rats

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Hepatocytes were prepared from rats fed a chow diet (control-fed) and from rats fed a similar diet in which the drinking water contained 10% (wt/vol) fructose (fructose-fed). Both types of hepatocyte preparations were cultured for ≤48 hours in supplemented Waymouth's medium containing increasing concentrations of bovine insulin (0 to 780 nmol/L). During the first 24 hours of culture, hepatocytes from fructose-fed rats secreted more very–low-density lipoprotein (VLDL) triacylglycerol (TAG) than hepatocytes from control-fed rats. This difference persisted at all concentrations of insulin. There was no difference in the rate of secretion of apolipoprotein B (apo B). In both control-fed and fructose-fed animals, the inhibitory effect of insulin on the secretion of VLDL was greater on the second versus the first day of culture. Under these conditions, hepatocytes from fructose-fed groups were less sensitive to insulin inhibition as compared with those from the control-fed group. This was evidenced by the following: (1) the decreased inhibitory effect of insulin on the secretion of both total and newly synthesized VLDL TAG, (2) the attenuated inhibitory effect of insulin on the secretion of VLDL apo B, (3) the decreased potency of insulin in suppressing the secretion of VLDL TAG in TAG-depleted hepatocytes from fructose-fed as compared with control-fed animals, and (4) the larger proportion of newly synthesized TAG secreted as VLDL in hepatocytes from fructose-fed rats as compared with controls. This difference was exacerbated at higher concentrations of insulin. These results suggest that the increased rate of secretion of VLDL from livers of fructose-fed rats is due to (1) an increased basal rate of secretion and (2) a decreased sensitivity to the inhibitory effect of insulin.

IT HAS BEEN ESTABLISHED for some time that rats consuming diets rich in fructose or sucrose become hyperinsulinemic[1-3] and develop hypertriglyceridemia.[4-9] These rats also develop resistance to the effect of insulin in stimulating peripheral glucose disposal.[2,10] The hypertriglyceridemia observed in fructose-fed rats is due to a combination of increased hepatic triacylglycerol (TAG) output[5,6,11,12] and decreased peripheral lipolysis via lipoprotein lipase.[13,14] In human subjects and experimental animals, treatment with high concentrations of insulin for periods of less than 24 hours suppresses the secretion of hepatic very–low-density lipoprotein (VLDL) both in vivo[15] and in vitro.[16-20] However, when hepatocytes are chronically exposed (>24 hours) to a high insulin concentration in vitro, the inhibitory effect on the secretion of lipids is attenuated[21,22] or abolished.[23,24] Under these conditions, hepatic insulin receptor activity becomes downregulated.[21,25] In view of these observations, it has previously been suggested[26-28] that the increased hepatic output of VLDL under conditions characterized by insulin resistance in vivo (see Howard,[29] Reaven and Chen,[30] and Kostner and Karádi[31] for review) is not due to a direct stimulation of VLDL output by insulin, but rather to resistance to the normal inhibitory effect of insulin on this process. This occurs despite the prevailing hyperinsulinemia. The major objective of the present study was to test this hypothesis by studying the ability of insulin to suppress directly in vitro the secretion of VLDL from hepatocytes of animals in which hepatic resistance to the effects of insulin on carbohydrate metabolism has been induced in vivo by long-term fructose intake.[10]

MATERIALS AND METHODS

Male Wistar rats were housed one per cage and maintained under conditions described previously.[32] For a period of 7 days before the start of the experiment, one group of rats had drinking water replaced with a solution of 10% (wt/vol) fructose (fructose-fed rats). The control group (control-fed rats) had drinking water only. Both groups had access to a pelleted chow diet ad libitum. Consumption of food and fructose solution and weights of the animals were recorded daily.

Preparation and Maintenance of Hepatocyte Cultures

Hepatocytes were prepared under sterile conditions and plated out into dishes as a suspension in Waymouth's medium containing 10% (vol/vol) fetal calf serum.[23] After 4 hours, the serum-containing medium was removed, the cell monolayer was washed, and cells were cultured for ≤48 hours in supplemented Waymouth's medium containing ^{3}H-oleate (0.75 mmol/L, 0.98×10^6 dpm/μmol) as described previously.[33] Secretion of VLDL TAG and apolipoprotein (apo) B was determined during successive 24-hour periods (ie, day 1 and day 2[33]). Insulin was present (where appropriate) for either the first or second 24-hour period only. In no case was insulin present throughout the whole of the 48-hour period.

Harvesting of Cells, Preparation of VLDL, and Measurement of TAG

At the end of each culture period (day 1 or day 2), the medium was removed. Cells were harvested and the secreted VLDL was obtained as described previously.[33] In control experiments in which ^{3}H-TAG–labeled VLDL was added exogenously to the medium, only 5.8% ± 0.3% (n = 3) was removed from the medium by the cells. Total lipid fractions of the VLDL and of the cell pellet were obtained as previously described.[23] TAG in the extracts was measured and assayed[34] using a kit (Triglycerides GPO-PAP; Boehringer, Mannheim, Germany). For measurement of [^{3}H]

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Submitted January 26, 1994; accepted November 8, 1994.

Supported by a grant from the Medical Research Council of the United Kingdom.

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0026-0495/95/4407-0003$03.00/0

content of labeled TAG, the total lipid fraction was separated by thin-layer chromatography.[35]

Determination of VLDL Apo B

Rat plasma VLDL apo B was used to prepare standard curves for these assays. This was obtained by treatment of rats with 1.0 mL of Triton WR1339 (10% wt/vol in 0.9% saline) injected via the tail vein. VLDL-enriched plasma was obtained 3.5 hours later, and the VLDL fraction was obtained by ultracentrifugation. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of an aliquot of the VLDL fraction using a 15% gel showed that the major apolipoproteins present were apo B_{100} and apo B_{48}, with the latter present in the greatest amount (data not shown). Neither apo E nor apo C were present in this preparation. Thus, protein content of the VLDL is exclusively apo B, and this was determined by a modification of the Lowry procedure.[36] An aliquot of the VLDL standard was diluted with phosphate-buffered saline ([PBS] 10 mmol/L Na_2HPO_4, 3.7 mmol/L NaH_2PO_4, 0.15 mol/L NaCl, and 1.5 mmol/L NaN_3) containing Tween 20 (0.05%) to produce a protein concentration of approximately 5 μg/mL. A range of dilutions was prepared, and 0.1 mL of each dilution (containing ~20 to 200 ng apo B) was transferred in triplicate to the wells of an enzyme-linked immunosorbent assay plate (Nunc-Immuno; Inter-Med, Roskilde, Denmark). PBS containing Tween 20 was added to 0.3 mL of hepatocyte-secreted VLDL to produce an identical concentration to that in the standards. Secreted VLDL samples (0.1 mL of each) were then pipetted in duplicate into the wells of the plate. The samples were kept at 4°C overnight to allow binding of apo B to the plate. Excess unbound material was removed by sharply inverting the plate, and the coated wells were washed three times with 0.05% Tween-PBS. Anti–human apo B antiserum (raised in sheep) was diluted 100-fold with 0.05% Tween-PBS; 0.1 mL of this solution was added to each well, and the plates were kept at room temperature for 2 hours. Excess unbound antibody was removed, and the wells were washed three times as described earlier. Antisheep IgG (raised in donkey) linked to alkaline phosphatase was diluted 1,000-fold with 0.05% Tween-PBS; 0.1 mL of this solution was added to each well, and the plate was kept at room temperature for 2 hours. Excess antibody was removed, and the plates were washed three times with PBS only. Finally, 200 μL substrate solution (1 mg/mL *p*-nitrophenyl phosphate in 0.1 mol/L carbonate buffer, pH 9.6, containing 0.5 mmol/L $MgCl_2$) was added to each well. After 30 minutes at room temperature, absorbance of each well was measured at a wavelength of 405 nm (reference filter at 620 nm) using an Anthos Reader 2001 (Anthos Labtec Instruments, Salzburg, Austria). This procedure results in measurements of apo B secretion greater than those reported previously using an anti–rat apo B antibody in which purified VLDL apo B was used as a standard.[20,33] It is possible that these differences arise from the small amounts of Triton associated with the apo B used as a standard in the present study. However, this will not affect the relative changes observed.

Other Methods

Cellular protein level was measured using the method reported by Lowry et al.[37] Oleate bound to bovine serum albumin (essentially fatty acid–free) was prepared using the method reported by Van Harken et al.[38] Bovine serum albumin concentration in the culture medium was 0.5% (wt/vol).

Materials

All tissue culture media were obtained from Gibco (Paisley, Scotland, UK). Radiochemicals were obtained from Amersham International (Aylesbury, Bucks, UK). Anti–human apo B antiserum was obtained from Boehringer-Mannheim (Lewes, UK), and anti–sheep IgG antibody was obtained from Sigma (Poole, UK).

Statistical Methods

All values are presented as the mean ± SEM of several independent experiments. Significant differences were obtained using a paired or unpaired Student's *t* test. Repeated-measures ANOVAs were performed to determine overall differences in the inhibition curves resulting from insulin over a concentration range of 0 to 780 nmol/L.

RESULTS

Both groups of animals showed a similar weight increase during the 7-day feeding period (Table 1). However, fructose-fed animals consumed significantly less solid food. This was balanced by a fructose intake equivalent to 9.7 ± 1.6 g/d (33% wt/wt of total food intake). Plasma and liver TAG contents of fructose-fed animals were significantly greater than those of control-fed rats (Table 1).

During the first 24 hours (day 1) of culture in the absence of insulin, secretion of VLDL TAG from hepatocytes of fructose-fed rats was considerably greater than that from hepatocytes of control-fed rats ($P < .01$; Fig 1). At each concentration of insulin, the output of VLDL TAG remained greater in hepatocytes from fructose-fed animals versus controls. Furthermore, between 78 and 780 nmol/L insulin, whereas TAG secretion from hepatocytes of the control-fed group continued to decrease ($P < .05$ for output at 780 *v* 78 nmol/L), there was no further significant decrease in secretion of TAG from hepatocytes of the fructose-fed group (Fig 1). A similar pattern was also observed for secretion of TAG newly synthesized from exogenous ^{3}H-oleate. In this case, the secretion rate was calculated on the basis of specific radioactivity of the exogenous ^{3}H-oleate (0.98×10^6 dpm/μmol), and this therefore represented TAG synthesized exclusively from this source. The total mass of TAG secreted (ie, measured enzymically) was greater than that calculated above because it includes secretion of endogenous, unlabeled TAG present in the liver before cells were exposed to exogenous ^{3}H-oleate. The inhibitory effect of insulin, expressed as a percentage of the secretion rate of TAG in the absence of the hormone, was less pronounced in hepatocytes from the fructose-fed group versus the control-fed group (Figs 2 and 3). This was the case irrespective of whether the total mass (Fig 2) or the newly synthesized mass (Fig 3) of VLDL TAG was measured. In hepatocytes of control-fed rats, at

Table 1. Characteristics of Control-Fed and Fructose-Fed Rats

	Control-Fed	Fructose-Fed
Weight gain (g)	34 ± 6	34 ± 5
Final weight (g)	238 ± 10	288 ± 13
Food intake per day (g)	27 ± 1	20 ± 2*
Fructose intake per day (g)	—	9.7 ± 1.6
Plasma TAG (mg/mL)	0.69 ± 0.07	1.43 ± 0.2*
Liver TAG (mg/g)	4.23 ± 0.50	7.16 ± 0.57*

*$P < .05$ *v* control-fed.

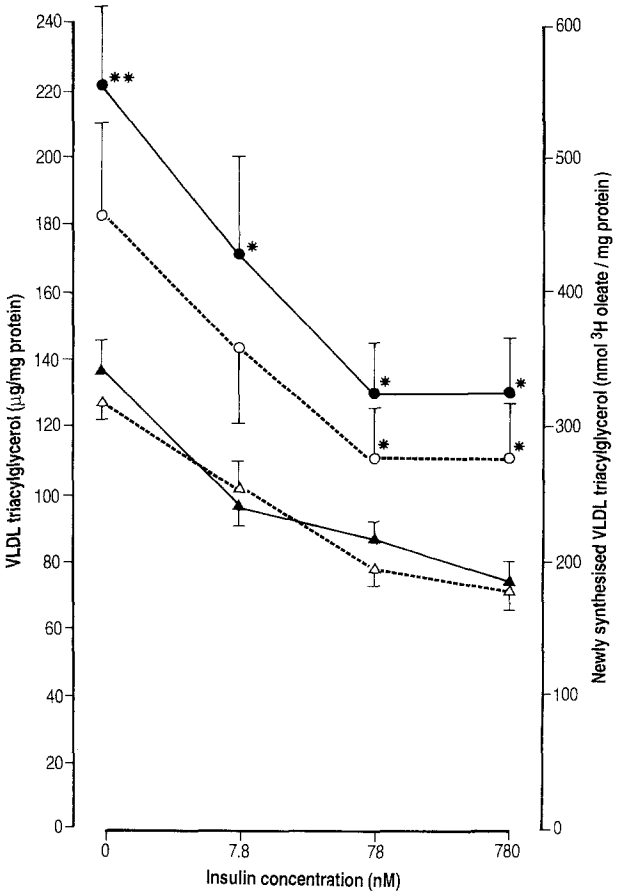

Fig 1. Effects of insulin on secretion of VLDL TAG in hepatocytes from (▲, △) control-fed and (●, ○) fructose-fed rats. Each point is the mean ± SEM of 7 independent experiments. *$P < .05$, **$P < .01$: *v* control-fed. (—), Total mass; (----) ^{3}H-oleate–labeled.

780 nmol/L insulin there was a further significant decrease in secretion of both total and newly synthesized VLDL TAG versus 78 nmol/L insulin ($P < .05$). This continued decline was not observed in hepatocytes of fructose-fed rats.

Although fructose feeding had no effect on TAG synthesis in vitro from exogenous oleate versus the control condition (576 ± 98 *v* 692 ± 85 nmol ^{3}H-oleate incorporated/24 h/mg cell protein, respectively), a greater proportion of the newly synthesized material emerged as VLDL in hepatocytes from fructose-fed rats ($P < .05$; Fig 4). Insulin decreased the proportion secreted to a greater extent in hepatocytes of control-fed versus fructose-fed rats ($P < .05$; Fig 4), again suggesting that the latter group was less sensitive to the inhibitory effect of insulin. The higher rate of VLDL TAG secretion by hepatocytes of fructose-fed rats could not be maintained during the second 24-hour period (day 2 of culture). During this period, whereas the rate observed in cells of control-fed rats remained unchanged or increased somewhat (138 ± 9 μg/mg on day 1 and 146 ± 15 on day 2), the rate in cells of fructose-fed rats declined by approximately 50% (222 ± 23 μg/mg on day 1 and 114 ± 14

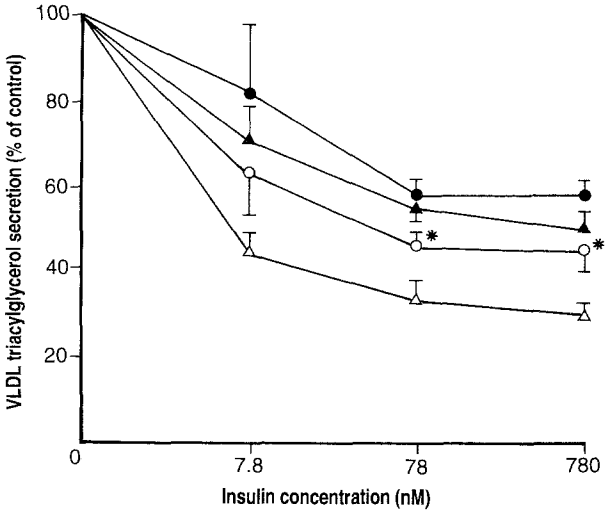

Fig 2. Relative suppression of VLDL TAG secretion by insulin in hepatocytes from (▲, △) control-fed and (●, ○) fructose-fed rats. Effects of insulin on secretion of VLDL TAG (total mass) are expressed as percentages of the rates of secretion in the absence of insulin in each type of hepatocyte preparation. In hepatocytes cultured for 2 days, cells were exposed to insulin only during the final 24 hours of culture. Each point is the mean ± SEM of 7 independent experiments. *$P < .05$ *v* control-fed. The line resulting from fructose-fed rats on day 2 is significantly different (ANOVA: $P = .019$, $F = 7.53$) *v* control-fed. (●, ▲) Day 1 of culture; (○, △) day 2. In the absence of insulin, hepatocytes from fructose-fed animals secreted 222 ± 23 μg/mg protein on day 1 and 114 ± on day 2; those from control-fed animals secreted 138 ± 9 on day 1 and 146 ± 15 on day 2.

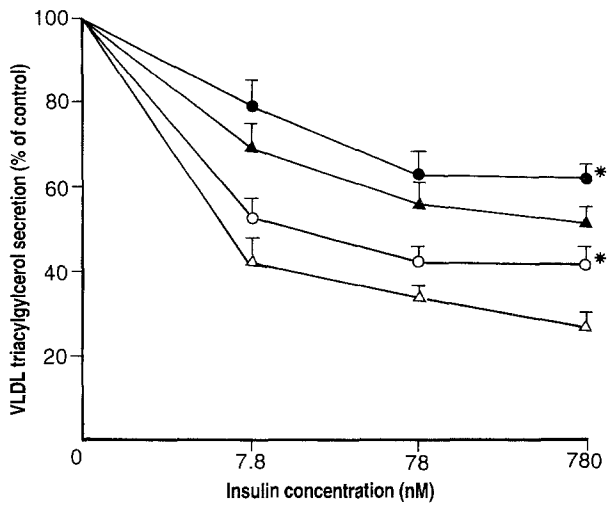

Fig 3. Relative suppression of newly synthesized VLDL TAG secretion by insulin in (▲, △) control-fed and (●, ○) fructose-fed rats. Each point is the mean ± SEM of 7 independent experiments. *$P < .05$ *v* control-fed. The line resulting from fructose-fed rats on day 2 is significantly different (ANOVA: $P = .04$, $F = 5.42$) *v* control-fed. (●, ▲), Day 1 of culture; (○, △) day 2. In the absence of insulin, hepatocytes from fructose-fed animals secreted (in nmol ^{3}H-oleate incorporated per mg protein) 457 ± 69 on day 1 and 235 ± 38 on day 2; those from control-fed animals secreted 321 ± 16 on day 1 and 302 ± 52 on day 2.

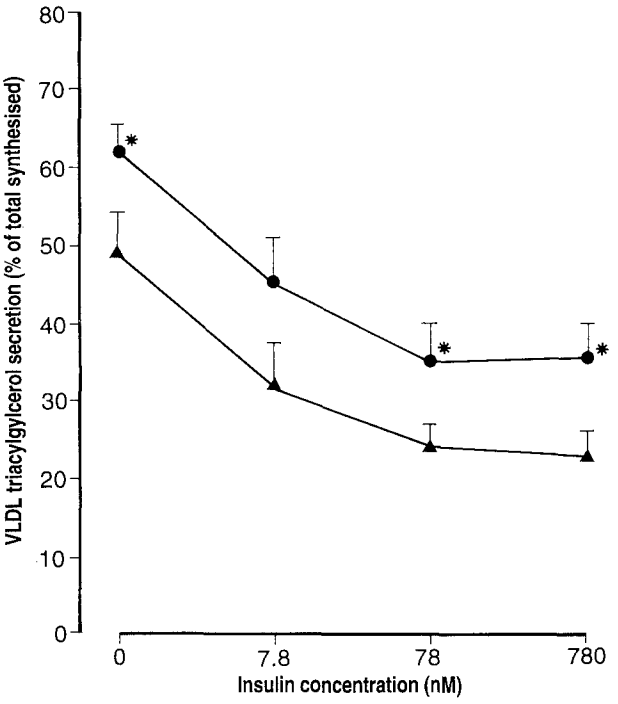

Fig 4. Effect of insulin on proportion of newly synthesized TAG secreted as VLDL. The sum of labeled cellular and VLDL TAG represented the total amount of newly synthesized TAG. Secretion of TAG at each insulin concentration is expressed as a percentage of the total synthesized. Each point is the mean ± SEM of 7 independent preparations. *$P < .05$ *v* control-fed. (●) Fructose-fed; (▲) control-fed.

on day 2). With each type of hepatocyte preparation, the inhibitory effect of insulin on the secretion of TAG was more pronounced on day 2 versus day 1 of culture (Figs 2 and 3). Thus, the ability of insulin to promote intracellular storage of TAG was consequently greater during the second 24 hours of culture. This occurred despite an increase in the basal concentration of intracellular TAG from the outset of day 2 versus day 1. It would therefore appear that an increase in cellular TAG concentration per se is not sufficient to desensitize the hepatocyte to the inhibitory effect of insulin. However, as occurred during the first day of culture, the inhibitory effect of insulin on secretion of TAG was less pronounced in hepatocytes from fructose-fed rats versus control-fed rats. This was the case for secretion of both total (Fig 2) and newly synthesized (Fig 3) TAG.

During the first 24 hours of culture, despite the stimulatory effect of fructose feeding on secretion of VLDL TAG, there was no corresponding effect on secretion of VLDL apo B (Table 2). Neither was there any difference between the two types of hepatocyte preparations in the extent to which insulin suppressed apo B secretion. During the next 24 hours of culture, there also was no difference in apo B secretion rates between the two types of cell preparations in the absence of insulin. However, the greater inhibitory response to insulin in hepatocytes from control-fed animals (Table 2 and Fig 5) resulted in a lower rate of apo B secretion at 780 nmol/L insulin in hepatocytes from the

Table 2. Effects of Insulin on Secretion of VLDL Apo B in Hepatocyte Cultures From Control-Fed and Fructose-Fed Rats

Insulin Concentration (nmol/L)	VLDL Apo B Secretion (μg/mg cell protein)	
	Day 1 (4-28 h)	Day 2 (24-48 h)
Control-fed		
0	4.24 ± 0.61	1.72 ± 0.12
7.8	3.76 ± 0.56	1.00 ± 0.17
78	3.71 ± 0.65	0.89 ± 0.17
780	3.63 ± 0.54	0.65 ± 0.10
Fructose-fed		
0	4.18 ± 0.64	1.81 ± 0.35
7.8	4.18 ± 0.60	1.08 ± 0.16
78	3.39 ± 0.53	1.08 ± 0.23
780	3.14 ± 0.44	1.12 ± 0.18*

NOTE. For control-fed rats, n = 8 for day 1 and n = 7 for day 2. For fructose-fed rats, n = 7 for day 1 and n = 5 for day 2.
P < .05 *v* control-fed.

control group versus the fructose-fed group ($P < .05$; Table 2).

DISCUSSION

The present results confirm earlier reports[5,6,11,12] that long-term feeding of fructose or sucrose increases the rate of hepatic VLDL output. This has previously been attributed to an increased rate of de novo lipogenesis[39] and/or increased channeling of exogenous fatty acids toward esterification at the expense of oxidation.[11] In the present study, fructose feeding had no effect on the synthesis of TAG from exogenous oleate by derived hepatocytes either in the absence or presence of 780 nmol/L insulin. During the first 24 hours of culture (day 1), these values were (in nanomoles ^{3}H-oleate incorporated per milligram cell protein) 692 ± 85

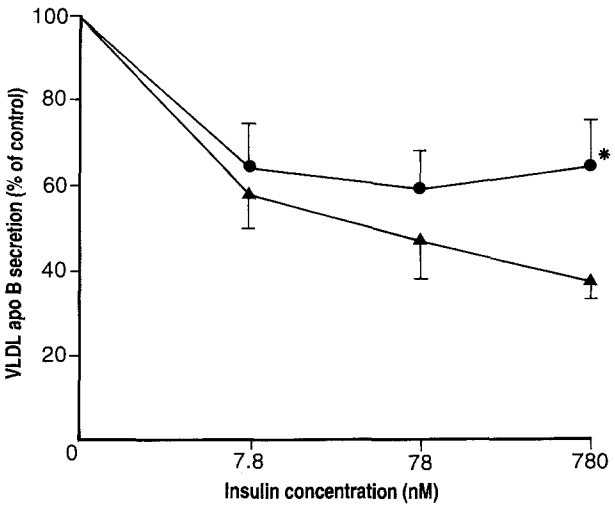

Fig 5. Relative suppression of VLDL apo B by insulin in hepatocytes from (▲) control-fed and (●) fructose-fed rats. In the absence of insulin, hepatocytes from control-fed animals secreted 1.72 ± 0.12 μg apo B/mg protein during day 2; the corresponding value for fructose-fed animals was 1.81 ± 0.35. Each point is the mean ± SEM of 7 animals in the control group and 5 in the fructose-fed group. *$P < .05$ *v* control-fed.

Table 3. Time-Dependent Changes in Specific Radioactivity of VLDL TAG and Cellular TAG

Culture Period (h)	Specific Radioactivity of ^{3}H-TAG (nmol ^{3}H-oleate/μg)	
	VLDL	Cellular
Control-fed		
6 (n = 5)	0.72 ± 0.19	1.32 ± 0.09*
24 (n = 5)	1.57 ± 0.08	1.64 ± 0.14
Fructose-fed		
6 (n = 3)	1.19 ± 0.23	1.30 ± 0.04
24 (n = 5)	2.50 ± 0.21	1.48 ± 0.17

*$P < .05$ *v* VLDL.

for control-fed, versus 576 ± 98 for fructose-fed and 768 ± 85 for control-fed versus 727 ± 74 for fructose-fed, respectively. The increased rate of secretion of TAG in hepatocytes from the fructose-fed group resulted from an increase in the proportion of this total newly synthesized TAG that entered the secretory pathway (Fig 4). In this respect, it has been shown previously[40] that in hepatocytes from chow-fed rats, TAG newly synthesized from exogenous oleate does not enter directly into the secretory pathway, but is initially transferred into a cytosolic pool from which it is subsequently mobilized for the assembly of VLDL. The existence of this indirect route is supported by the present observation that after 6 hours culture in the presence of ^{3}H-oleate, specific radioactivity of VLDL TAG secreted by control hepatocytes was only approximately 50% ($P < .05$) that of the cellular material. However, at this time, there was no difference in specific radioactivities of cellular and VLDL TAG in hepatocytes from fructose-fed rats (Table 3). These observations indicate either that hepatocellular TAG is more rapidly mobilized for assembly of VLDL in fructose-fed rats, as previously proposed[11] for sucrose-fed animals, or that a greater proportion of newly synthesized TAG is channeled directly into the secretory pathway without first entering the cytosolic pool. The somewhat higher specific radioactivity of VLDL TAG as compared with cellular TAG ($P < .09$) in hepatocytes from fructose-fed animals after 24 hours culture (Table 3) suggests that this may be a contributory factor. If this is correct, it would suggest that in fructose-fed animals, the cytosolic storage pool of TAG is a relatively poor contributor to VLDL TAG as compared with that in control-fed animals. This may explain, at least in part, the higher steady-state levels of hepatic TAG in fructose-fed versus control-fed animals (Table 1). Whatever the case, the increased secretion of VLDL TAG does not appear to result from an increased availability of apo B in hepatocytes from fructose-fed animals (Table 2).

Not only was there a higher rate of VLDL TAG secretion in hepatocytes from fructose-fed rats, but this process was less susceptible to inhibition by insulin (Figs 2 and 3), especially on the second day of culture. It is clear from Figs 2 and 3 that in hepatocytes from both fructose-fed and control-fed animals, the inhibitory effects of insulin are more pronounced on the second day, versus the first day of culture. This may result from partial damage to insulin receptors by the initial collagenase treatment, from which the cells recover by the second day. Further evidence for the decreased sensitivity to insulin of hepatocytes from fructose-fed rats came from the observation that although in both groups of rats insulin suppressed the proportion of newly synthesized TAG that was secreted, this effect was less pronounced in hepatocytes from the fructose-fed group (Fig 4).

The reason(s) for this resistance to the normal inhibitory effect of insulin is obscure, but may be related to the prevailing hyperinsulinemia in vivo[1-3] resulting in a downregulation of insulin receptor activity.[21,25] Thus, the high rates of secretion of VLDL TAG in vivo[5,6] in rats fed sucrose or fructose may result from an enhanced basal rate (perhaps caused by an increased rate of fatty acid synthesis de novo) and a decreased sensitivity to downregulation by insulin. In this regard, it should be noted that in fructose-fed rats, at any given concentration of insulin, suppression of VLDL secretion to levels observed in control animals could not be achieved (Fig 1).

It has been shown previously that fructose feeding of rats also leads to resistance to the normal stimulatory effect of insulin on whole-body glucose disposal.[9,10] Of possible relevance to this issue is the report that in muscle the decreased sensitivity of metabolic processes to manipulation by insulin is related to the TAG content of the cell,[41] possibly via the operation of a fatty acid/glucose cycle.[42] We therefore considered the possibility that the decreased sensitivity of liver cells from fructose-fed animals to inhibition of VLDL output by insulin was associated with an increase in TAG content (Table 1). To test this, cells from control-fed and fructose-fed rats were depleted in intracellular TAG by culturing them overnight in the absence of extracellular oleate.[40] In control-fed rats, this treatment led to a decrease in cellular TAG content from 204 ± 33 to 51 ± 8 μg/mg protein. Although these cells secreted less TAG than normally treated cells that contained higher amounts of TAG, there was no difference in the extent of inhibition by insulin (Table 4). The same was true of cells

Table 4. Effects of Fructose Feeding and Cellular TAG Depletion on the Response of VLDL Secretion to Insulin

Insulin Concentration (nmol/L)	Culture Condition	
	Normal	TAG-Depleted
Chow-fed		
0	100	100
7.8	45.7 ± 5.0	51.0 ± 1.8
78	34.4 ± 2.6	39.8 ± 4.7
780	30.2 ± 2.2	31.8 ± 5.3
Fructose-fed		
0	100	100
7.8	64.6 ± 11.3	70.7 ± 8.4*
78	46.4 ± 3.2*	70.7 ± 13.4*
780	45.2 ± 5.1*	60.0 ± 8.3*

NOTE. For control-fed rats, n = 8 for normal and n = 5 for TAG-depleted. For fructose-fed rats, n = 7 for normal and n = 5 for TAG-depleted. Each value is expressed as a percentage of the corresponding amount of TAG secreted in the absence of insulin. Control values: chow-fed, 146 ± 15 and 87 ± 18 μg/mg cell protein for normal and TAG-depleted cells, respectively; fructose-fed, 114 ± 14 and 44 ± 14, respectively.

*$P < .05$ *v* chow-fed.

from fructose-fed rats. It was nevertheless of interest that even under these conditions of TAG depletion, cells from fructose-fed rats remained less sensitive to the inhibitory effects of insulin on the secretion of VLDL TAG (Table 4).

The effects of fructose and sucrose feeding on hepatic apo B secretion are controversial. For instance, although a high sucrose intake led to an increased secretion of newly synthesized apo B in cultured hepatocytes,[39] no changes in the secretion of total apo B were detected in isolated liver preparations from either sucrose-fed[43] or fructose-fed[44] rats. Instead, there was an increase in particle size of the VLDL secreted,[44,45] a suggestion supported by the present observation of an increase in the content of TAG (Fig 1) relative to apo B (Table 2) in VLDL particles secreted by hepatocytes from fructose-fed rats. However, it should be pointed out that the present investigation was limited to studies of changes in VLDL secretion. It remains possible that changes also occurred in the secretion of particles of density greater than 1.006.

The increased secretion of VLDL TAG by hepatocytes from fructose-fed rats could not be maintained during the second day of culture. In the absence of insulin, these rates were (in micrograms per milligram cell protein) 146 ± 15 for control-fed and 114 ± 14 for fructose-fed rats. The relatively low rate of TAG secretion on day 2 in fructose-fed rats did not reflect a depletion in cellular TAG mass (166 ± 20 μg/mg for day 1 *v* 466 ± 67 for day 2). More likely, it resulted from the absence in vitro of factors that in vivo mediate metabolic effects of fructose feeding and that are maintained for a certain period after isolation and culture of the cells. Nevertheless, resistance to the normal inhibitory effects of insulin on the secretion of VLDL TAG (Figs 2 and 3) and of apo B (Table 2 and Fig 5) was retained by these cells during the prolonged culture period. It would therefore appear that this latter effect of fructose feeding is long-lasting and not dependent on continuous exposure to conditions that in vivo initiate the original change.

If these in vitro observations are applicable at the level of the intact animal, they would suggest that in fructose-fed animals insulin was incapable of producing the same fractional decrease in VLDL output as occurred in chow-fed controls. Thus, at any given insulin concentration, fructose-fed animals secrete more VLDL relative to controls than when insulin was not present. It is this relative insensitivity to insulin that contributes to the elevated levels of hepatic VLDL output in fructose-fed animals. A similar insensitivity or resistance to the normal inhibitory effect of insulin on the secretion of VLDL may also explain the high rates of VLDL secretion commonly observed in human subjects with metabolic disturbances associated with insulin resistance, such as obesity and non–insulin-dependent diabetes.[10,46-48] Of particular interest in this respect is the recent report[49] that short-term insulin administration failed to downregulate apo B_{100} production in chronically hyperinsulinemic obese subjects, although there was a 50% decrease in apo B production under the same conditions in normal subjects.

ACKNOWLEDGMENT

We would like to thank M. Barber for typing the manuscript and the Staff of the Medical Illustration Department, John Radcliffe Hospital, Oxford, UK, for preparing the diagrams. Dr C. Bourgeois gave helpful advice on the use of the ANOVA test for statistical analysis of observed differences. G.F.G. is a member of the External Scientific Staff of the Medical Research Council (United Kingdom) at the University of Oxford.

REFERENCES

1. Wright DW, Hansen RI, Mondon CE, et al: Sucrose-induced insulin resistance in the rat: Modulation by exercise and diet. Am J Clin Nutr 38:879-883, 1983

2. Zavaroni I, Sander S, Scott S, et al: Effect of fructose feeding on insulin secretion and insulin action in the rat. Metabolism 29:970-973, 1980

3. Hwang I-S, Ho H, Hoffman BB, et al: Fructose induced insulin resistance and hypertension in rats. Hypertension 10:512-516, 1987

4. Bar-On H, Stein Y: Effect of glucose and fructose administration on lipid metabolism in the rat. J Nutr 94:95-105, 1968

5. Kannan R, Baker N, Bruckdorder KR: Secretion and turnover of very–low-density lipoprotein triacylglycerols in rats fed chronically diets rich in glucose and fructose. J Nutr 111:1216-1223, 1981

6. Kazumi T, Vranic M, Steiner G: Triglyceride kinetics: Effects of dietary glucose, sucrose or fructose alone or with hyperinsulinaemia. Am J Physiol 250:E325-E330, 1986

7. Bruckdorfer KR, Baker N: Relationships among hepatic lipogenesis, hepatic triacylglycerol secretion and hypertriglyceridaemia in rats fed chronically on fructose- or glucose-rich fat-free diets. Biochem Soc Trans 15:940-951, 1987

8. Sleder J, Chen Y-DI, Cully MD, et al: Hyperinsulinemia in fructose-induced hypertriglyceridemia in the rat. Metabolism 29: 303-305, 1980

9. Zavaroni I, Chen Y-DI, Mondon CE, et al: Ability of exercise to inhibit carbohydrate-induced hypertriglyceridemia in rats. Metabolism 30:476-480, 1981

10. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

11. Yamamato M, Yamamoto I, Tanaka Y, et al: Fatty acid metabolism and lipid secretion by perfused livers from rats fed laboratory stock and sucrose-rich diets. J Lipid Res 28:1156-1165, 1987

12. Gibbons GF, Burnham FJ: Effect of nutritional state on the utilization of fatty acids for hepatic triacylglycerol synthesis and secretion as very–low-density lipoprotein. Biochem J 275:87-92, 1991

13. Hirano T, Mamo JCL, Poapst ME, et al: Impaired very–low-density lipoprotein triglyceride catabolism in acute and chronic fructose-fed rats. Am J Physiol 256:E559-E565, 1989

14. Mamo JCL, Hirano T, James L, et al: Partial characterization of the fructose-induced defect in very–low-density lipoprotein triglyceride metabolism. Metabolism 40:888-893, 1991

15. Vogelberg KH, Gries FA, Moschinski D: Hepatic production of VLDL triglycerides. Dependence of portal substrate and insulin concentration. Horm Metab Res 12:688-694, 1980

16. Durrington PN, Newton RS, Weinstein DB, et al: Effects of insulin and glucose on very–low-density lipoprotein triglyceride secretion by cultured rat hepatocytes. J Clin Invest 70:63-73, 1982

17. Patsch W, Franz S, Schonfeld G: Role of insulin in lipopro-

tein secretion by cultured hepatocytes. J Clin Invest 71:1161-1174, 1983

18. Pullinger CR, Gibbons GF: Effects of hormones and pyruvate on the secretion of very–low-density lipoprotein triacylglycerol and cholesterol by rat hepatocytes. Biochim Biophys Acta 833:44-51, 1985

19. Mangiapane EH, Brindley DN: Effects of dexamethasone and insulin on the synthesis of triacylglycerols and phosphatidylcholine and the secretion of very–low-density lipoproteins and lysophosphatidylcholine by monolayer cultures of rat hepatocytes. Biochem J 233:151-160, 1986

20. Sparks CE, Sparks JD, Bolognino M, et al: Insulin effects on apolipoprotein B lipoprotein synthesis and secretion by primary cultures of rat hepatocytes. Metabolism 35:1128-1136, 1986

21. Patsch W, Gotto AM Jr, Patsch JR: Effects of insulin on lipoprotein secretion in rat hepatocyte cultures. The role of the insulin receptor. J Biol Chem 261:9603-9606, 1986

22. Dashti N, Williams DL, Alaupovic P: Effects of oleate and insulin on the production rates and cellular mRNA concentration of lipoproteins in HEP-G2 cells. J Lipid Res 30:1365-1373, 1989

23. Bartlett SM, Gibbons GF: Short- and longer-term regulation of very–low-density lipoprotein secretion by insulin, dexamethasone and lipogenic substrates in cultured hepatocytes. A biphasic effect of insulin. Biochem J 249:37-43, 1988

25. Amatruda JM, Newmeyer HW, Chang CL: Insulin-induced alterations in insulin-binding and insulin action in primary cultures of rat hepatocytes. Diabetes 31:145-148, 1982

26. Gibbons GF: Insulin, diabetes and very–low-density lipoprotein metabolism. Biochem Soc Trans 17:49-51, 1989

27. Gibbons GF: Assembly and secretion of hepatic very–low-density lipoprotein. Biochem J 268:1-13, 1990

28. Jackson TK, Salhanick AI, Elovson J, et al: Insulin regulates apolipoprotein B turnover and phosphorylation in rat hepatocytes. J Clin Invest 86:1746-1751, 1990

29. Howard BV: Lipid metabolism in diabetes mellitus. J Lipid Res 28:613-627, 1987

30. Reaven GM, Chen Y-DI: Role of insulin in regulation of lipoprotein metabolism in diabetes. Diabetes Metab Rev 4:639-652, 1988

31. Kostner GM, Karádi I: Lipoprotein alterations in diabetes mellitus. Diabetologia 31:717-722, 1988

32. Duerden JM, Bartlett SM, Gibbons GF: Long-term maintenance of high rates of very–low-density lipoprotein secretion in hepatocyte cultures. A model for studying the direct effects of insulin and insulin deficiency in vitro. Biochem J 263:937-943, 1989

33. Björnsson OG, Duerden JM, Bartlett SM, et al: The role of pancreatic hormones in the regulation of lipid storage, oxidation and secretion in primary cultures of rat hepatocytes. Short- and long-term effects. Biochem J 281:381-386, 1992

34. Trinder P: Determination of glucose in blood using glucose-oxidase with an alternative oxygen acceptor. Ann Clin Biochem 6:24-30, 1969

35. Goldfarb S, Barber TA, Pariza MA, et al: Lipid synthesis and ultrastructure of adult rat hepatocytes during their first twenty-four hours in culture. Exp Cell Res 117:39-46, 1978

36. Markwell MAK, Haas SM, Bieber LL, et al: A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 87:206-210, 1978

37. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951

38. Van Harken DR, Dixon CW, Heimberg M: Hepatic lipid metabolism in experimental diabetes. The effect of concentration of oleate on metabolism of triglyceride and on ketogenesis. J Biol Chem 244:2278-2285, 1969

39. Boogaerts JR, Malone-McNeal M, Archambault-Schexnayder J, et al: Dietary carbohydrate induces lipogenesis and very–low-density lipoprotein synthesis. Am J Physiol 246:E77-E83, 1984

40. Gibbons GF, Bartlett SM, Sparks CE, et al: Extracellular fatty acids are not utilized directly for the synthesis of very–low-density lipoprotein in primary cultures of rat hepatocytes. Biochem J 287:749-753, 1992

41. Storlien LH, Jenkins AB, Chisholm DL, et al: Relationships of muscle triglyceride and ω-3 fatty acids in muscle phospholipid. Diabetes 40:280-289, 1991

42. Randle PJ, Kerbey AL, Espinal J: Mechanisms in decreasing glucose oxidation in diabetes and starvation. Role of lipid fuels and hormones. Diabetes Metab Rev 4:623-638, 1988

43. Strobl W, Gorder NL, Fienup GA, et al: Effects of sucrose diet on apolipoprotein biosynthesis in rat liver. J Biol Chem 264:1190-1194, 1989

44. Witztum JL, Schonfeld G: Carbohydrate diet–induced changes in very–low-density lipoprotein composition and structure. Diabetes 27:1215-1229, 1978

45. Kazumi T, Yoshino G, Matsuba K, et al: Effects of dietary glucose or fructose on the secretion rate and particle size of triglyceride-rich lipoprotein in Zucker fatty rats. Metabolism 40:962-966, 1991

46. Reaven GM, Greenfield MS: Diabetic hypertriglyceridaemia. Evidence for three clinical syndromes. Diabetes 30:66-75, 1981

47. Kesäniemi YA, Beltz WF, Grundy SM: Comparison of metabolism of apoprotein B in normal subjects, obese patients, and patients with coronary heart disease. J Clin Invest 76:586-595, 1985

48. Abbott WGM, Lillioja S, Young AA, et al: Relationships between plasma lipoprotein concentrations and insulin action in an obese hyperinsulinaemic population. Diabetes 36:897-904, 1987

49. Lewis GF, Uffelman KD, Szeto LW, et al: The effects of acute hyperinsulinaemia on very low density lipoprotein (VLDL) triglyceride and VLDL apolipoprotein (apo) B production in normal weight and obese individuals. Diabetes 42:833-842, 1993