Inhibition of Hepatic Very–Low-Density Lipoprotein Secretion in Obese Zucker Rats Adapted to a High-Protein Diet

Athina-Despina Kalopissis, Geneviève Griffaton, and Daniel Fau

The effect of a high-protein (HP) diet on hepatic very–low-density lipoprotein (VLDL) secretion was studied in obese and lean Zucker rats. With the control (C) diet, isolated hepatocytes from obese as compared with lean rats displayed higher uptake of [1-^{14}C]oleate 0.7 mmol/L, 95% of which was esterified to glycerolipids; greater oleate incorporation into VLDL-triacylglycerol (TG); 2.6 times higher total VLDL-TG secretion; and 11-fold higher de novo fatty acid synthesis. Adaptation to HP feeding decreased weight gains in both phenotypes and hepatocyte TG content in obese rats. Oleate uptake by hepatocytes was appreciably reduced in the obese phenotype only. Despite esterification rates similar to those for the C diet, oleate incorporation into VLDL-TG decreased by 34% and 55% in obese and lean rats, respectively. Total (mass) VLDL-TG secretion was drastically decreased by 65% and 48% in obese and lean rat hepatocytes, respectively. HP feeding combined with overnight fasting accentuated the above decreases. Fatty acid synthesis was 50% lower in cells from HP-fed obese rats, but increased 1.7-fold in lean ones. Plasma glucagon increased in both phenotypes under HP feeding, whereas plasma insulin either increased (obese) or decreased (lean), with the insulin to glucagon ratio slightly decreasing. Thus, HP feeding drastically inhibited hepatic VLDL secretion in obese and lean Zucker rats by an undefined mechanism that was apparently related neither to de novo fatty acid synthesis nor to changes in oleate partitioning between esterification and oxidation.

HUMAN OBESITY is often accompanied by hypertriglyceridemia, and both metabolic disorders are considered potential risk factors for coronary artery disease. High-protein (HP) diets have been administered in the past two decades to combat obesity,[1,2] since they place a large energy demand on the organism consuming them. Some of their metabolic consequences, such as increased ureagenesis and gluconeogenesis,[3-6] decreased lipogenesis,[7] and increased plasma glucagon,[4,5,7] have been assessed in rats. Surprisingly, the effects of HP diets on lipoprotein metabolism remain largely unknown.

In the present study we used the Zucker rat strain as an animal model combining obesity and hypertriglyceridemia. The hepatic lipid metabolism of obese Zucker rats displays severe abnormalities such as extremely high rates of de novo fatty acid synthesis,[8-10] very low ketogenesis,[8-12] and twofold to threefold enhanced very–low-density lipoprotein (VLDL) secretion,[10,12,13] which is the main cause of their hypertriglyceridemia, since their intestinal VLDL production is normal[14] and their lipolytic system is normally functional.[15] Our aim was to investigate whether adaptation to a HP diet would decrease the excessive hepatic VLDL production of obese Zucker rats, resulting in the lower plasma triacylglycerol (TG) concentration reported by Peret et al.[16] Obese and lean Zucker rats were therefore adapted for 2 weeks to either a control (C) or HP diet, and VLDL secretion rates were measured on freshly isolated hepatocytes. Cell incubations in the presence of [1-^{14}C]oleate 0.7 mmol/L (the plasma concentration of obese rats fed a standard diet) or tritiated water permitted measurements of exogenous fatty acid metabolism or de novo fatty acid synthesis, respectively, as well as evaluation of relative contributions of exogenous and endogenous fatty acids to VLDL-TG.

MATERIALS AND METHODS

Animals and Diets

Male obese (fa/fa) and lean (Fa/?) rats of the Zucker strain were purchased from CSEAL-CNRS (Orléans-la-Source, France) at 12 weeks of age (weight of lean rats, 240 to 260 g; weight of obese rats, 280 to 300 g). On arrival, they were housed in individual anodized wire-bottomed cages at 23 ± 2°C under a 12-hour light cycle (lights on at 7 AM). They were divided into two groups on the basis of comparable weights, with one group being allotted to a C (high-carbohydrate) diet and the other to a HP (carbohydrate-free) diet. The C diet contained 24% casein, 63% corn starch, 2% peanut oil, 6% salt mixture, 1% vitamin mixture, and 4% cellulose. The HP diet contained 85% casein, 8% peanut oil, 4% salt mixture, 1% vitamin mixture, and 2% cellulose. Both diets were administered ad libitum for 2 weeks. Half the animals maintained on the HP diet were fasted overnight (16 hours) before the hepatocytes were isolated. Thus, a total of 12 obese and 12 lean rats were used as liver donors, namely four rats of each phenotype fed the C diet, four fed the HP diet ad libitum, and four fed the HP diet and fasted overnight.

Hepatocyte Incubations

Two hepatocyte preparations, one from an obese and one from a lean rat with the same nutritional regimen and state, were studied in parallel on the same day. Rats were anesthetized with sodium pentobarbital (5 mg/100 g body weight). Hepatocytes were isolated by liver perfusion with collagenase (Boehringer, Mannheim, Germany) according to the method of Seglen.[17] After appropriate washes to eliminate cell debris, endothelial cells, and Kupffer's cells, hepatocytes were suspended in Krebs-Henseleit bicarbonate buffer containing 1% dialyzed fatty acid–free albumin (Sigma, St Louis, MO), pH 7.45, which was used as the incubation medium. The cell viability (85% to 95% trypan blue exclusion) and yield of cells were the same for the six groups of rats. Incubation conditions have been previously described.[18,19] Briefly, 20 × 10^6 hepatocytes were incubated in duplicate in 4 mL incubation medium supplemented with glucose (20 mmol/L) and aprotinin (0.7 TIU). A

From INSERM U 177, Institut Biomédical des Cordeliers, Paris; and INSERM U 24, Hôpital Beaujon, Clichy, France.

Submitted October 5, 1992; accepted April 9, 1994.

Supported in part by the Centre National de la Recherche Scientifique (D.F.).

Address reprint requests to Athina-Despina Kalopissis, PhD, URA 1283 CNRS, Institut Biomédical des Cordeliers, 15, rue de l'Ecole de Médecine, Paris 75006.

0026-0495/95/4401-0006$03.00/0

portion of the cells was incubated with [1-^{14}C]oleic acid (specific activity, 55 mCi/mmol; CEA, Saclay, France). [1-^{14}C]oleate was mixed with sodium oleate (Sigma) and added once in the medium at the start of incubations (0 minutes) to obtain an initial concentration of 2 μCi/0.7 mmol/L. The molar ratio of oleate to albumin was 4.7. In another series of vials, tritiated water (specific activity, 10.8 mCi/mL; CEA) was added at 0 minutes (0.5 mCi per vial) to measure de novo fatty acid synthesis (in the absence of oleate). The supernatant from one of the duplicate vials for each time point was directly extracted for lipids (as described below), and the other was ultracentrifuged at $1.4 \times 10^8 \times g \cdot$ min to obtain VLDL. The specific activity of the 0.7-mmol/L [1-^{14}C]oleate added into the incubation medium was used to convert the disintegrations per minute of ^{14}C-labeled substrates into nanomoles of oleate. The specific activity of tritiated water added into the medium was used to convert the disintegrations per minute of ^{3}H-labeled lipids into nanomoles of palmitate (the end product of de novo fatty acid synthesis) as previously described.[18]

Analytical Methods

The washed cell pellets, incubation media, and VLDL fractions were extracted separately for lipids, and the lipid phase was washed extensively with methanol/water (1:1 vol/vol) to remove aqueous contamination as previously described.[18] [1-^{14}C]oleate-labeled lipid extracts were subjected to thin-layer chromatography,[19] and the radioactivity of lipid spots was counted in toluene scintillation medium. ^{3}H-labeled lipid levels were measured after a 2-hour saponification with ethanolic KOH at 80°C followed by extraction with petroleum ether.[18] Hepatocyte TG content was determined enzymatically (Boehringer kit no. 124032) after phospholipid (PL) elimination using silicic acid (325 mesh, Sigma; activated 2 hours at 110°C). We also measured cellular PL[20] and free and esterified cholesterol (CS)[21] levels after elution through Sep-pak columns (Waters, Millipore, Bedford, MA).

Hormonal Assays

The methods for determining insulin and glucagon levels have been described.[16] These hormone levels were measured in obese and lean rats fed the C or HP diet ad libitum. Blood was withdrawn from the abdominal aorta of rats under sodium pentobarbital anesthesia and centrifuged at 4°C (2,500 × *g*, 20 minutes) in the presence of 400 UIP/mL iniprol (Sanofi-Winthrop, Unité Choay, Gentilly, France) and 2 mg/mL EDTA. Aliquots of the plasma were stored at −80°C until needed for the assays. The radioimmunoassay for insulin was performed with ^{125}I-insulin (CIS-BioInternational, Gif-sur-Yvette, France), and for glucagon, with ^{125}I-glucagon (Pharmacia-Serano Diagnostics, St Quentin en Yvelines, France).

Statistical Analysis

Results are expressed as the mean ± SE. Data were submitted to ANOVA after assessing the normality of distribution and homogeneity of variance between the six hepatocyte groups studied, and comparisons were made by Fisher's F test. Linear regression analyses were performed for the various parameters studied as a function of time.

RESULTS

Body Weight and Hepatocyte Lipid Composition

Lean and particularly obese rats gained weight rapidly on the C diet. On the contrary, modest weight gains were noted on the HP diet (Table 1). Under any dietary condition, body weights of lean rats were lower than those of the corresponding obese animals. HP feeding significantly decreased body weights of fed and fasted lean rats and fasted obese rats.

The major differences in hepatocyte lipid composition concerned TG content (Table 1). As expected, cellular TG was considerably greater in obese as compared with lean animals under the three nutritional conditions studied. Adaptation to the HP diet greatly reduced hepatocyte TG of obese rats ($P < .01$). Surprisingly, in the lean phenotype cellular TG was higher when animals were fed the HP diet ad libitum and was reduced to control levels by overnight fasting.

Hepatocyte PL and free CS contents were similar between the six groups of cells. Esterified CS was higher in obese as compared with lean rats under any of the three dietary conditions studied.

Hepatocyte Metabolism of [1-^{14}C]oleate

To study the effect of the HP diet on the metabolic fate of oleate, a representative fatty acid, its cellular uptake, esterification to neutral lipids (TG, PL, and CS esters), and incorporation into VLDL were measured in hepatocytes from the six experimental groups and are illustrated in Figs 1 to 3. The results were calculated as nanomoles of oleate by using the specific activity of the 0.7-mmol/L [1-^{14}C]ole-

Table 1. Effect of the HP Diet on Body Weight and Hepatocyte Lipid Composition

	Body Weight (g)		μg per 10^6 Cells			
Phenotype	Starting	Final	TG	PL	Free CS	CS Ester
C diet, fed state						
Obese (n = 4)	289.4 ± 10.2	405.0 ± 24.1	312.9 ± 18.0	340.5 ± 14.5	19.2 ± 1.4	3.8 ± 0.4
Lean (n = 4)	250.0 ± 7.9*	331.0 ± 10.7*	30.9 ± 2.2‡	270.6 ± 12.1*	15.6 ± 1.3	1.2 ± 0.4†
HP diet, fed state						
Obese (n = 4)	290.0 ± 1.01	341.0 ± 14.4	176.5 ± 24.3‖	277.1 ± 25.2	14.2 ± 0.9§	3.5 ± 1.1
Lean (n = 4)	251.0 ± 7.4*	286.5 ± 8.8*§	50.4 ± 2.7‡‖	325.7 ± 17.3§	14.4 ± 0.5	1.7 ± 0.9
HP diet, fasted state						
Obese (n = 4)	290.4 ± 8.2	329.5 ± 7.3§	195.5 ± 10.8‖	294.4 ± 25.6	15.3 ± 2.0	2.9 ± 0.3
Lean (n = 4)	249.5 ± 7.7*	263.3 ± 8.3‡‖	24.9 ± 1.0‡	310.3 ± 14.4	14.3 ± 1.7	1.6 ± 0.3*

NOTE. Values for TG, PL, free CS, and CS ester were measured at 0 minutes and are the mean ± SE. Statistical significance was calculated by Fisher's F test after ANOVA.

*$P < .05$, †$P < .01$, ‡$P < .001$: obese *v* lean rats with the same nutritional state.

§$P < .05$, ‖$P < .01$, ¶$P < .001$: for the same phenotype, as compared with the C diet.

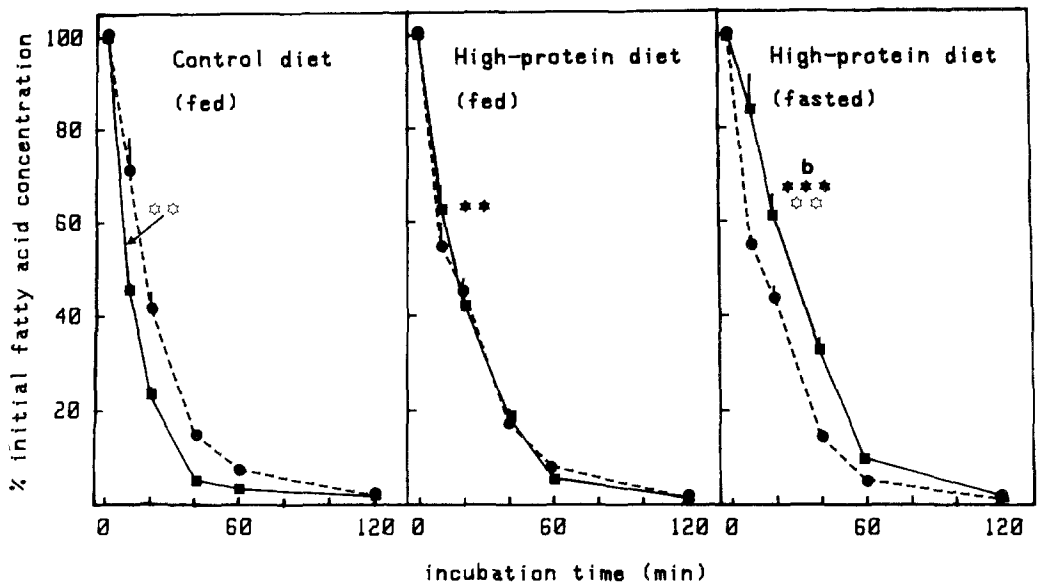

Fig 1. [1-^{14}C]oleate disappearance from the incubation medium. Hepatocytes of obese and lean Zucker rats fed ad libitum the C diet, HP diet, or HP diet followed by an overnight fast were incubated up to 120 minutes in the presence of [1-^{14}C]oleate 0.7 mmol/L. Results are expressed as the percentage of initial oleate concentration (100% at 0 minutes) and are the mean ± SE of four individual hepatocyte preparations. When not shown, SE values were smaller than the symbols. (■) Obese rats; (●) lean rats. Statistically significant differences between the six experimental groups were calculated by the F test after ANOVA: ☆, obese *v* lean rats under the same dietary condition; ★, obese (or lean) rats fed the HP diet ± overnight fast as compared with the C diet. *$P < .05$; **$P < .01$; *** $P < .001$. [a]$P < .05$; [b]$P < .01$; [c]$P < .001$: Obese (or lean) rats fed the HP diet and fasted overnight *v* obese (or lean) rats fed the HP diet ad libitum.

ate preparation. To normalize results between individual experiments with separate oleate preparations, all results presented in Figs 1 through 3 were expressed as a percentage of the initial (0 minutes) medium oleate concentration in each group.

[1-^{14}C]oleate uptake. The disappearance of [1-^{14}C]oleate from the incubation medium (Fig 1) served as an indirect estimation of its uptake by the cells. It was thus considered that oleate uptake at a given time point corresponded to the difference between the fatty acid concentration in the medium at 0 minutes and the concentration at the given time point. Since hepatocytes took up exogenous fatty acid fairly rapidly, comparisons between phenotypes and diets were meaningful and clear-cut essentially up to 40 minutes. Oleate uptake by lean rat cells was similar under all dietary conditions. On the contrary, oleate uptake by hepatocytes of obese rats displayed two unusual features: (1) with the C diet it was higher than that of lean rat cells ($P < .01$); and (2) it decreased considerably with the HP diet administered ad libitum ($P < .01$ relative to the C diet) and even more after the overnight fast ($P < .001$ as compared with the C and HP diets administered ad libitum). Thus, hepatocytes from obese rats fed the C diet, HP diet, or HP diet + overnight fasting took up over 20 minutes of incubation 78%, 58%, or 39%, respectively, of the initial oleate content in the medium. The greater fatty acid uptake of obese rat cells with the C diet was therefore completely abolished by the HP diet.

[1-^{14}C]oleate incorporation into hepatocyte glycerolipids. All six groups of hepatocytes rapidly metabolized oleate, since less than 1.5% of total lipid label was in the form of free fatty acids at all time points. This observation is indicative of the normal metabolic capacities of our cell preparations. Table 2 shows the percentage of incorporation of [1-^{14}C]oleate into total glycerolipids and its partition between lipid classes separated by thin-layer chromatography. Figure 2 shows the nanomoles of 0.7-mmol/L [1-^{14}C]oleate incorporated into TG and PL (expressed as a percentage of the nanomoles of oleate present in the medium at 0 minutes).

Table 2. Incorporation of [1-^{14}C]Oleate Into Hepatocyte Glycerolipids (%)

Phenotype	Total Glycerolipids*	PL†	MG + DG†	TG†	CS Ester†
C diet, fed state					
Obese (n = 4)	95	8.2	2.6	88.3	0.9
Lean (n = 4)	65	17.9	3.1	78.0	1.0
HP diet, fed state					
Obese (n = 4)	95	10.4	3.9	85.1	0.6
Lean (n = 4)	55	20.0	4.0	75.0	1.0
HP diet, fasted state					
Obese (n = 4)	70	12.0	2.4	84.8	0.8
Lean (n = 4)	40	30.9	2.4	65.7	1.0

Abbreviations: MG, monoacylglycerol; DG, discylglycerol.

*Values are the sum of all cellular labeled glycerolipids (PL, MG, DG, TG, and CS ester) after 10- and 20-minute incubations and are expressed as the percentage of [1-^{14}C]oleate taken up by the cells. Since secretion of labeled VLDL begins at 20 minutes, subsequent time points were not used for this calculation.

†Values are the percentages of the total cellular glycerolipids. They were also calculated after 10- and 20-minute incubations, so as to be minimally affected by the different turnover rates of TG and PL in the hepatocytes and thus reflect more accurately oleate partitioning between TG and PL.

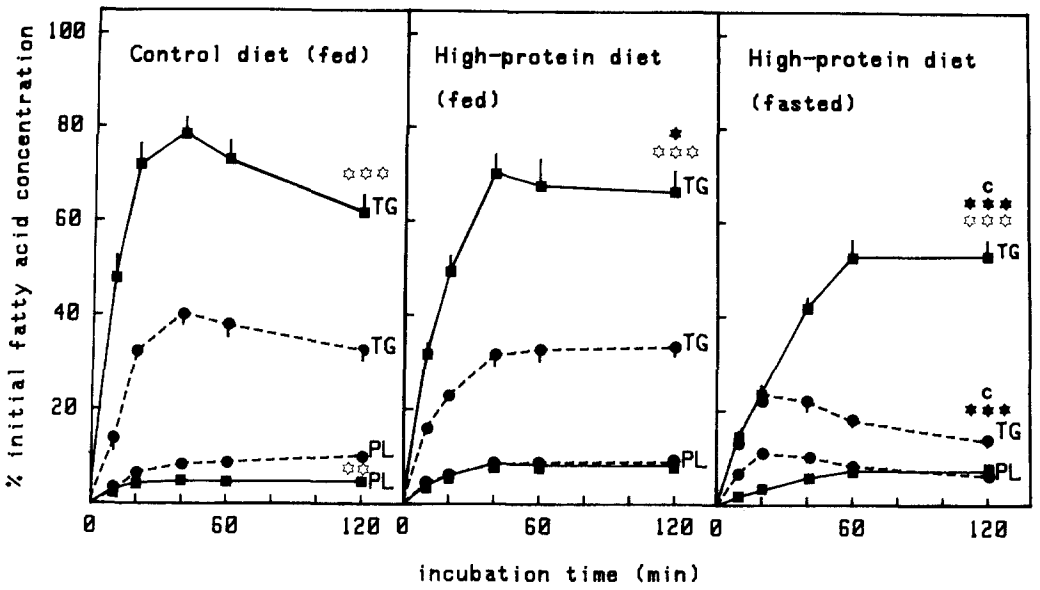

Fig 2. Incorporation of [1-^{14}C]oleate into cellular TG and PL. Experiments are the same as those described in Fig 1. Descriptions, statistical analyses, and symbols for statistically significant differences are as in Fig 1.

Hepatocytes from obese animals are characterized by an exacerbated capacity to esterify fatty acids. In the present experiments, 95% of [1-^{14}C]oleate taken up was esterified to glycerolipids when obese rats were fed the C or HP diet ad libitum (Table 2). Moreover, these cells converted the major part of oleate to TG (85% to 89%) at the expense of PL. Overnight fasting of HP-fed obese rats reduced total glycerolipid synthesis to 70% of the amount taken up, but did not affect oleate partitioning between TG and PL.

On the contrary, hepatocytes from lean rats fed the C diet esterified only 65% of the oleate taken up and partitioned it more equally between TG and PL. When lean rats were fed the HP diet ad libitum or fasted overnight, total glycerolipid formation was decreased, essentially through a lower TG synthesis (which decreased in the order C diet > HP diet > HP diet + overnight fast), whereas PL synthesis from exogenous oleate was similar in the three groups of lean rat cells.

Figure 2 shows the incorporation of [1-^{14}C]oleate into hepatocyte TG and PL over the 2-hour incubations. Hepatocytes from obese rats synthesized at least two times more radioactive TG than those from corresponding lean animals under all three nutritional conditions ($P < .001$), but similar or lower amounts of labeled PL. With the C diet, the sharp increase of radioactive TG and PL between 0 and 20 minutes concords well with the rapid disappearance of oleate from the medium as shown in Fig 1. By 40 minutes, practically all oleate has disappeared from the medium and cellular TG begins to decline due to its concomitant secretion as VLDL-TG into the medium (Fig 3), while radioactive PL (accounting for only 3.4% of radioactive VLDL lipids, as described below) has plateaued. With the HP diet, esterification of [1-^{14}C]oleate to TG and PL was clearly delayed in cells from fed obese rats over the first 20 minutes ($P < .01$) and in cells from fasted rats over the first 40 minutes ($P < .001$), probably as a consequence of decreased uptake (Fig 1). On the other hand, the slower decrease of hepatocyte TG levels after 60 minutes may result from their lower incorporation into VLDL (described below). In lean rat hepatocytes, the synthesis of radioactive TG and PL reflected essentially their esterification capacity, since they displayed similar oleate uptake rates irrespective of diet (Fig 1).

[1-^{14}C]oleate incorporation into VLDL. VLDL of obese rats adapted to either the C or HP diet contained more labeled TG and less labeled PL (94% and 3.4%, respec-

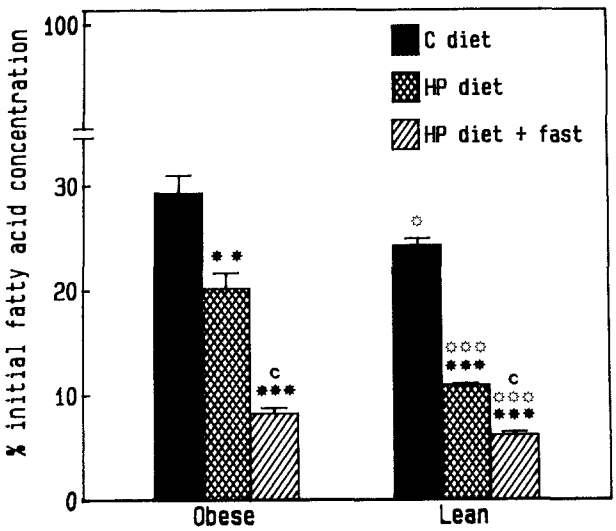

Fig 3. Incorporation of [1-^{14}C]oleate into VLDL-TG. Experiments are the same as those described in Fig 1. Here we represent ^{14}C-labeled VLDL-TG after 120-minute incubations. Statistical significance of differences between means was calculated by Student's *t* test. Symbols for statistical significance are as in Fig 1.

tively, of total VLDL lipids) than VLDL of corresponding lean animals (91% TG and 6.7% PL). VLDL isolated by ultracentrifugation from the medium after 10-minute incubations contained no trace of radioactivity. Radioactive VLDL appeared in the medium from 20 minutes onward and was secreted linearly throughout the 120-minute incubations in all experimental groups, as expected.[17] Since TG is by far the major VLDL lipid, we have estimated VLDL secretion rates by their TG. The calculated regression lines for labeled VLDL-TG were as follows for obese rats fed ad libitum: C diet, $y = 0.280x - 3.082$ ($r = .971$); HP diet, $y = 0.200x - 3.764$ ($r = .995$); HP diet followed by overnight fasting, $y = 0.080x - 1.150$ ($r = .948$). The calculated regression lines for radioactive VLDL-TG were as follows for lean animals fed ad libitum: C diet, $y = 0.236x - 3.310$ ($r = .986$); HP diet, $y = 0.108x - 1.778$ ($r = .987$); HP diet followed by overnight fasting, $y = 0.058x - 0.711$ ($r = .977$).

With the C diet, hepatocytes from obese and lean rats exported over 120 minutes into the medium as VLDL-TG 30% and 24%, respectively, of the initial medium oleate content (Fig 3). Although statistically significant ($P < .05$), this difference between the two phenotypes is of small magnitude.

The HP diet decreased the incorporation of exogenous oleate into VLDL-TG of obese animals by 34% ($P < .01$) and 72% ($P < .001$) when they were fed ad libitum or fasted overnight, respectively (Fig 3). The inhibition of VLDL production by HP feeding was more pronounced in lean rat cells. Thus, a 55% ($P < .001$) and 75% ($P < .001$) reduction in labeled VLDL-TG secretion was measured under ad libitum feeding and after overnight fasting, respectively.

Total VLDL Secretion

Total (labeled + unlabeled) VLDL-TG was present in the medium from the beginning of the incubations (0 minutes) and increased linearly with time in all groups of hepatocytes (Fig 4).

Hepatocytes from obese animals fed the C diet secreted 2.6 times more VLDL-TG than cells from lean ones, as expected ($P < .001$). Adaptation to the HP diet induced a pronounced decrease of total VLDL secretion in both phenotypes. VLDL secretion was inhibited by 65% ($P < .001$) and 71% ($P < .001$) in obese animals fed ad libitum and fasted overnight, respectively. Corresponding decreases in the lean phenotype were 48% ($P < .05$) and 78% ($P < .001$). Obviously, obese rat cells were more affected by the HP diet than by the 16-hour fast, whereas lean ones were apparently equally affected by the HP diet and food deprivation.

De Novo Fatty Acid Synthesis

De novo fatty acid synthesis was measured only in rats fed ad libitum the C or HP diet. With the C diet, obese rat cells synthesized 11 times more fatty acids than lean ones over 120 minutes (Table 3). Adaptation to the HP diet resulted in a 47% decreased de novo fatty acid synthesis in hepatocytes from obese rats. Surprisingly, the HP diet increased this pathway 1.7-fold in lean rat cells. All the same, the phenotype effect prevailed, since hepatocytes from obese animals still synthesized 3.5-fold more fatty acids than cells from lean rats.

The proportion of newly synthesized tritiated fatty acids appearing in the medium as VLDL-TG relative to total

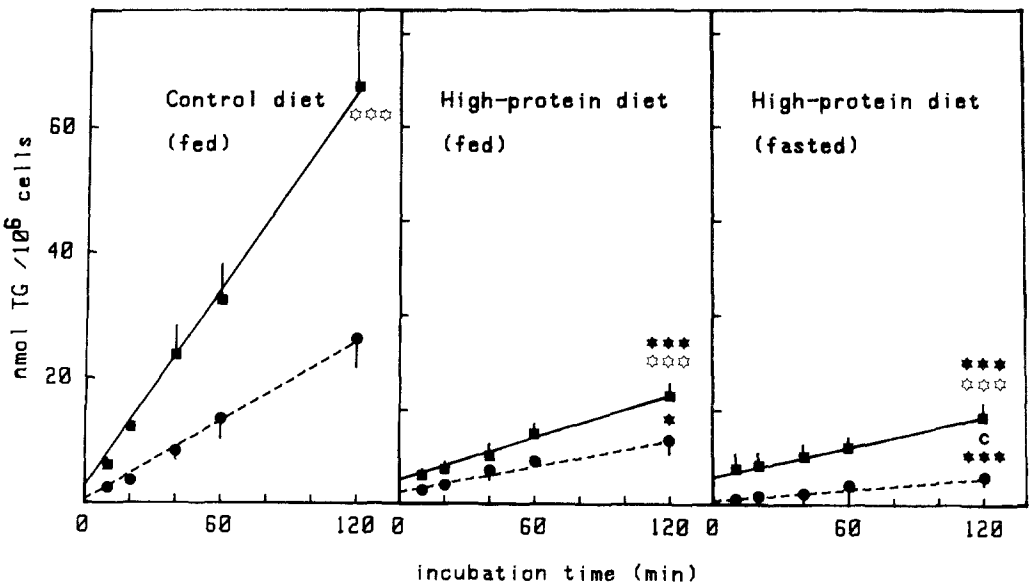

Fig 4. Total VLDL-TG secretion. The total VLDL-TG level was measured with the same hepatocyte preparations as in the three preceding figures. Here we represent the calculated regression lines and experimental values (mean ± SE) for each time point. Descriptions, statistical analyses, and symbols for statistically significant differences are as in Fig 1. Equations for regression lines are as follows for the (●) lean rats: C diet, $y = 0.210x + 0.568$ ($r = .909$); HP diet administered ad libitum, $y = 0.090x + 2.623$ ($r = .853$); HP diet + overnight fast, $y = 0.040x + 0.955$ ($r = .783$). For the (■) obese rats, regression line equations are as follows: C diet, $y = 0.524x + 2.692$ ($r = .906$); HP diet administered ad libitum, $y = 0.147x + 5.233$ ($r = .882$); HP diet + overnight fast, $y = 0.108x + 5.946$ ($r = .719$).

Table 3. Total Hepatocyte De Novo Fatty Acid Synthesis

Phenotype	C Diet	HP Diet
Obese	45.1 ± 2.8 (4)	23.7 ± 5.5 (4)‡
Lean	4.0 ± 0.4 (4)†	6.8 ± 1.0 (4)*‡

NOTE. Hepatocytes from rats fed ad libitum the C or HP diet were incubated for 2 hours in the presence of 0.5 mCi tritiated water. ^{3}H-labeled lipid levels were measured separately on cell pellets and VLDL fractions. Here we represent the sums of ^{3}H-lipids in [cells + VLDL]. Results are presented as nmol palmitate/10^6 cells/120 min. Values are the mean ± SE for four rats used as hepatocyte donors. Statistical significance was calculated by ANOVA followed by Fisher's F test: *P < .05, †P < .001: obese *v* lean rats fed the same diet.

‡P < .05 for the same phenotype with the HP as compared with the C diet.

tritiated fatty acids (in medium + cells) was also higher in obese rats (11.5% and 10.6% with the C and HP diets, respectively, as compared with 3.2% and 5.6% in lean rats fed the C and HP diets, respectively).

Contribution of Exogenous and Endogenous Fatty Acids to VLDL-TG

The respective contributions of exogenous ^{14}C-oleate and endogenous ^{3}H-fatty acids to VLDL-TG were assessed by the ratio of ^{14}C- or ^{3}H-labeled VLDL-TG fatty acids to total VLDL-TG × 3. VLDL-TG was multiplied by 3, since each TG molecule carries three fatty acid molecules.

In lean rats, the endogenous fatty acid contribution to VLDL-TG did not exceed 1%, whereas exogenous fatty acids accounted for 30% of VLDL-TG irrespective of the diet. In obese rats, the endogenous fatty acid contribution to VLDL-TG was 3% to 4%, and [1-^{14}C]oleate accounted for 30% of VLDL-TG after HP feeding but only for 15% with the C diet. Obviously, the majority of VLDL-TG in both phenotypes and diets originated from the cytoplasmic TG storage pool.

Plasma Insulin and Glucagon

As expected, obese rats had very high plasma insulin concentrations with the C diet and slightly higher ones with the HP diet (Table 4). In lean rats, insulinemia decreased after HP feeding. Plasma glucagon increased appreciably after adaptation to the HP diet in both phenotypes, particularly in obese rats (P < .01). Thus, the insulin to glucagon molar ratio decreased in both obese and lean rats adapted to the HP diet.

DISCUSSION

In the present study, we investigated the effect of a HP diet on the hepatic VLDL production of obese and lean Zucker rats and demonstrated that it was drastically reduced in both phenotypes. Thus, the 40% lower plasma TG reported for HP-fed obese Zucker rats by Peret et al[16] can be accounted for by the 65% decreased hepatic VLDL-TG secretion measured in this study. Although an additional effect of HP feeding on plasma VLDL clearance cannot be ruled out, our data point to a direct inhibitory effect of HP feeding on hepatocyte VLDL synthesis and/or secretion, by an as-yet-unknown mechanism. In severely obese subjects, protein-supplemented fasting (ie, a low-calorie protein diet) also decreased serum TG by 35% and serum cholesterol by 26% despite an increased plasma free fatty acid concentration, suggesting that VLDL secretion was also reduced in humans ingesting high protein amounts.[2]

The experimental system used was that of freshly isolated hepatocytes in suspension and not in primary culture, for the following reasons: First, isolated hepatocytes are the system of choice for studies of short incubation periods (such as the 10- to 120-minute kinetic study reported here). Conversely, cultured hepatocytes have to be plated for at least 4 hours before use and are thus more appropriate for longer-term studies such as hormonal studies often conducted for 3 days. Second, hepatocytes in suspension maintain a high capacity to synthesize and secrete VLDL,[18,19] whereas cells in primary culture secrete less VLDL, with this secretion further decreasing with increasing age of the culture.[22] Third, the incubation medium of hepatocytes in suspension is a simple Krebs-Ringer bicarbonate-buffered saline containing 20 mmol/L glucose. On the contrary, hepatocytes in primary culture de-differentiate rapidly and are therefore plated and maintained in a more complex medium containing hormones (insulin, glucocorticoids). Thus, some results obtained with cultured hepatocytes are more difficult to interpret, since they may have been due to the presence of hormones.

Several metabolic parameters affected by the HP diet may be related to the hepatic VLDL synthetic and secretory pathway. Regarding the food/caloric intake, both obese and lean animals gained less weight on the HP diet (as also reported by Peret et al[16]), probably through decreased food consumption and increased energy demand.[1-4] According to Wangsness et al,[23] obese Zucker rats reduce their food intake to a somewhat greater extent (by 40%) than lean

Table 4. Plasma Insulin and Glucagon Concentrations

	C Diet		HP Diet	
Plasma Hormone	Obese	Lean	Obese	Lean
Insulin (μU/mL)	317.9 ± 32.0 (4)†	60.5 ± 4.3 (3)	388.6 ± 34.0 (4)†	50.2 ± 5.2 (4)
Glucagon (pg/mL)	292.5 ± 18.7 (4)*	211.7 ± 17.1 (3)	383.3 ± 28.0 (4)†‡	236.0 ± 13.4 (4)
Insulin to glucagon molar ratio	25.6	6.7	23.9	5.0

NOTE. Data were analyzed by ANOVA. Values are the mean ± SE for the number of animals indicated in parentheses. Statistical significance was calculated by Fisher's F test.

*P < .05, †P < .001: obese *v* lean rats fed the same diet.

‡P < .01 for obese rats fed the HP *v* C diet.

littermates (by 31%) upon adaptation to a HP diet as compared with a C or high-fat diet. Even so, the daily food intake of obese rats remains 1.3-fold greater than that of lean HP-fed rats.[23] The impact of decreased food/caloric consumption on the metabolic parameters of the present study cannot be directly assessed, since the amount of food ingested by the C diet groups was not adjusted to that of the HP diet groups. The decreased food intake with the HP diet probably contributed to several changes in hepatic lipid metabolism such as the lower cellular TG content and de novo fatty acid synthesis in obese animals and the decreased VLDL production in both phenotypes. However, it should be noted that mass VLDL production was inhibited by 65% in obese and by 48% in lean HP-fed rats, with these decreases being greater than those for the corresponding food intakes. HP feeding combined with overnight fasting resulted in decreases of 71% and 78% in VLDL secretion rates by obese and lean rat cells, respectively, relative to the rates for corresponding animals fed the C diet. However, differences in VLDL secretion between the two phenotypes persisted. It is noteworthy that the hepatic VLDL secretion of obese rats was more affected by the HP diet than by the overnight fast, whereas in lean rats it was equally affected by the HP diet and food deprivation. The decreased oleate uptake by hepatocytes was apparently not correlated with lower food intake, since it was observed in HP-fed obese rats only whether under ad libitum feeding or after overnight fasting.

On the other hand, the drastically altered hormonal profile and hepatic lipid metabolism of obese rats on a standard diet cannot be solely accounted for by hyperphagia, since all these abnormalities persist upon pair-feeding obese and lean littermates.[24] Futhermore, Barry and Bray[25] clearly showed that when the food intake of obese Zucker rats and albino rats rendered hyperphagic by hypothalamic lesions was restricted to that of lean Zucker animals (pair-feeding), only the genetically obese Zucker rats maintained significantly higher levels of plasma TG than the other two groups. Conversely, when either Wistar[18] or obese and lean Zucker rats (L. Oussadou, G. Griffaton, and A.D. Kalopissis, manuscript in preparation) were adapted to a high-fat diet that does not affect food intake, VLDL secretion by isolated hepatocytes from all groups of fat-fed animals was consistently decreased by at least 50%. It would thus appear that the hepatic VLDL production is more related to the genotype and the nature of the diet and less to the food and/or caloric intake.

Because insulin and glucagon have often been implicated in the regulation of hepatic VLDL production, their plasma concentrations were measured in the present study. Administration of the HP diet to both lean and obese Zucker rats increased plasma glucagon, whereas plasma insulin either increased (obese) or decreased (lean), with the insulin to glucagon ratio being somewhat decreased. Studies on normolipemic rat strains showed higher plasma glucagon and similar[4] or slightly lower[5,7] plasma insulin, with the net result also being a decreased insulin to glucagon ratio.[4,5,7] Differences in circulating insulin concentrations between the different studies probably result from the known circadian fluctuations of the hormone, partially depending on feeding schedules.

To what extent may these hormonal modifications induce a decrease in hepatic VLDL secretion? The effect of insulin on hepatic VLDL production is a controversial issue, with some researchers reporting a stimulating effect,[26] and most others an inhibitory effect, but one of small magnitude in normal rats.[22,27,28] However, in diabetic rats the inhibitory effect of insulin is observed during the first 24 hours of culture, but not after longer-term exposure to the hormone.[22] In obese Zucker rats displaying concomitantly hyperinsulinemia and VLDL hypersecretion under standard diets, an inhibitory role of insulin on hepatic VLDL secretion seems unlikely, unless hepatic insulin "resistance" renders the liver of these animals completely insensitive to insulin. In this study, changes in plasma insulin were small and not consistently correlated with changes in VLDL secretion.

On the other hand, several studies clearly showed that glucagon decreases in vitro liver TG synthesis and VLDL secretion,[27-29] although the exact mechanism of action has not been established to our knowledge. Moreover, induced hyperglucagonemia in obese Zucker rats results in 42%-decreased plasma TG,[30] suggesting that VLDL output was decreased. Also, the marked increase of plasma glucagon in dichloroacetate-treated obese Zucker rats[31] was accompanied by twofold lower TG concentrations in liver and plasma. Conversely, essential fatty acid–deficient rats concomitantly displayed reduced plasma glucagon concentrations and increased TG secretion rates.[32] In the present study, plasma glucagon was significantly increased after HP feeding, particularly in obese rats. However, lean rats fed either diet displayed a lower glucagonemia than obese rats fed the C diet. Thus, it is difficult at present to relate the decreased hepatic VLDL secretion of HP-fed obese and lean rats solely to changes in plasma glucagon.

Although the regulation of the VLDL biosynthetic pathway in the liver remains largely unknown, it has been shown to be modulated by nutritional factors: it is enhanced by high-sucrose diets,[33,34] decreased by high-fat diets[18,34] and fasting,[34] and again increased after fasting followed by refeeding.[35] Thus, a high VLDL secretion rate has been linked to high de novo fatty acid synthesis (such as occurs in sucrose-fed rats, particularly after fasting and refeeding,[35] and in obese Zucker rats,[10] an elevated concentration of exogenous fatty acids (when added in vitro[29]), and an altered partition of exogenous fatty acids favoring esterification at the expense of oxidation (such as has been described in obese Zucker rats[12]). On the other hand, the hepatic esterification capacity is apparently never limiting.[33] We will first consider de novo synthesized and exogenous fatty acids as precursors of VLDL-TG, and then discuss their respective roles as potential regulatory factors of hepatic VLDL secretion rates depending on how they fit the experimental results of the present study obtained after adaptation to the HP diet.

De novo fatty acid synthesis was very active in hepatocytes of C-fed obese rats, as expected,[8-10] and decreased by half with the HP diet. Still, it proceeded at 3.5-fold higher

rates in HP-fed obese as compared with HP-fed lean rats, probably because obese rat livers synthesize more fatty acids than livers of their lean littermates from all of the lipogenic precursors including amino acids.[36] The decreased fatty acid synthesis was probably due to inhibition of the glycolytic pathway (which provides the acetylcoenzyme A substrate for fatty acid synthesis[8,37]) and to concomitant decreases in the activities of key enzymes such as acetylcoenzyme A carboxylase, fatty acid synthetase, and malic enzyme.[16] To what extent may VLDL secretion depend on fatty acid synthesis, as originally suggested by Windmueller and Spaeth?[35] Despite the frequent occurrence of a close parallelism between these two pathways,[8-10,34] no evidence has been obtained as yet for a direct stimulatory effect of fatty acid synthesis on VLDL production.[18,27,34] Results on lean rats in this study argue against a stimulatory effect of de novo fatty acid synthesis on VLDL secretion, since the first was increased and the second decreased by HP feeding. From a quantitative point of view, at most 11% and 5% of newly synthesized tritiated fatty acids were secreted in this study as VLDL-TG by obese and lean rat cells, respectively. Moreover, they accounted only for 3% to 4% of the total VLDL-TG production in obese rats and for 1% in lean animals. This confirms previous observations that de novo–synthesized fatty acids are a minor VLDL-TG precursor in obese Zucker rats,[10] as well as in Wistar rats.[18,34]

On the contrary, exogenous fatty acids are a quantitatively important source of VLDL-TG, accounting for 20% to 30% of total VLDL-TG in Wistar rats[18] and for 15% to 30% in Zucker rats (present study). Peret et al[16] reported that plasma nonesterified fatty acids are reduced in HP-fed obese (but not lean) Zucker rats, and attributed the decreased plasma TG to the plasma fatty acid decrease. Heimberg et al[29] also linked hepatic VLDL secretion to exogenous fatty acid flux. However, under several in vivo conditions the concentration of plasma nonesterified fatty acids and VLDL secretion rates do not vary in parallel. Thus, rats adapted to a high-fat diet display a 40%- to 45%-decreased post-Triton VLDL-TG secretion as compared with rats fed a standard diet at 8 AM and 2 PM when both groups of animals have similar plasma fatty acid levels. Conversely, at 8 PM the fat-fed rats have higher plasma nonesterified fatty acids but a post-Triton VLDL-TG secretion similar to that of control rats.[38] In man, diets rich in fish oil markedly suppress VLDL-TG and VLDL apolipoprotein (apo) B formation without affecting plasma nonesterified fatty acid transport.[39] In essential fatty acid–deficient rats, post-Triton TG secretion is increased, whereas the plasma fatty acid concentration is unchanged.[32] Long-term glucagon administration results in significant decreases in plasma TG in both obese and lean Zucker rats, whereas it increases plasma nonesterified fatty acids in lean rats and decreases them in obese animals.[30] In isolated hepatocytes from fat-fed Wistar rats, the decrease in the secretion of VLDL-TG could not be reversed by addition of 0.7 mmol/L oleate.[28] Liver perfusion experiments using oleate concentrations ranging from 0.18 to 1.8 mmol/L clearly showed that livers from fructose-fed rats took up similar oleate amounts but consistently secreted two times more VLDL-TG than livers from control rats at all concentrations of oleate in the perfusate.[33] Interestingly, TG secretion became constant and TG accumulated in the liver at fatty acid uptakes exceeding 13 μEq/g/h, suggesting saturation of the VLDL secretory process. Moreover, in the present study hepatocytes from all six groups of rats were incubated with the same 0.7-mmol/L oleate concentration, but VLDL production rates varied considerably between groups. Thus, the inhibition of hepatic VLDL production under HP feeding was probably not due to changes in plasma nonesterified fatty acid levels.

Another factor that may influence the VLDL production rate is exogenous fatty acid uptake by hepatocytes. We previously observed that the hepatic uptake of a representative fatty acid, oleate, was decreased in Wistar rats adapted to high-fat feeding.[18] With the C diet in this study, obese rat hepatocytes took up oleate more rapidly than cells from lean rats, particularly up to 20 minutes when the medium oleate concentration was elevated. This higher uptake rate was abolished by HP feeding (present study) or high-fat feeding (L. Oussadou, G. Griffaton, and A.D. Kalopissis, manuscript in preparation). Increased hepatic fatty acid uptake in obese as compared with lean Zucker rats has in fact been observed in several studies,[9,11,12] but was not always statistically significant. The difficulty in establishing clear-cut differences may reside in the rapidity of fatty acid uptake, so that only early incubation times produce more conclusive results.

Although the mechanism(s) responsible for the increased fatty acid uptake by cells of obese rats fed the C diet remain(s) obscure, we did not find any published evidence indicating parallel variations in fatty acid uptake and VLDL secretion. Sex steroids[40] and clofibrate treatment[41] enhanced hepatic fatty acid uptake. However, VLDL secretion increased in female animals,[40] but decreased in clofibrate-treated ones.[41] In the present study, a high fatty acid uptake and a high VLDL output were measured in obese rats fed the C diet, with the opposite occurring with the HP diet. On the contrary, lean rat cells took up similar oleate amounts irrespective of the diet and nutritional state, whereas their VLDL secretion decreased in the order C diet > HP diet > HP diet + overnight fast. Thus, fatty acid uptake and VLDL secretion rates were not closely related in Zucker rats.

On the other hand, the HP diet in this study did not affect hepatic metabolism of oleate, a representative exogenous fatty acid added at a physiological concentration (0.7 mmol/L). Thus, hepatocytes from HP-fed obese rats esterified 95% of the oleate taken up and converted 87% of it into TG and 10% into PL, similar to the C diet. Therefore, exogenous fatty acid oxidation could hardly have been increased in hepatocytes from HP-fed obese rats. Thus, the increased ketone body synthesis of HP-fed obese Zucker rats[16] stemmed from mitochondrial catabolism of ketogenic amino acids provided by the diet rather than fatty acids. Despite a similar esterification rate, oleate incorporation into VLDL-TG decreased by 34% and total VLDL-TG secretion by 65% as compared with the C diet. When

HP-fed obese rats were subjected to an overnight fast, their hepatocyte esterification capacity remained high (75% of the oleate taken up) and the relative proportions of newly formed TG and PL persisted. Despite that, a drastic inhibition of VLDL secretion was measured. Oleate esterification in lean rat cells was only slightly decreased with the HP diet relative to the C diet, whereas oleate incorporation into VLDL-TG decreased by 55% and total VLDL secretion by 48%. Fukuda et al.[12] invoked the altered partition of exogenous fatty acids, ie, increased esterification and decreased oxidation, as one of the mechanisms underlying VLDL hypersecretion by livers of obese Zucker rats fed a standard diet. In view of the present results, it is unlikely that exogenous fatty acid partition per se is an important regulatory factor of hepatic VLDL synthesis and/or secretion, at least under HP feeding. Rather, hepatocytes probably increase mitochondrial oxidation of the substrate provided in greater amounts by the diet, ie, fatty acids under high-fat feeding[11] and amino acids under HP feeding.

We will now briefly summarize for each phenotype how modifications of oleate metabolism affected VLDL production under HP feeding. In lean rats, [1-^{14}C]oleate uptake was identical under the three nutritional conditions studied. Oleate esterification to TG decreased by 15%, whereas VLDL-TG output decreased much more, with ^{14}C-VLDL-TG decreasing by 55% and total (mass) VLDL-TG by 48%. Although de novo fatty acid synthesis was 1.7-fold greater with the HP diet, the absolute fatty acid amounts synthesized by this pathway are too small to increase appreciably the total VLDL-TG production. When HP feeding was combined with overnight fasting, cellular ^{14}C-TG was reduced by 40% as a result of the decreased balance of esterification to oxidation and the lower conversion to TG. Again, VLDL-TG secretion declined more, by 75% for ^{14}C-labeled and by 78% for total VLDL-TG. Since lean rats had comparable plasma nonesterified fatty acids under C and HP feeding,[16] one would expect a similar degree of inhibition of hepatic VLDL production in vivo and in vitro.

In obese rats, [1-^{14}C]oleate uptake decreased in the order C diet > HP diet > HP diet + overnight fast. Under ad libitum HP feeding, the balance of esterification to oxidation of oleate and its conversion to TG were the same as with the C diet, indicating that the small 5% to 10% decrease in cellular ^{14}C-TG was solely due to decreased oleate uptake. However, this was accompanied by a 34%-reduced secretion of ^{14}C-VLDL-TG and a 65%-reduced secretion of total VLDL-TG. The twofold lower de novo fatty acid synthesis may in part account for the greater reduction in mass relative to ^{14}C-labeled VLDL-TG. However, the absolute amounts of ^{3}H-fatty acids synthesized were small and accounted at most for 3% to 4% of total VLDL-TG. Thus, the drastic inhibition of total VLDL secretion cannot be accounted for solely by availability of precursor fatty acids, either endogenous or exogenous. The combination of HP feeding with an overnight fast effectively reduced the balance of oleate esterification to oxidation, but not its preferential conversion to TG. Now the 35% decrease in cellular ^{14}C-TG, resulting from decreases in the uptake and esterification of oleate, was followed by a 72%-lower ^{14}C-VLDL-TG output and a 71%-lower total VLDL-TG production relative to the C diet. Since plasma nonesterified fatty acids are lower in HP-fed as compared with C-fed obese rats (0.37 *v* 0.49 mmol/L, respectively[16]), hepatic VLDL production may be even more inhibited in vivo than in vitro through decreased flux of the exogenous fatty acid source.

It thus appears that de novo–synthesized and exogenous fatty acids are, respectively, minor and quantitatively important precursors of VLDL-TG. Concerning exogenous fatty acids, neither their hepatic uptake nor their partitioning between esterification and oxidation were closely related to VLDL secretion rates in HP-fed or C-fed Zucker rats. Thus, the results of the present study do not support previous hypotheses[12,29,35] that either exogenous or de novo–synthesized fatty acids act as prime determinants of hepatic VLDL secretion rates, at least under HP feeding. The observation that the relative contributions to VLDL-TG of exogenous (^{14}C-labeled) and endogenous (^{3}H-labeled) fatty acids were little modified by HP feeding, whereas the main inhibitory effect of this diet was on the output of labeled and unlabeled VLDL-TG, suggests that the overall VLDL biosynthetic pathway was inhibited by the HP diet.

TG, the major lipid component of VLDL, also originates from a third source, namely TG temporarily stored in cytoplasmic lipid droplets. Experimental evidence suggests the following sequence of events for VLDL assembly and secretion: fatty acids synthesized de novo in the hepatocyte or taken up from the circulation become esterified to TG on smooth endoplasmic reticulum (ER) membranes, which possess the necessary enzymatic equipment (reviewed in Gibbons[42]). Part of the newly formed TG is assembled with apo B to form nascent VLDL destined for export after translocation through the secretory pathway. Those TG molecules not incorporated into VLDL in a relatively short period of time (since the half-life of ER-TG is < 1 hour) are temporarily stored in the cytoplasm in a lipid droplet form. Cytoplasmic TG is subsequently mobilized through a lysosomal hydrolysis step, with the resulting fatty acids being partially oxidized and partially reesterified to TG on smooth ER membranes and incorporated into VLDL.[19,43] The following statements are regarding the regulation of the movements of TG into and out of the cytoplasmic pool: (1) Several studies (summarized in Francone et al[43]) have shown that TG formed on ER membranes in excess of the liver's capacity to synthesize and secrete VLDL is rapidly channeled to the cytoplasmic storage pool. (2) The proportion of ER-TG entering the secretory pathway versus the storage pool is affected by the nutritional state, being increased in sucrose-fed rats[44] and decreased in fat-fed rats.[43] (3) Specific labeling of the cytoplasmic TG pool showed that the mobilization of stored TG and its incorporation into VLDL is decreased by a high-fat diet concomitantly with total VLDL production.[43] On the contrary, there is indirect evidence that sucrose feeding induces a greater mobilization of stored TG and increases VLDL secretion.[44] (4) The size of the cytoplasmic TG pool is probably not directly related to the VLDL production rate, since Wistar rats adapted to a high-fat diet displayed a sevenfold greater

cytoplasmic TG content and a 50%-decreased hepatic VLDL secretion.[18,43]

Apo B availability at the site of VLDL assembly is another potential regulatory factor of hepatic VLDL production. The nutritional status (such as sucrose feeding[45] and high-fat feeding[46]) apparently affects concomitantly hepatic VLDL-TG and apolipoprotein (especially apo B-48) secretion. These effects are produced in vivo, but cannot be mimicked in vitro by addition of glucose or fatty acids to the incubation medium of hepatocytes,[45] suggesting an interplay of hormones and various effectors. Since in the present study VLDL secretion was inhibited after in vivo administration of the HP diet, VLDL apo B production (not measured here) may have been decreased in proportion to VLDL-TG. Despite the parallelism observed between VLDL-TG and apo B secretion after in vivo nutritional adaptations, it remains unclear whether the primary effect of diets is exerted on apolipoprotein synthesis, in turn regulating the VLDL production rate, or whether VLDL production is regulated in the first place by the diet, in turn modifying apolipoprotein synthesis and secretion.

The contribution of cytoplasmic TG to VLDL-TG was not directly assessed in this study. It can be indirectly estimated by subtracting the sum of VLDL-[^{14}C]-TG + VLDL-[^{3}H]-TG from the total (mass) VLDL-TG. This calculation yields cytoplasmic TG contributions varying from 66% to 81%, suggesting that stored TG was the major precursor of VLDL-TG under all conditions studied. This concords well with studies in obese Zucker rats[10] and in Wistar rats fed a C diet, high-fat diet,[18] or high-sucrose diet.[34]

In conclusion, we demonstrated in this study that the HP diet drastically reduced hepatocyte VLDL secretion of obese and lean Zucker rats. The reduced food/caloric intake under HP feeding may partially account for the decreased VLDL production. HP feeding decreased oleate uptake by cells of obese rats only, whereas hepatocytes from both phenotypes maintained the same capacities to esterify oleate as with the C diet. Thus, VLDL inhibition was probably neither due to a decreased fatty acid uptake nor to an altered fatty acid partitioning between esterification and oxidation. A correlation with de novo fatty acid synthesis is also improbable, since this pathway decreased in obese but increased in lean animals and de novo–synthesized fatty acids are a minor precursor of VLDL-TG. Rather, HP feeding inhibited total VLDL secretion, as reflected by the drastically decreased mass VLDL-TG over 2 hours, by a mechanism that remains to be determined.

ACKNOWLEDGMENT

We thank Isabelle Leniobey for excellent secretarial assistance and Michel Joliff for help with the graphics.

REFERENCES

1. Apfelbaum M: The effects of very restrictive high protein diets. Clin Endocrinol Metab 5:417-430, 1976

2. Scheen AJ, Luyckx AS, Scheen-Lavigne MC, et al: Hormonal and metabolic adaptation to protein-supplemented fasting in obese subjects. Int J Obes 6:165-174, 1982

3. Freedland RA, Szepesi B: Control of enzyme activity: Nutritional factors, in Rechcigl M Jr (ed): Enzyme Synthesis and Degradation in Mammalian Systems. Basel, Switzerland, Karger, 1971, pp 103-140

4. Eisenstein AB, Strack I, Steiner A: Glucagon stimulation of hepatic gluconeogenesis in rats fed a high-protein, carbohydrate-free diet. Metabolism 23:15-23, 1974

5. Peret J, Foustock S, Chanez M, et al: Plasma glucagon and insulin concentrations and hepatic phosphoenolpyruvate carboxykinase activities during and upon adaptation of rats to a high-protein diet. J Nutr 111:1173-1184, 1981

6. Peret J, Foustock S, Chanez M, et al: Hepatic metabolites and amino acid levels during adaptation of rats to a high-protein, carbohydrate-free diet. J Nutr 111:1704-1710, 1981

7. Katsurada A, Fukuda H, Iritani N: Effects of dietary nutrients on substrate and effector levels of lipogenic enzymes, and lipogenesis from tritiated water in rat liver. Biochim Biophys Acta 878:200-208, 1986

8. McCune SA, Durant PJ, Jenkins PA, et al: Comparative studies on fatty acid synthesis, glycogen metabolism, and gluconeogenesis by hepatocytes isolated from lean and obese Zucker rats. Metabolism 30:1170-1178, 1981

9. Azain MJ, Martin RJ: Effect of genetic obesity on the regulation of hepatic fatty acid metabolism. Am J Physiol 244:R400-R406, 1983

10. Azain MJ, Fukuda N, Chao F-F, et al: Contributions of fatty acid and sterol synthesis to triglyceride and cholesterol secretion by the perfused rat liver in genetic hyperlipemia and obesity. J Biol Chem 260:174-181, 1985

11. Malewiak MI, Griglio S, Kalopissis AD, et al: Oleate metabolism in isolated hepatocytes from lean and obese Zucker rats. Influence of a high-fat diet and in vitro response to glucagon. Metabolism 32:661-668, 1983

12. Fukuda N, Azain MJ, Ontko JA: Altered hepatic metabolism of free fatty acids underlying hypersecretion of very low density lipoproteins in the genetically obese Zucker rat. J Biol Chem 257:14066-14072, 1982

13. Schonfeld G, Pfleger B: Overproduction of very low density lipoproteins by livers of genetically obese rats. Am J Physiol 220:1178-1181, 1971

14. Oussadou L, Kalopissis AD, Francone OL, et al: Intestinal very–low-density lipoprotein secretion in the genetically obese Zucker rat. Biochim Biophys Acta 959:76-83, 1988

15. Wang C-S, Fukuda N, Ontko JA: Studies on the mechanism of hypertriglyceridemia in the genetically obese Zucker rat. J Lipid Res 25:571-579, 1984

16. Peret J, Bach AC, Delhomme B, et al: Metabolic effects of high-protein diets in Zucker rats. Metabolism 33:200-207, 1984

17. Seglen PO: Preparation of rat liver cells. Exp Cell Res 74:450-454, 1972

18. Kalopissis AD, Griglio S, Malewiak MI, et al: Very–low-density-lipoprotein secretion by isolated hepatocytes of fat-fed rats. Biochem J 198:373-377, 1981

19. Francone OL, Kalopissis AD, Griffaton G: Contribution of cytoplasmic storage triacylglycerol to VLDL-triacylglycerol in isolated rat hepatocytes. Biochim Biophys Acta 1002:28-36, 1989

20. Bartlett CR: Phosphorus assay in column chromatography. J Biol Chem 234:466-468, 1959

21. Rudel LL, Morris MD: Determination of cholesterol using *o*-phthalaldehyde. J Lipid Res 14:364-366, 1973

22. Duerden JM, Bartlett SM, Gibbons GF: Regulation of very–low-density-lipoprotein lipid secretion in hepatocyte cultures derived from diabetic animals. Biochem J 262:313-319, 1989

23. Wangsness PJ, Dilettuso BA, Martin RJ: Dietary effects on body weight, feed intake and diurnal feeding behavior of genetically obese rats. J Nutr 108:256-264, 1978

24. Martin RJ, Gahagan J: Serum hormone levels and tissue metabolism in pair-fed lean and obese Zucker rats. Horm Metab Res 9:181-186, 1977

25. Barry WS, Bray GA: Plasma triglycerides in genetically obese rats. Metabolism 18:833-839, 1969

26. Laker ME, Mayes PA: Investigations into the direct effects of insulin on hepatic ketogenesis, lipoprotein secretion and pyruvate dehydrogenase activity. Biochim Biophys Acta 795:427-430, 1984

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

28. Gibbons GF, Pullinger CR: Regulation of hepatic very–low-density lipoprotein secretion in rats fed on a diet high in unsaturated fat. Biochem J 243:487-492, 1987

29. Heimberg M, Weinstein I, Kohout M: The effects of glucagon, dibutyryl cyclic adenosine 3′,5′-monophosphate and concentration of free fatty acid on hepatic lipid metabolism. J Biol Chem 244:5131-5139, 1969

30. Mahmood HA, Wood PJ, Marks V: The effect of induced hyperglucagonaemia on the Zucker fatty rat. Diabetologia 14:405-412, 1978

31. Hayek A, Woodside WF: Short-term influences of dichloroacetate on genetically hyperlipemic rats. Metabolism 29:120-124, 1980

32. Bird MI, Williams MA: Triacylglycerol secretion in rats: Effects of essential fatty acids and influence of dietary sucrose, glucose or fructose. J Nutr 112:2267-2278, 1982

33. Schonfeld G, Pfleger B: Utilization of exogenous free fatty acids for the production of VLDL-triglyceride by livers of carbohydrate-fed rats. J Lipid Res 12:614-621, 1971

34. 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

35. Windmueller HG, Spaeth AE: De novo synthesis of fatty acid in perfused rat liver as a determinant of plasma lipoprotein production. Arch Biochem Biophys 122:362-369, 1967

36. Haggarty P, Reeds PJ, Fletcher JM, et al: The fate of ^{14}C derived from radioactively labelled dietary precursors in young rats of the Zucker strain (Fa/- and fa/fa). Biochem J 235:323-327, 1986

37. Lane MD, Mooney RA: Tricarboxylic acid cycle intermediates and the control of fatty acid synthesis and ketogenesis. Curr Top Cell Regul 18:221-242, 1981

38. Kalopissis AD, Griglio S, Malewiak MI, et al: Effect of a high-fat diet on very low density lipoprotein secretion. Biochim Biophys Acta 620:111-119, 1980

39. Nestel PJ, Connor WE, Reardon MF, et al: Suppression by diets rich in fish oil of very low density lipoprotein production in man. J Clin Invest 74:82-89, 1984

40. Ockner RK, Burnett DA, Lysenko N, et al: Sex differences in long chain fatty acid utilization and fatty acid binding protein concentration in rat liver. J Clin Invest 64:172-181, 1979

41. Renaud G, Foliot A, Infante R: Increased uptake of fatty acids by the isolated rat liver after raising the fatty acid binding protein concentration with clofibrate. Biochem Biophys Res Commun 80:327-334, 1978

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

43. Francone OL, Griffaton G, Kalopissis AD: Effect of a high-fat diet on the contribution of cytoplasmic TG to VLDL-TG. Am J Physiol 263:E615-E623, 1992

44. Yamamoto 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

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

46. Francone OL, Kalopissis AD, Griffaton G: Concomitant inhibition of VLDL triglyceride and apoprotein secretion by hepatocytes of rats adapted to a high-fat diet, in Malmendier CL (ed): Hypercholesterolemia, Hypocholesterolemia, Hypertriglyceridemia. New York, NY, Plenum, 1990, pp 325-328