Interaction between Free Fatty Acids and Insulin in the Acute Control of Very Low Density Lipoprotein Production in Humans

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Abstract

Changes in VLDL triglyceride and VLDL apo B production were determined semiquantitatively in healthy young men by examining the effect of altering plasma insulin and/or FFA levels on the change in the slopes of the specific activity of VLDL [^{3}H]triglyceride glycerol or the ^{131}I-VLDL apo B versus time curves.

In one study (n = 8) insulin was infused for 5 h using the euglycemic hyperinsulinemic clamp technique. Plasma FFA levels declined by ~ 80% (0.52±0.01 to 0.11±0.02 mmol/liter), VLDL triglyceride production decreased by 66.7±4.2% (P = 0.0001) and VLDL apo B production decreased by 51.7±10.6% (P = 0.003). In a second study (n = 8) heparin and Intralipid (Baxter Corp., Toronto, Canada) were infused with insulin to prevent the insulin-mediated fall in plasma FFA levels. Plasma FFA increased approximately twofold (0.43±0.05 to 0.82 + 0.13 mmol/liter), VLDL triglyceride production decreased to a lesser extent than with insulin alone (P = 0.006) (−31.8±9.5%, decrease from baseline P = 0.03) and VLDL apo B production did not decrease significantly (−6.3±13.6%, P = NS). In a third study (n = 8) when heparin and Intralipid were infused without insulin, FFA levels rose approximately twofold (0.53±0.04 to 0.85±0.1 mmol/liter), VLDL triglyceride production increased by 180.1±45.7% (P = 0.008) and VLDL apo B production increased by 94.2±28.7% (P = 0.05).

We confirm our previous observation that acute hyperinsulinemia suppresses VLDL triglyceride and VLDL apo B production in healthy humans. In addition, we have demonstrated that elevation of plasma FFA levels acutely stimulates VLDL production in vivo in healthy young males. Elevating plasma FFA during hyperinsulinemia attenuates but does not completely abolish the suppressive effect of insulin on VLDL production, at least with respect to VLDL triglycerides. Therefore, in normal individuals the acute inhibition of VLDL production by insulin in vivo is only partly due to the suppression of plasma FFA, and may also be due to an FFA-independent process. (*J. Clin. Invest.* 1995. 95:158–166.) Key words: very low density lipoprotein • apolipoprotein B • free fatty acid • triglyceride • insulin

Introduction

Chronic hyperinsulinemia has been shown to stimulate triglyceride turnover in animal models (1) and insulin itself has been postulated to play a facilitatory role in the overproduction of hepatic (VLDL) particles in certain insulin-resistant states (2). Short-term addition of insulin to culture media, on the other hand, has been shown to inhibit VLDL triglyceride and apo B production in vitro in cultured rat and human hepatocytes (3–10) and $HepG_2$ cells (11–15). By contrast, studies using perfused rat livers have generally shown an acute stimulatory effect of insulin (16–20). We have previously provided some of the first in vivo evidence that acute hyperinsulinemia suppresses VLDL apo B and triglyceride production in humans (21, 22).

Free fatty acids, when added to the hepatocyte or Hep G_2 cell culture medium, have been shown to stimulate production of VLDL triglyceride and apo B in vitro (11–13, 15, 23–28) and to attenuate the acute inhibitory effect of insulin (11, 13, 15). Plasma FFA levels are exquisitely sensitive to acute changes in the ambient insulin concentration in healthy insulin-sensitive individuals (29), hence insulin, in vivo, may also indirectly regulate VLDL production by reducing plasma levels of FFA as a consequence of its inhibitory effect on adipose tissue hormone sensitive lipase (30).

In our previous studies examining the acute in vivo effect of insulin on VLDL production we noted a dramatic reduction in plasma FFA concentration within 1 h of the onset of acute hyperinsulinemia (21, 22). In those studies it was not possible to determine whether the acute inhibitory effect of insulin on VLDL production was entirely mediated by the suppression of substrate (FFA) availability for VLDL synthesis. The present study examines the role played by FFA in mediating insulin's effect on VLDL production. Currently described mathematical modeling techniques designed to quantify absolute VLDL production rates are limited to steady-state conditions and require prolonged sampling periods. Therefore we have used a semiquantitative radiolabeled VLDL tracer technique as previously described to estimate the relative changes in VLDL triglyceride and VLDL apo B production rates compared to baseline (21). This approach involves examining the change in slopes of the specific activity of VLDL [^{3}H]triglyceride glycerol (VLDL [^{3}H]TGG)[1] and ^{131}I-VLDL apo B versus time curves, respec-

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1. *Abbreviation used in this paper.* [^{3}H]TGG, [^{3}H]triglyceride glycerol.

tively, occurring with acute hyperinsulinemia or change in plasma FFA concentration.

Using this technique, in this paper we have tested the hypothesis that insulin has an acute suppressive effect on VLDL production independent of its effects on FFA. Eight healthy young male volunteers were studied on two occasions each. On one occasion, plasma FFA levels were allowed to fall during acute hyperinsulinemia. On the subsequent occasion plasma FFA levels were acutely raised approximately twofold during hyperinsulinemia by infusing heparin and Intralipid (Baxter Corp.). This provided an opportunity to compare the effects of two different levels of FFA while peripheral insulin levels were matched. Eight additional subjects were studied at basal insulin levels while infusing heparin and Intralipid to raise plasma FFA levels twofold. The latter study permitted a comparison of the effects of two different levels of peripheral insulinemia while matching plasma FFA concentrations.

Methods

Subjects

16 healthy male volunteers (age = 26.1±1.4 yr, body mass index = 23.6±0.5 kg/m^2) participated in the study. Subjects with hyperlipidemia or any systemic illness were excluded from the study. No subject was taking any medication at the time of the study, and subjects were asked to refrain from drinking alcohol for 1 wk before the study. All had stable body weight for the month before the study. Subjects were instructed to consume their regular diet until 1800 h of the evening before the study, and then to fast. Informed written consent was obtained from all participants in accordance with the guidelines of the University of Toronto Human Subjects Review Committee. All studies were conducted in The Toronto Hospital Clinical Investigation Unit.

Experimental protocol

Each of eight subjects was studied on two occasions, 8 wk apart. Each underwent a euglycemic hyperinsulinemic clamp on one occasion (insulin infusion study) and the same clamp plus heparin and Intralipid on another (insulin-heparin-Intralipid study). The order of these studies was randomly assigned. In an attempt to limit the exposure to radioactive iodine, each subject was studied on a maximum of two occasions each. Therefore an additional eight subjects participated in the third study, receiving heparin and Intralipid without the clamp (heparin-intralipid study).

5–7 d before the study, ~ 450 ml of blood was drawn, the plasma separated, and the red cells returned to the subject. 250 ml plasma was separated from the blood and VLDL ($d < 1.006$ g/ml, Svedberg flotation constant [S_f] 20–400) isolated from the plasma under sterile conditions by spinning in a 50.2 Ti rotor for 16 h at 32,000 rpm (108,000 g) and 10°C. The VLDL was washed once. Apo B was then iodinated with ^{131}I using sterile techniques by modification of the method described by McFarlane (31). The ^{131}I-VLDL apoB was checked for sterility and freedom from pyrogens.

The volunteers consumed their usual diet until 1800 h of the evening before the study. At 0330 h, a bolus of 200 μCi 2-[^{3}H]glycerol was injected intravenously to label endogenously synthesized VLDL TGG. 4 h later, at 0730 h, the ^{131}I-VLDL previously isolated from the subject was injected intravenously and began a 3-h baseline sampling period.

(a) Insulin infusion study

At 1030 h hyperinsulinemia was induced by infusing 840 mU/m^2 of crystalline human insulin (Connaught Novo Nordisk, Toronto, Canada) over 10 min into a catheter placed in a forearm vein. This was followed by a constant insulin infusion of 40 mU/m^2 per min and continued for 5 h until the end of the clamp period at 1530 h. Plasma glucose levels were measured during the hyperinsulinemic period every 5 min in samples of arterialized venous blood and the values used to adjust the rate of a 20% dextrose infusion to maintain constant euglycemia (5.0–5.5 mmol/liter). Serum potassium was replaced at 10 meq/h throughout the insulin infusion.

(b) Insulin-heparin-Intralipid study

This study was similar to the insulin study described above but in addition, when the insulin infusion was started, Intralipid 10% solution and heparin sodium (Organon Tieknika, Toronto, Canada) were infused at 25 ml/h and 250 U/h, respectively, and continued for 5 h. The doses of heparin and Intralipid were empirically determined in pilot studies to raise plasma FFA levels approximately twofold during the insulin clamp. Intralipid 10% is a sterile fat emulsion containing 10% soybean oil, 1.2% egg phospholipids, and 2.25% glycerin in water. Soybean oil is a mixture of predominantly unsaturated fatty acids. The fatty acids forming the major component of the emulsions are linoleic (50%), oleic (26.5%), palmitic (10.5%), linolenic (8.5%), and stearic (3.5%). The emulsified fat particles are similar to naturally occurring chylomicrons.

(c) Heparin-Intralipid study

This study was similar to study *b* above, with Intralipid 10% solution infused at 25 ml/h and heparin at 90 ml/h. Insulin and dextrose were not infused. The doses of heparin and Intralipid were empirically determined to raise plasma FFA levels approximately twofold in the absence of an exogenous insulin infusion, thus matching the levels seen in study *b* above.

Blood sampling

At 30-min intervals throughout the study, arterialized blood samples were withdrawn into NaEDTA-containing vacutainer tubes through a catheter placed in a superficial forearm vein which was maintained in a warming pad. Throughout the study, all samples were immediately chilled to 4°C, plasma separated and then frozen or held at 4°C as appropriate for later analyses. Blood levels of lipids, lipoproteins, apo B, FFA, glycerol, and insulin were analyzed.

Calculation of changes in VLDL-triglyceride and VLDL-apo B production rates

The semiquantitative method used in this study has been described in detail previously (21). We have shown that, under steady-state conditions, the decline in VLDL [^{3}H]TGG sp act and ^{131}I-VLDL apo B sp act for the length of time examined in the present study is monoexponential and that fasting VLDL is a valid tracer for hyperinsulinemic VLDL. The 4-h time lapse between the administration of [^{3}H]glycerol and the beginning of the baseline sampling period allowed the VLDL [^{3}H]TGG sp act to peak and then begin declining, such that the rate of decline of sp act in the VLDL fraction could be examined. Apo B sp act, on the other hand, declines almost immediately after administration and the slope of the decline is predictable for at least 9 h.

Under steady-state conditions, the slope of the sp act versus time curve reflects the input of unlabeled (i.e., newly made) VLDL triglyceride and apo B into the circulating VLDL triglyceride and apo B pools, respectively, as a fraction of that pool. Although changes in removal, which are likely to occur during the initial 1–3 h of hyperinsulinemia (when VLDL is not in steady state), may also affect the slope of the curve, the slope is only examined once a new steady state is reached, and therefore a change in the slope reflects a change in production. We were therefore able to determine the change in endogenous VLDL triglyceride and apo B production relative to baseline by calculating the change in slope occurring with an acute manipulation such as the infusion of insulin or Intralipid plus heparin. The slopes for VLDL [^{3}H]TGG sp act and ^{131}I-VLDL apo B sp act versus time were calculated during the 3-h baseline period and again after the manipulation, when a new steady state was reached (generally within 1–3 h). The slope after the manipulation was corrected for the actual change in VLDL triglyceride or VLDL apo B pool size, to allow for the change in slope that would result from a change in pool size alone. Since the volume of distribution

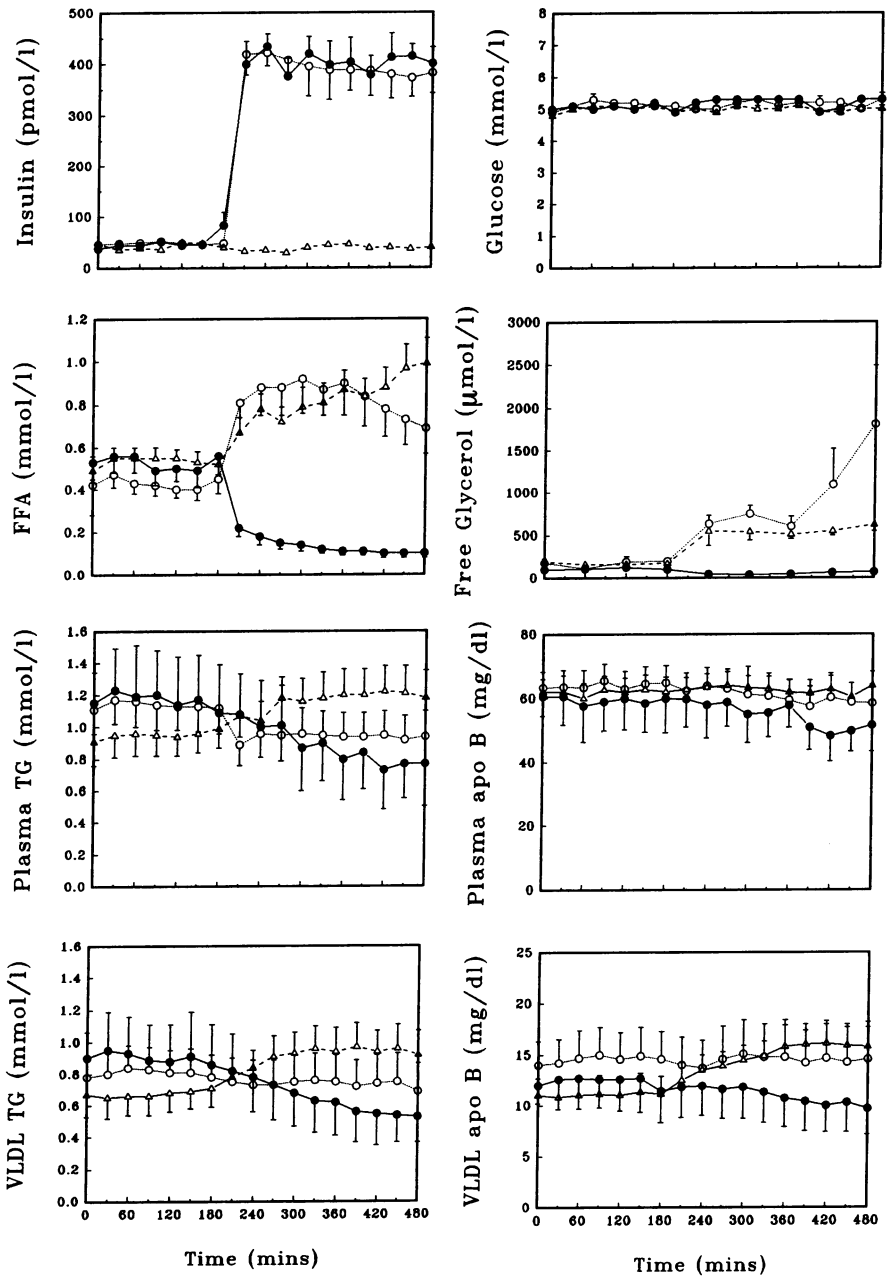

Figure 1. Plasma levels of insulin, glucose, FFA, free glycerol, triglycerides (TG) and apo B, and VLDL triglycerides and apo B, are illustrated for the three studies; insulin infusion alone (*filled circles*), insulin plus heparin and Intralipid (*open circles*), heparin and Intralipid (*open triangles*). Time 0–180 min represents the baseline sampling period. Insulin, heparin, and Intralipid infusions began at 180 min and continued for 5 h until 480 min. Bars represent SEM.

of VLDL (intravascular volume) is not felt to change appreciably with acute hyperinsulinemia, this calculation was made using the difference between the mean basal and mean postmanipulation steady state VLDL triglyceride or apo B concentration for that individual. For example, if an individual's VLDL apo B concentration decreased by 35% with hyperinsulinemia, the ^{131}I-VLDL apo B sp act-vs-time slope during hyperinsulinemia after a new steady state was reached was adjusted by decreasing (flattening) the slope by 35% to account for the steepening that occurred as a consequence of the change in pool size alone.

If endogenous VLDL triglyceride or apo B production increases, the second slope (adjusted for change in pool size) will be steeper than the baseline slope. If production decreases, the slope will flatten. Since we studied a relatively homogeneous group of subjects, all of whom had normal fasting triglyceride levels, the interindividual variation in basal VLDL production rate would be expected to be rather small. Therefore, expression of the results as percent change in VLDL production from baseline permits a valid comparison between the individuals participating in the three studies.

Laboratory methods

Glucose was analyzed enzymatically using a Glucose Analyzer II (Beckman Instruments Corp., Fullerton, CA). Insulin was measured by radioimmunoassay using a double antibody separation method (kit supplied by Pharmacia Diagnostic, Uppsala, Sweden) (32) (intraassay coefficient of variation [cv] = 6.7%, interassay cv = 4.6%). Cholesterol was measured using the CHOD-PAP enzymatic colorimetric kit (Boehringer Mannheim GambH Diagnostica, Montreal, Canada) (intraassay cv = 1.4%, interassay cv = 2.9%). Triglycerides were measured as esterified glycerol using the enzymatic colorimetric kit (Boehringer Mannheim GmbH Diagnostica). Free glycerol was eliminated from the sample in a preliminary reaction followed by enzymatic hydrolysis of triglyceride with subsequent determination of the liberated glycerol by

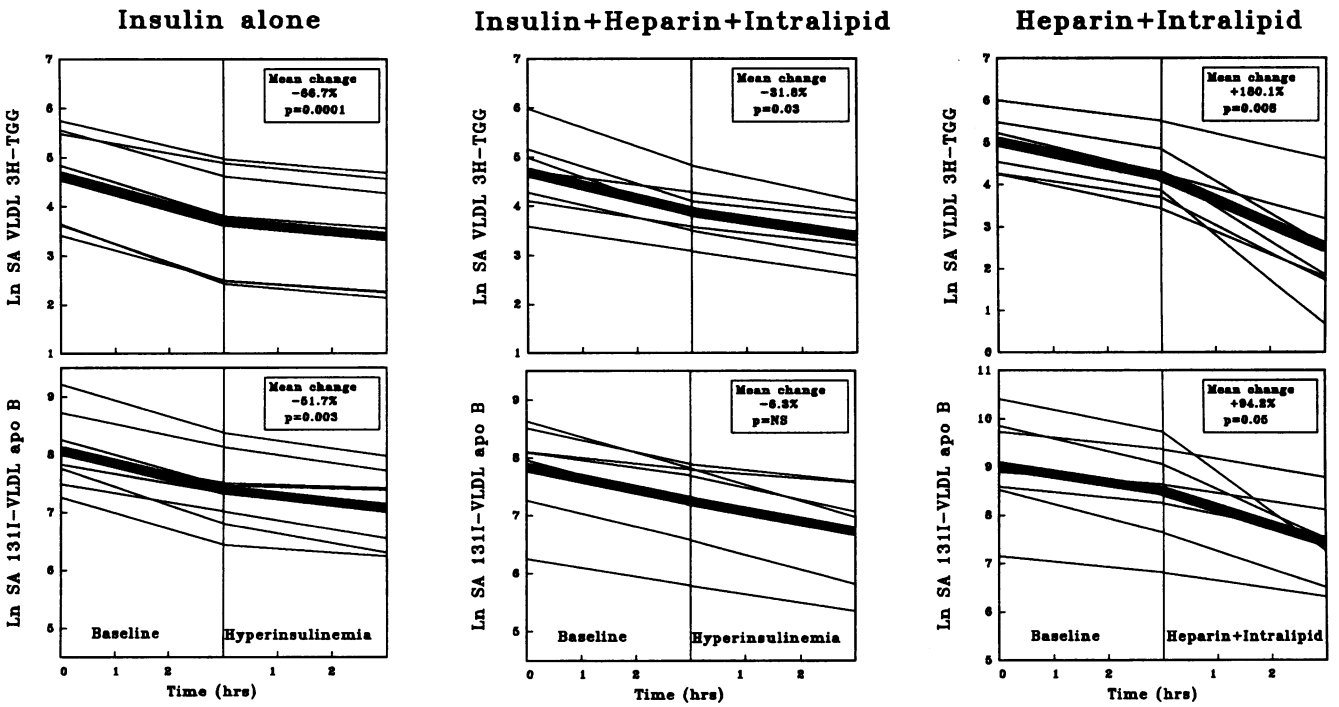

Figure 2. The natural logarithm of the specific activity of VLDL [^{3}H]TGG (*three top panels*) and ^{131}I-VLDL apo B (*three lower panels*) versus time curves are shown for the three studies. The baseline slope between 0 and 180 min is illustrated by the slope during the first 3 h in each panel. The slope illustrated during the latter 3 h represents the sp act versus time curve after a new steady state of VLDL triglyceride or VLDL apo B pool size has been reached (usually after 1–3 h). These latter slopes have been corrected for any change in pool size that may have occurred before the new steady state. The dense lines represent the group means. Statistics shown are by paired *t* test comparing the baseline slope to the corrected slope in the latter 3 h of the study. A flattening of the slope after perturbing the system indicates a reduction in nascent (unlabeled) VLDL production, while a steepening of the slope indicates an increase in production. Bars represent SEM.

colorimetry (intraassay cv = 3.0%, interassay cv = 4.9%). FFA were measured by the colorimetric method described by Bergmann et al. (33) (intra-assay cv = 4.3%, interassay cv = 2.9%). Plasma free glycerol was measured by the glycerol ultraviolet method (kit supplied by Boehringer Mannheim GmbH Diagnostica) (intraassay cv = 2.9%, interassay cv = 2.3%). Apo B was measured by an electroimmunoassay method developed to permit assay of this apoprotein in triglyceride-rich lipoproteins (34) (intraassay cv = 2.4%, interassay cv = 3.8%).

Lipoprotein ultracentrifugation. VLDL (S_f20–400) for lipoprotein analysis was separated from 6–8 ml of plasma by overlaying with a *d* = 1.006 g/ml solution and spinning in a 70.1 Ti rotor for 16 h, 39,000 rpm (108,000 *g*) at 10°C.

Measurement of ^{131}I-VLDL apo B and VLDL [^{3}H]TGG sp act. ^{131}I-VLDL apo B and VLDL [^{3}H]TGG were measured in isolated VLDL and expressed as sp act of VLDL apo B and triglyceride mass, respectively. VLDL Apo B was precipitated by the method of Le et al. (35) and counted in a gamma 5500 counter (Beckman Instruments). VLDL [^{3}H]TGG was extracted by the method of Folch et al. (36) and counted in an scintillation counter (LKB Instruments, Inc., Gaithersburg, MD) after allowing 8 wk for the ^{131}I radioactivity to decay.

Statistical analysis

All results are expressed as the mean±SEM. Differences between the three studies were tested for significance using general linear Modeling and Tukey's Studentized Range post hoc test. The significance of differences in the post hoc test was tested at the P = 0.05, 0.01, 0.005, and 0.001 levels. Differences were regarded as significant if the corresponding P value was < 0.05. Comparisons were made between the mean level of measured parameters during the baseline sampling period (0–180 min) and the final 3 h of the study (300–480 min) using the paired *t* test. Pearson correlation coefficients were calculated with linear regression analysis. Slopes of specific activity versus time were calculated by least squares. The baseline and the postmanipulation slopes were compared in each individual by paired *t* test.

Data analysis was performed using the Statistical Analysis System (SAS Version 6.04 edition for personal computers; SAS Institute Inc., Cary, NC).

Results

Glucose and insulin concentrations (Fig. 1) and dextrose infusion rates. In both euglycemic hyperinsulinemic clamp studies, with or without the addition of heparin and Intralipid, plasma glucose levels were maintained in the euglycemic range with a coefficient of variation of 8.8% and 8.7%, respectively. Plasma insulin levels were elevated to a similar extent in both studies (409.5±13.3 and 381.5±16.8 pmol/liter, respectively, mean±SEM). In the study in which heparin and Intralipid were infused without exogenous insulin, glucose and insulin remained at basal concentrations.

20% dextrose infusion rates, calculated for the final 30 min of the euglycemic hyperinsulinemic clamp, were similar in the two studies in which insulin was infused (237±15 ml/h with insulin alone vs. 229±21 ml/h with insulin plus Intralipid and heparin, P = NS). Since hepatic glucose production has been shown to be > 95% suppressed in normal insulin-sensitive individuals at the levels of peripheral hyperinsulinemia achieved in

the present study (37), these data indicate that the infusion of Intralipid, with elevation of FFA levels in the 1 mM range, did not grossly affect insulin sensitivity.

Plasma lipids and apo B (Fig. 1). All changes in concentrations described below compare the 0–180-min mean baseline level to the 300–480-min time period mean, and P values shown with these concentrations represent statistics for within-group changes over time. Plasma FFA declined when insulin was infused alone (0.52±0.01 to 0.11±0.02 mmol/liter, $P = 0.0003$). This represents approximately an 80% reduction in plasma FFA, the majority of the decline occurring within 1 h of the onset of the insulin infusion. In contrast, when heparin and Intralipid were infused with insulin, FFA levels rose from 0.43±0.05 to 0.82±0.13 mmol/liter ($P = 0.02$). FFA levels increased to a similar extent when heparin and Intralipid were infused in the absence of exogenous insulin, from a basal level of 0.53±0.04 to 0.85±0.1 mmol/liter.

Plasma free glycerol levels were extremely low at baseline in all studies. Levels did not change appreciably in the insulin infusion study (110.0±10.5 to 82.5±28.3 μmol/liter). Free glycerol increased when insulin was infused with heparin and Intralipid (180.0±23.5 to 1,050±255.7 μmol/liter, $P = 0.009$) as well as when heparin and Intralipid were infused without insulin (172.9±11.5 to 545.7±68.3 μmol/liter, $P = 0.002$). The increase in glycerol in the insulin-heparin-Intralipid study was greater than in the heparin-Intralipid study ($P = 0.01$).

Plasma triglyceride concentrations decreased with acute hyperinsulinemia when insulin was infused alone (1.22±0.26 to 0.85±0.23 mmol/liter, $P = 0.003$) and when heparin and Intralipid were infused during hyperinsulinemia (1.15±0.15 to 0.94±0.15 mmol/liter, $P = 0.01$). During the Intralipid/heparin infusion without exogenous insulin, triglycerides increased from 0.84±0.09 to 1.11±0.15 mmol/liter, $P = 0.03$).

Plasma apo B declined in both the insulin infusion study (60.8±8.4 to 52.7±7.2 mg/dl, $P = 0.009$) and in the insulin/heparin/Intralipid study (64.3±5.2 to 59.5±6.4 mg/dl, $P = 0.03$). When heparin and Intralipid were infused without insulin, apo B levels remained constant (60.5±4.8 to 60.1±4.1 mg/dl, P = NS).

VLDL composition (Fig. 1). VLDL triglyceride concentration decreased with insulin alone (0.91±0.23 to 0.57±0.19 mmol/liter, $P = 0.004$), remained unchanged with insulin/heparin/Intralipid (0.81±0.14 to 0.74±0.17 mmol/liter, P = NS) and increased with heparin/Intralipid (0.58±0.08 to 0.86±0.13 mmol/liter, $P = 0.007$). Differences between all three studies between 300 and 480 min were significant at $P = 0.01$.

VLDL apo B decreased with insulin alone (12.5±2.9 to 10.6±2.8 mg/dl, $P = 0.03$), did not change with insulin/heparin/Intralipid (14.6±2.6 to 14.9±3.4 mg/dl, P = NS) and increased with heparin/Intralipid (9.6±1.3 to 14.5±1.7 mg/dl, $P = 0.002$).

VLDL triglyceride and apo B levels increased or decreased in individual subjects, reaching a new steady state by 30 min to 3 h after the onset of hyperinsulinemia or the Intralipid infusion. Any further change was < 5% in the majority and < 10% in all subjects.

VLDL triglyceride and apo B production rates (Figs. 2 and 3, Tables I, II, and III). With the exogenous insulin infusion alone, VLDL triglyceride production decreased by 66.7±4.2% ($P = 0.0001$) and VLDL apo B production decreased by 51.7±10.6% ($P = 0.003$).

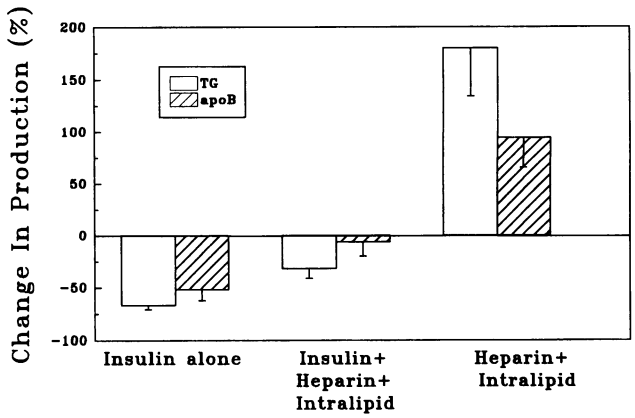

Figure 3. Summary of changes in VLDL triglyceride (TG, *clear bars*) and VLDL apolipoprotein B (apo B, *hatched bars*) production, expressed as percent decrease (negative) or increase (positive) compared to baseline in each of the three studies. The ~ 50% reduction in VLDL TG and VLDL apo B production with hyperinsulinemia is attenuated somewhat but not completely abolished when plasma FFA are raised by infusing heparin and Intralipid with insulin. When FFA are raised to similar levels by infusing heparin and Intralipid in the absence of insulin, there is a marked stimulation of VLDL TG and VLDL apo B production. Bars represent SEM. Differences between studies for apo B production are: $P = 0.04$ for insulin alone vs insulin plus Intralipid and heparin; $P < 0.001$ for insulin alone vs heparin plus Intralipid; $P < 0.005$ for insulin plus Intralipid and heparin vs heparin plus Intralipid. Differences between studies for triglyceride production are: $P = 0.006$ for insulin alone vs insulin plus Intralipid and heparin; $P < 0.001$ for insulin alone vs heparin plus Intralipid; $P < 0.001$ for insulin plus Intralipid and heparin vs heparin plus Intralipid.

When plasma FFA levels were raised twofold by infusing heparin and Intralipid with insulin, VLDL triglyceride production decreased by 31.8±9.5% ($P = 0.03$) and apo B production did not decrease significantly (−6.3±13.6%, P = NS). The decreases in production of both VLDL triglyceride and VLDL apo B were greater in the insulin infusion study than in the insulin/heparin/Intralipid study (paired t test, $P = 0.006$ and $P = 0.04$, respectively). The triglyceride data from one subject were not included in the analysis due to the variability of the data, and the radioactivity of the apo B was not included from another as it was < 1.5 times background.

When heparin and Intralipid were infused without insulin, VLDL triglyceride production increased by 180.1±45.7% ($P = 0.008$) and apo B production increased by 94.2±28.7% ($P = 0.05$). The VLDL triglyceride data from one individual and apo B data from a second individual were omitted from the analysis for reasons cited above.

There were no significant correlations between area under the FFA curve (after 180 min) and the magnitude of change in VLDL production.

Discussion

In the present study we have tested the hypothesis that insulin has an acute suppressive effect on VLDL production in humans independent of the effect of FFA. We have confirmed our previous findings that insulin acutely suppresses VLDL triglyceride and VLDL apo B production in lean healthy individuals (21,

Table I. Insulin Infusion Study: Changes in VLDL apo B and Triglyceride Pool Size and Slopes of Decay of Specific Activity of VLDL ^{131}I-apo B and VLDL [^{3}H]TGG versus Time

Subject	Percent change in pool size of VLDL apo B	VLDL ^{131}I-apo B sp act slope during baseline	VLDL ^{131}I-apo B sp act slope during hyperinsulinemia: Measured	VLDL ^{131}I-apo B sp act slope during hyperinsulinemia: Adjusted for change in pool size	Percent change in slope between baseline and adjusted hyperinsulinemia
1	−17.2	−0.0054	−0.0026	−0.0027	−50.1
2	−23.9	−0.0044	−0.0005	−0.0004	−90.0
3	−14.4	−0.0028	−0.0021	−0.0018	−34.4
4	−12.4	−0.0033	−0.0026	−0.0023	−30.2
5	−33.1	−0.0028	−0.0007	−0.0005	−81.7
6	−3.7	−0.0026	−0.0025	−0.0026	−1.3
7	−51.6	−0.0046	−0.0017	−0.0011	−76.0
8	−34.3	−0.0046	−0.0031	−0.0023	−49.7
				Mean change	−51.7±10.6% (P = 0.003)

Subject	Percent change in pool size of VLDL TG	VLDL ^{3}H-TGG sp act slope during baseline	VLDL ^{3}H-TGG sp act slope during hyperinsulinemia: Measured	VLDL ^{3}H-TGG sp act slope during hyperinsulinemia: Adjusted for change in pool size	Percent change in slope between baseline and adjusted hyperinsulinemia
1	−44.6	−0.0057	−0.0020	−0.0014	−75.4
2	−64.0	−0.0063	−0.0022	−0.0014	−78.3
3	−34.6	−0.0033	−0.0025	−0.0019	−43.5
4	−43.3	−0.0049	−0.0029	−0.0020	−58.7
5	−64.9	−0.0067	−0.0027	−0.0017	−75.4
6	−8.7	−0.0043	−0.0017	−0.0016	−63.1
7	−75.3	−0.0051	−0.0022	−0.0012	−75.7
8	−65.5	−0.0052	−0.0032	−0.0019	−63.1
				Mean change	−66.7±4.2% (P = 0.0001)

Statistics are by paired t test comparing adjusted slope during hyperinsulinemia to the baseline slope.

22). We have also demonstrated, for the first time, that an acute elevation of plasma FFA stimulates VLDL production in humans. When both plasma FFA and insulin levels were raised simultaneously, there remained a significant reduction in VLDL triglyceride production (although VLDL apo B production did not increase or decrease significantly). The magnitude of this reduction was less than with insulin alone. We have therefore shown that insulin has an acute suppressive effect on VLDL triglyceride production in young healthy males, independent of the effect of a reduction in plasma FFA. However, at least part of the acute suppressive effect of insulin on VLDL production can be explained by the decrease in plasma FFA that occurs with acute hyperinsulinemia.

Byrne et al. (13) reported similar effects in $HepG_2$ cells. Supplementing the cell culture medium with oleic acid diminished the insulin-induced inhibition of triglyceride secretion from 18.2 to 7.8%. Others (11, 12, 15) have also studied the interaction of oleate and insulin on secretion of lipoproteins in $HepG_2$ cells. Oleate resulted in a marked stimulation of VLDL secretion whereas insulin caused a significant decrease. Insulin limited the oleate-induced stimulation of VLDL secretion (11). However, in the latter experiment (11) the combination of insulin and oleate in the medium still resulted in a net increase in VLDL secretion above basal. The interpretation of these studies depends on the basal VLDL secretion rate.

In the present studies, when the hyperinsulinemia-induced decline in FFA was prevented, the reduction in VLDL triglyceride production was still reduced but this was less than during hyperinsulinemia alone. Under the same conditions insulin no longer inhibited VLDL apo B production. However, one must caution the reader against overinterpreting the changes in apo B as there was considerable variability in the apo B production data in the insulin-heparin-Intralipid study. Our data would suggest that the acute inhibitory effect of insulin on VLDL production in vivo is due in part to the suppression of plasma FFA. However, at least with respect to VLDL triglyceride production, insulin appears to have some additional effect, perhaps acting directly on the hepatocyte to inhibit VLDL production.

FFA are rapidly taken up by hepatocytes and esterified before entry into the cytoplasmic pool of triglyceride. Gibbons et al. (27) have suggested that the size of this cytoplasmic triglyceride pool, rather than the availability of extracellular oleic acid, correlated with VLDL secretion. In rat hepatocytes, ~ 70% of VLDL triglyceride secreted is derived by the lipolysis of cytoplasmic triglycerides with re-esterification near the site of VLDL assembly with apo B (38). This proportion is not affected by insulin and intracellular lipolysis of stored triglyceride does not appear to be rate-limiting for VLDL assembly (38).

The assembly and secretion of VLDL requires apo B synthesis and a metabolically accessible pool of VLDL lipid components, including triglycerides and cholesteryl esters (39). A number of investigators have shown that insulin leads to en-

Table II. Insulin/Heparin/Intralipid Infusion Study: Changes in VLDL apo B and Triglyceride Pool Size and Slopes of Decay of Specific Activity of VLDL ^{131}I-apo B and VLDL [^{3}H]TGG versus Time

Subject	Percent change in pool size of VLDL apo B	VLDL ^{131}I-apo B sp act slope during baseline	VLDL ^{131}I-apo B sp act slope during hyperinsulinemia: Measured	VLDL ^{131}I-apo B sp act slope during hyperinsulinemia: Adjusted for change in pool size	Percent change in slope between baseline and adjusted hyperinsulinemia
2	−9.5	−0.0022	−0.0038	−0.0034	+55.7
3	−13.8	−0.0026	−0.0021	−0.0025	−4.0
4	+24.3	−0.0045	−0.0036	−0.0047	+5.9
5	−22.2	−0.0017	−0.0015	−0.0012	−28.2
6	−5.6	−0.0038	−0.0045	−0.0043	+13.1
7	−21.3	−0.0035	−0.0020	−0.0017	−52.2
8	−20.8	−0.0042	−0.0034	−0.0028	−34.4
				Mean change	−6.3±13.6% (P = NS)

Subject	Percent change in pool size of VLDL TG	VLDL ^{3}H-TGG sp act slope during baseline	VLDL ^{3}H-TGG sp act slope during hyperinsulinemia: Measured	VLDL ^{3}H-TGG sp act slope during hyperinsulinemia: Adjusted for change in pool size	Percent change in slope between baseline and adjusted hyperinsulinemia
1	−1.4	−0.0059	−0.0019	−0.0019	−67.5
2	−35.9	−0.0066	−0.0039	−0.0028	−56.8
3	+13.1	−0.0029	−0.0018	−0.0020	−31.0
4	+26.4	−0.0024	−0.0018	−0.0024	−1.5
5	−42.4	−0.0028	−0.0039	−0.0028	−1.6
6	+6.8	−0.0043	−0.0030	−0.0032	−26.8
7	−34.0	−0.0064	−0.0054	−0.0040	−37.1
				Mean change	−31.8±9.5% (P = 0.03)

Statistics are by paired t test comparing adjusted slope during hyperinsulinemia to the baseline slope. Note that apo B sp act data for subject 1 and TG sp act data for subject 8 were not included because sp act was either not >1.5 × background activity or poor regressions for decay curves.

hanced intracellular apo B degradation (6, 40) and accumulation of intracellular triglyceride (5–8, 40), suggesting an "uncoupling" effect of insulin on VLDL assembly. The exact cellular mechanism of this "uncoupling" effect of insulin is the focus of intensive investigation and much debate. Evidence from several laboratories indicate that apo B mRNA levels do not change in response to acute insulin (12, 14, 41) or FFA exposure (12, 39, 42), implying that insulin suppression of cellular and secreted apo B occurs by a co- or posttranslational mechanism. Studies in $HepG_2$ cells indicate that the stimulatory effect of oleate on apo B has been shown to be mediated through an inhibition of posttranslational protein degradation (25). While it is not known whether insulin directly enhances apo B proteolysis, with subsequent reduction in VLDL assembly and intracellular triglyceride accumulation, it does appear that apo B proteolysis is also enhanced when lipid availability becomes rate-limiting for VLDL particle synthesis (43). Fatty acids may become rate-limiting for VLDL formation and Arbeeny et al. (44) showed that inhibition of fatty acid synthesis decreases VLDL secretion in primary hamster hepatocytes (44). However, in both $HepG_2$ cells and cultured rat hepatocytes insulin effects occur even in the presence of an extracellular supply of oleic acid (12, 45, 46).

In the present study we raised and sustained elevated plasma FFA levels by infusing heparin to stimulate endogenous lipoprotein lipase activity and Intralipid to provide adequate triglyceride substrate for lipolysis. Intralipid provides a mixture of poly-, mono-, and unsaturated fatty acids and so the effect on VLDL production is the net effect of these fatty acids. Various fatty acids may differ in their effects on lipoprotein apo B assembly and inhibition of apo B degradation (47, 48), and this should be borne in mind when interpreting our results.

Because heparin was administered with the Intralipid, we suspect that there was a rapid intravascular lipolysis of the Intralipid particles. This is supported by the minimal increase in plasma triglyceride concentration (Fig. 1) when Intralipid and heparin were administered without insulin, and an actual decrease in plasma triglycerides when insulin plus Intralipid and heparin were infused. In addition, there was no difference in the dextrose infusion rate required to maintain euglycemia in the insulin clamp studies performed with or without Intralipid, suggesting no gross change in insulin sensitivity due to the triglycerides or FFA from the Intralipid. It is unlikely, therefore, that there was a confounding effect of triglycerides or FFA affecting tissue insulin sensitivity.

Subjects fasted for ~ 16.5 h before the start of the glucose or Intralipid infusions. Since counterregulatory hormones may be elevated after this length of fast, it is possible that the in vivo effects of insulin on VLDL production would be different if studies were performed in the postprandial as opposed to the postabsorptive state. In addition, the ability of FFA to stimulate VLDL production may depend on whether the individual is in the fed or fasted state. Oleate added to isolated perfused liver of fed rats does not stimulate secretion of apo B, while apo B

Table III. Heparin/Intralipid Infusion Study: Changes in VLDL apo B and Triglyceride Pool Size and Slopes of Decay of Specific Activity of VLDL ^{131}I-apo B and VLDL [^{3}H]TGG versus Time

Subject	Percent change in pool size of VLDL apo B	VLDL ^{131}I-apo B sp act slope during baseline	VLDL ^{131}I-apo B sp act slope during Intralipid: Measured	VLDL ^{131}I-apo B sp act slope during Intralipid: Adjusted for change in pool size	Percent change in slope between baseline and adjusted Intralipid slope
9	+40.0	−0.0050	−0.0038	−0.0063	+25.9
10	+23.6	−0.0020	−0.0021	−0.0027	+38.2
11	+28.7	−0.0021	−0.0023	−0.0032	+56.0
13	+5.4	−0.0019	−0.0037	−0.0039	+100.7
14	+28.2	−0.0015	−0.0021	−0.0029	+91.5
15	+89.0	−0.0039	−0.0015	−0.0136	+252.9
16	+71.9	−0.0044	−0.0024	−0.0086	+94.5
				Mean change	+94.2±28.7% (P = 0.05)

Subject	Percent change in pool size of VLDL TG	VLDL ^{3}H-TGG sp act slope during baseline	VLDL ^{3}H-TGG sp act slope during Intralipid: Measured	VLDL ^{3}H-TGG sp act slope during Intralipid: Adjusted for change in pool size	Percent change in slope between baseline and adjusted Intralipid slope
9	+89.1	−0.0038	−0.0019	−0.0018	+364.0
10	+8.3	−0.0046	−0.0083	−0.0090	+95.7
11	+78.7	−0.0032	−0.0023	−0.0109	+234.1
12	+73.1	−0.0042	−0.0036	−0.0135	+219.9
13	+14.6	−0.0027	−0.0043	−0.0050	+82.6
15	+54.2	−0.0036	−0.0058	−0.0013	+250.7
16	+21.1	−0.0053	−0.0048	−0.0061	+13.5
				Mean change	+180.1±45.7% (P = 0.008)

Statistics are by paired t test comparing adjusted slope during hyperinsulinemia to the baseline slope. Note that apo B sp act data for subject 12 and TG sp act data for subject 14 were not included because sp act was either not >1.5 × background activity or poor regressions for decay curves.

secretion is increased from perfused liver of fasted rats (49). One should exercise some caution in extrapolating the results of the present study to explain the physiological role of postprandial hyperinsulinemia in controlling VLDL production.

Apo B and triglyceride each reflect different components of VLDL. The parallel changes in the relative production rates for each strengthens the conclusions that can be drawn from these studies.

In conclusion we have shown that insulin inhibits VLDL production acutely in healthy young males, both via its ability to suppress FFA mobilization from peripheral tissues as well as by some other mechanism, perhaps a direct hepatic effect. As suggested by Sparks and Sparks (39) this suppressive effect of insulin may play an important role in limiting the postprandial competition between hepatic and intestinal triglyceride-rich lipoprotein particles for clearance during feeding while at the same time promoting hepatic triglyceride storage, thus smoothing out the transition from fed-to-fasting states.

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