Chronic Physiologic Hyperinsulinemia Impairs Suppression of Plasma Free Fatty Acids and Increases De Novo Lipogenesis But Does Not Cause Dyslipidemia in Conscious Normal Rats

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Type 2 diabetes mellitus and obesity are characterized by fasting hyperinsulinemia, insulin resistance with respect to glucose metabolism, elevated plasma free fatty acid (FFA) levels, hypertriglyceridemia, and decreased high-density lipoprotein (HDL) cholesterol. An association between hyperinsulinemia and dyslipidemia has been suggested, but the causality of the relationship remains uncertain. Therefore, we infused eight 12-week-old male catheterized conscious normal rats with insulin (1 mU/min) for 7 days while maintaining euglycemia using a modification of the glucose clamp technique. Control rats (n = 8) received vehicle infusion. Baseline FFAs were 1.07 ± 0.13 mmol/L, decreased to 0.57 ± 0.10 ($P < .05$) upon initiation of the insulin infusion, and gradually increased to 0.95 ± 0.12 by day 7 (P = NS *v* baseline). On day 7 after a 6-hour fast, plasma insulin, glucose, and FFA levels in control and chronically hyperinsulinemic rats were 32 ± 5 versus 116 ± 21 mU/L ($P < .005$), 122 ± 4 versus 129 ± 8 mg/dL (P = NS), and 1.13 ± 0.18 versus 0.95 ± 0.12 mmol/L (P = NS); total plasma triglyceride and cholesterol levels were 78 ± 7 versus 66 ± 9 mg/dL (P = NS) and 50 ± 3 versus 47 ± 2 mg/dL (P = NS), respectively. Very-low-density lipoprotein (VLDL) + intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and HDL2 and HDL3 subfractions of plasma triglyceride and cholesterol were similar in control and hyperinsulinemic rats. Plasma FFA correlated positively with total ($r = .61$, $P < .005$) triglycerides. On day 7 after an 8-hour fast, hyperinsulinemic-euglycemic clamps with 3-^{3}H-glucose infusion were performed in all rats. Chronically hyperinsulinemic rats showed peripheral insulin resistance (glucose uptake, 15.8 ± 0.8 *v* 19.3 ± 1.4 mg/kg · min, $P < .02$) but normal suppression of hepatic glucose production (HGP) compared with control rats (4.3 ± 1.0 *v* 5.6 ± 1.4 mg/kg · min, P = NS). De novo tissue lipogenesis (3-^{3}H-glucose incorporation into lipids) was increased in chronically hyperinsulinemic versus control rats (0.90 ± 0.10 *v* 0.44 ± 0.08 mg/kg · min, $P < .005$). In conclusion, chronic physiologic hyperinsulinemia (1) causes insulin resistance with regard to the suppression of plasma FFA levels and increases lipogenesis; (2) induces peripheral but not hepatic insulin resistance with respect to glucose metabolism; and (3) does not cause an elevation in VLDL-triglyceride or a reduction in HDL-cholesterol.

THE COMBINATION of type 2 diabetes mellitus, obesity, atherosclerotic cardiovascular disease, and hypertension is frequently found in the general population and is associated with hyperinsulinemia, insulin resistance for glucose metabolism, and dyslipidemia. The dyslipidemia is characterized by elevated plasma free fatty acid (FFA) levels, increased very-low-density lipoprotein (VLDL) triglyceride concentrations, and reduced high-density lipoprotein (HDL) cholesterol concentrations.[1-3] This constellation of abnormalities is part of a syndrome,[2] which has been referred to as the insulin resistance syndrome or syndrome X.[1]

A similar syndrome exists in rat models for type 2 diabetes mellitus and obesity.[4] Zucker,[5] Dahl,[6] hypertriglyceridemic,[7] and Wistar fatty[8] rats are characterized by a cluster of disorders including dyslipidemia, hyperinsulinemia, insulin resistance with respect to both glucose and lipid metabolism, and hypertension (in the hypertriglyceridemic rat). Because of the multiple metabolic and hemodynamic associations and interactions, it is difficult to know which components of the syndrome are primary and which are secondary. The combination of hyperinsulinemia and insulin resistance is thought by many investigators to be of primary pathogenic importance in the development of the cluster of abnormalities that comprise the insulin resistance syndrome.[3] Epidemiological and experimental data in humans and animals support a key role for hyperinsulinemia in the etiology of dyslipidemia.[4,9,10] However, most studies that have examined the effect of a perturbation in the plasma insulin concentration on glucose and lipid metabolism have studied the acute changes (several hours of hyperinsulinemia) and/or have failed to maintain the plasma glucose concentration constant.[11-17] Concerning the latter, it is well known that both hypoglycemia and hyperglycemia have important effects on insulin-mediated glucose metabolism, ie, hypoglycemia causes the release of counterregulatory hormones that stimulate lipolysis, elevate plasma FFA levels, and impair insulin sensitivity, whereas sustained hyperglycemia impairs insulin sensitivity via downregulation of the glucose transport system.[18] Consequently, it remains unclear whether chronic hyperinsulinemia, while maintaining euglycemia with a variable exogenous glucose infusion to prevent hypoglycemia or hyperglycemia, induces insulin resistance for glucose and lipid metabolism and/or causes dyslipidemia.

To investigate whether a causal relationship exists between hyperinsulinemia and dyslipidemia, we exposed catheterized conscious normal rats to 7 days of physiological hyperinsulinemia while maintaining strict euglycemia using a modification of the glucose clamp technique.

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MATERIALS AND METHODS

Animals, Housing, and Feeding

Sixteen male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 300 to 320 g were individually housed in metabolic cages (21 × 22 × 26 cm) under conditions of constant temperature (23°C) and a 12-hour light/dark photoperiod (lights on from 6 AM to 6 PM). Water was available ad libitum. Food (Teklad LM-485 rat sterilizable diet, Madison, WI) was available from 6 PM to 6 AM. Nutrient composition was as follows: protein 19.9%, fat 5.7%, fiber 4.4%, ash 6.5%, and nitrogen-free extract 52.5%. The gross energy content of the chow was 4.05 kcal/g.

Surgical and Experimental Procedure

The present study design used a modification of the euglycemic insulin clamp technique.[19] Rats were anesthetized with an intraperitoneal injection of phentobarbital (50 mg/kg body weight), and indwelling catheters were inserted in the right internal jugular vein and the left carotid artery as previously described.[20,21] The rats were permanently connected to a swivel insulin/glucose infusion system and placed in individual metabolic cages. After surgery, the animals were allowed to recover for 1 week, during which time they reached their preoperative weight. During this week, the rats were adapted to the entire experimental procedure, ie, blood sampling and vehicle fluid infusion.

After the 1-week adaptation period, insulin was infused (n = 8) for 1 week at a constant rate of 1 mU/min and a 25% glucose infusion was adjusted to maintain euglycemia (Fig 1). This glucose infusion profile was established empirically in a pilot study in five rats. The plasma glucose level was measured three times in the morning, twice in the afternoon, and once in the evening after the rats began to eat. Plasma glucose levels were maintained at about 7.2 mmol/L with a coefficient of variation less than 15%. In the pilot experiments, the range of plasma glucose levels was 5.0 to 8.5 mmol/L. Using the glucose infusion profile shown in Fig 1 as a guideline, only minimal individual adjustments of the 25% glucose infusion rate were required during the experiments. The control group (n = 8) received an equal daily volume of vehicle fluid (22 mL/d 0.3% NaCl) to mimic the water/electrolyte load in the insulin/glucose-infused group.

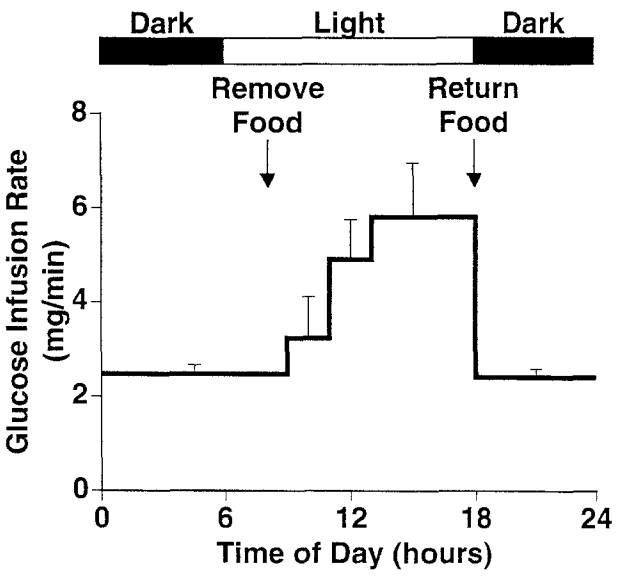

Fig 1. Mean 24-hour glucose infusion rate required to maintain euglycemia during the 7-day period of continuous insulin infusion.

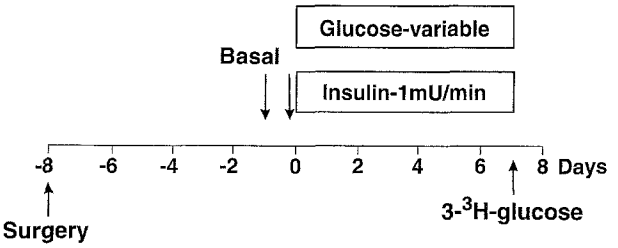

Fig 2. Schematic representation of the experimental protocol. Rats were infused for 7 days with vehicle or insulin (1 mU/min). On day 7, [3-^{3}H]-glucose was infused to quantify insulin-mediated glucose disposal in hyperinsulinemic rats. On day 7, control (vehicle-infused) rats received a euglycemic insulin clamp with [3-^{3}H]-glucose for 2 hours.

Daily Measurements

Water and food intake was quantified each day at 8 AM. At 1 PM, a 0.5-mL sample of citrated blood (10 μL, 150 mg/mL) was collected for measurement plasma insulin, glucose, and FFA. Blood loss was prevented by transfusing an equal volume of fresh prewarmed citrated (3 mg/mL) blood taken from a donor rat by heart puncture. The blood transfusion procedure has been shown not to influence the level of metabolites and hormones.[20]

Plasma Lipid Levels

A basal blood sample (2 mL) was collected on day 0 before insulin infusion, and a second sample was collected after 7 days of insulin or vehicle infusion. Blood loss was prevented by transfusing an equal volume of fresh prewarmed citrated blood as already described. Each blood sample was collected on citrate (40 μL, 150 mg/mL) and immediately centrifuged at low speed (3,000 rpm) to obtain plasma. Plasma lipoproteins were separated by density gradient ultracentrifugation at 40,000 rpm in an SW 41 Ti rotor at 6°C for 24 hours using Beckman ultracentrifuge model L8-70 (Beckman, Palo Alto, CA) as described previously.[22] The refractive index was measured, and the fractions were pooled on the basis of density as follows: VLDL plus low-density lipoprotein ([LDL]d < 1.019 g/mL), LDL (d = 1.019 to 1.063 g/mL), HDL2 (d = 1.063 to 1.10 g/mL), and HDL3 (d = 1.10 to 1.21 g/mL). Triglyceride (Stanbio Enzymatic Triglyceride Kit; Stanbio Laboratory, San Antonio, TX) and cholesterol (Cholesterol Kit; Wako Chemical, Richmond, VA) concentrations in plasma and in lipoproteins were measured by enzymatic methods. For quality control, a reference sample was included with each assay.

Hyperinsulinemic-Euglycemic Clamp With 3-^{3}H-Glucose Infusion

At 4 PM on day 7 following an 8-hour fast, a primed (4.7 μCi)–continuous (0.15 μCi/min) infusion of 3-^{3}H-glucose (DuPont-NEN, Boston, MA) was administered for 2 hours in rats receiving the long-term insulin infusion, and the plasma glucose concentration was maintained at 6.8 ± 0.3 mmol/L.[19-21] In rats receiving the vehicle infusion, at 4 PM on day 7, a primed (17 mU)–continuous (1 mU/min) insulin infusion was administered for 2 hours and a variable infusion of 25% glucose was adjusted to maintain the plasma glucose at 6.7 ± 0.2 mmol/L. A primed (4.7 μCi)–continuous (0.15 μCi/min) infusion of 3-^{3}H-glucose also was started with the insulin/glucose infusion to quantify the rate of glucose turnover in the vehicle-infused rats. A schematic representation of the experimental protocol is shown in Fig 2. In all studies, blood samples for determination of plasma tritiated glucose specific activity were obtained at 10-minute intervals throughout the study. Steady-state conditions for plasma [3-^{3}H]-glucose

specific activity were reached within 30 minutes after initiation of the [3-^{3}H]-glucose infusion. Plasma samples for insulin were collected at 80, 100, and 120 minutes. Blood loss was quantitatively replaced by a solution (1:1 vol/vol) of 3 mL fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 mU/mL). At the end of the study, rats were injected with sodium pentobarbital (60 mg/kg body weight intravenously), the abdomen was quickly opened, and the liver and rectus abdominal muscles were freeze-clamped in situ. Within 10 to 15 seconds, the psoas muscles and epididymal fat pads were freeze-clamped with aluminum tongs precooled in liquid nitrogen. All tissue samples were kept frozen at −80°C until analysis.

Whole-Body Glucose Uptake, Hepatic Glucose Production, Whole-Body Glycolysis, and Whole-Body Glucose Storage

Calculations were performed as described previously.[19-21,23,24] A steady-state plateau of plasma 3-^{3}H-glucose specific activity was achieved during the last 40 minutes of [3-^{3}H]-glucose infusion in each study. During this steady-state period, the glucose rate of appearance (Ra) equals the glucose rate of disappearance (Rd), and the glucose turnover rate was calculated by dividing the [3-^{3}H]-glucose infusion rate (dpm/min) by the steady-state plasma [3-^{3}H]-glucose specific activity (dpm/mg). In the insulin-stimulated state, the Ra equals the rate of hepatic glucose production (HGP) plus the rate of exogenous glucose infusion. Therefore, HGP = Ra − exogenous glucose infusion. In the insulin-stimulated state, the Rd equals the rate of whole-body glucose uptake.

The rates of whole-body glycolysis and whole-body glucose storage were determined as previously described.[23,24] Briefly, ^{3}H in the C-3 position of glucose is lost selectively to H_2O during glycolysis.[23,25] Therefore, plasma tritiated counts are present either as ^3H_2O or as [3-^{3}H]-glucose. Rates of whole-body glycolysis were estimated from the increment per unit time in ^3H_2O (dpm/L per minute) multiplied by the total body water mass (L) and divided by the [3-^{3}H]-glucose specific activity (dpm/mg). Plasma H_2O is assumed to be 93% of the total plasma volume, and the total body H_2O mass is assumed to be 65% of the body weight.[23] The rate of whole-body glycolysis was determined during the last 70 minutes of [3-^{3}H]-glucose infusion. The appearance of ^3H_2O in plasma over this period was linear in all studies as judged by linear regression analysis. The rate of whole-body glucose storage was calculated by subtracting the rate of whole-body glycolysis from the whole-body glucose Rd.

Muscle, Liver, and Adipocyte Glycogen Synthesis and Lipogenesis In Vivo

For each rat, the mean of two determinations per rectus abdominal and psoas muscle, liver, and epididymal fat pad was used to approximate the mean whole-body glycogen and lipid synthetic rates as described previously.[24] This extrapolation is based on the observation that the fiber composition of psoas (5% type I, 40% type IIa, and 55% type IIb) and rectus abdominal (20% type IIa and 80% type IIb) muscles is representative of the whole-body muscle fiber composition in the rat. The muscle mass of the rat consists mainly (95%) of type II fibers. When extrapolating from the tissue to the whole body, it was assumed that skeletal muscle, liver, and adipocytes contributed 40%, 4% (average liver weight in 40 rats; unpublished observations, Koopmans and DeFronzo, 1998), and 10% to whole-body weight, respectively.[23,24] The rate of glycogen or lipid synthesis was expressed as milligrams of glucose units in glycogen or lipid per kilogram (wet weight) of tissue per minute.

The rates of skeletal muscle, liver, and adipocyte glycogen or lipid synthesis were calculated by dividing the [3-^{3}H]-glucose counts in glycogen or lipid per kilogram tissue by the time-weighted mean plasma [3-^{3}H]-glucose specific activity (dpm/mg glucose) during the period of [3-^{3}H]-glucose infusion.[23,24] At the end of the insulin clamp studies, a portal vein blood sample was obtained simultaneously with the tissue collection procedure. [3-^{3}H]-glucose specific activity in portal blood was 99% ± 3% of the peripheral blood tritiated glucose specific activity. Since the tritiated glucose specific activity in peripheral blood during the insulin clamp closely reflects the tritiated glucose specific activity in the portal vein blood, it can be used to calculate the liver glycogen synthetic rate. Determination of the net rate of glycogen or lipid synthesis in the aforementioned way includes only the contribution of the direct pathway (from glucose); it does not include the contribution from indirect (C-3 fragments) pathways.

Chemical Determinations

The plasma glucose level was measured by the glucose oxidase method (Beckman Glucose Analyzer; Beckman Instruments, Fullerton, CA) and plasma insulin by radioimmunoassay using rat and porcine standards (Rat insulin kit; Linco, St. Louis, MO). The plasma lactate concentration was measured spectrophotometrically. Plasma FFAs were determined by an enzymatic calorimetric method using a commercial kit (NEFA C; Wako, Richmond, VA). Methods for the determination of plasma [3-^{3}H]-glucose specific activity have been previously described.[19-21,23,24] Briefly, plasma proteins were precipitated by $Ba(OH)_2$ and $ZnSO_4$ (Somogyi procedure) and the supernatants were evaporated to dryness at 55°C to eliminate ^3H_2O, reconstituted in 0.1 mL water, mixed with 3 mL Scintiverse II (Fisher, Pittsburgh, PA), and counted in a Beckman LS 6000 IC beta scintillation counter. All samples were assayed in duplicate. Plasma ^3H_2O radioactivity was calculated by subtracting the dpm in an aliquot of the Somogyi supernatant that was evaporated to dryness from an unevaporated aliquot.

The glycogen concentration was determined in triplicate in tissue homogenates after digestion with amyloglucosidase as previously described.[23,24] The intraassay and interassay coefficients of variation for muscle and liver glycogen were less than 10%. The tritiated glucose counts in muscle, liver, and adipocyte glycogen were determined as previously described.[23,24] Briefly, 250 mg tissue was homogenized in 1 mL 100-mmol/L phosphate buffer, pH 7.4, containing protease inhibitors, and a 125-μL aliquot of the supernatant of tissue homogenates was used to determine the amount of tritium label in glycogen. Glycogen was precipitated on Whatman #1 filter paper (Markson Labs, Hillsboro, OR) by washing in absolute ethanol at −20°C for 30 minutes. The washing procedure was repeated two more times, and the filter paper was placed in acetone for 5 minutes, air-dried, and counted in a beta scintillation counter.

To determine the tritiated glucose counts in the lipid of skeletal muscle, liver, and epididymal fat, approximately 1 g tissue was homogenized in 3 mL PBS. Six milliliters of a chloroform:methanol solution (1:2 vol/vol) was added and vortexed for 5 minutes. Subsequently, 2 mL chloroform was added and vortexed for 5 minutes; 2 mL water was then added and vortexed for 5 minutes. The samples were centrifuged for 10 minutes at 3,000 rpm. The upper aqueous and middle protein layers were separated from the lower lipid-containing chloroform fraction. The chloroform fraction was evaporated to dryness and counted.

Statistical Analysis

Multiple comparisons between two groups were performed using two-way ANOVA. When ANOVA showed a significant difference, Fisher's least-significant difference test was used to locate the differences. Single comparisons between two groups were made using the unpaired two-tailed Student's t test. The criterion for significance was set at P less than .05. All data are presented as the mean ± SEM.

RESULTS

Before (day 0) the start of the insulin infusion, plasma insulin, glucose, and FFA concentrations, body weight, and

food intake were similar in control and insulin-infused rats (Table 1). After 7 days of insulin or vehicle infusion, plasma insulin levels were increased fourfold in hyperinsulinemic versus control rats, whereas plasma glucose and FFA levels were similar in the two groups. Body weight increased similarly in both groups, but food intake was decreased 40% in chronically hyperinsulinemic rats compared with controls. The calories infused to maintain euglycemia compensated for the reduction in oral caloric intake (Fig 3).

The average 24-hour glucose infusion rate required to maintain euglycemia (between 6.1 and 7.8 mmol/L) during the 7 days of insulin infusion (1 mU/min) in the hyperinsulinemic group is shown in Fig 1. Each morning after removal of the food, the glucose infusion rate was increased stepwise, reaching a plateau of 5.9 ± 1.2 mg/min in the afternoon. After food was returned at the start of the nocturnal feeding period, the glucose infusion rate was decreased to 2.4 ± 0.2 mg/min. The glucose infusion rate remained fairly constant (decline <10% as a function of the duration of insulin infusion).

Plasma FFA and glucose levels during the 7-day period of insulin infusion are shown in Fig 4. On day 1 after initiation of the insulin/glucose infusion, plasma FFA concentrations decreased approximately 50% compared with basal (days −1 and 0) and returned to values not significantly different from baseline by day 7 (Table 1). Plasma FFA levels did not change in vehicle-infused control rats. Plasma glucose levels were similar in insulin/glucose-infused and vehicle-infused rats.

Figure 5 shows plasma lipoprotein concentrations after 7 days of insulin or vehicle infusion. Total plasma triglyceride and cholesterol levels were similar in hyperinsulinemic and control rats. The amount of triglyceride and cholesterol in VLDL + IDL, LDL, HDL2, and HDL3 subfractions also was similar in both groups of rats. After 7 days of insulin and vehicle infusion, plasma cholesterol and triglyceride profiles were unchanged from baseline in hyperinsulinemic and control rats (data not shown).

Plasma FFA correlated positively with total ($r = .61$, $P < .005$), VLDL + IDL ($r = .40$, $P < .05$), and LDL ($r = .57$, $P < .005$) triglyceride concentrations (Fig 6). No correlation was found between plasma FFA and HDL triglyceride concentrations and between plasma FFA and cholesterol subfractions. Plasma glucose and insulin levels did not correlate with triglyceride or cholesterol subfractions.

After 7 days of insulin or vehicle infusion, the effect of insulin on in vivo glucose metabolism was determined using a hyperinsulinemic-euglycemic clamp with 3-^{3}H-glucose infusion (Table 2). From comparable experiments performed after 8

Table 1. Plasma Glucose, Insulin, and FFA Concentrations, Body Weight, and Food Intake in Control and Chronically Hyperinsulinemic Rats

	Control		Hyperinsulinemic	
Parameter	Day 0	Day 7	Day 0	Day 7
Insulin (mU/L)	28 ± 4	37 ± 8	28 ± 2	116 ± 21*
Glucose (mmol/L)	6.8 ± 0.1	6.8 ± 0.2	6.7 ± 0.2	7.2 ± 0.5
FFA (mmol/L)	0.97 ± 0.10	1.13 ± 0.18	0.98 ± 0.14	0.95 ± 0.12
Body weight (g)	331 ± 5	361 ± 10	342 ± 6	367 ± 10
Food intake (g/d)	17 ± 2	20 ± 2	20 ± 3	12 ± 2*

*$P < .005$ *v* control.

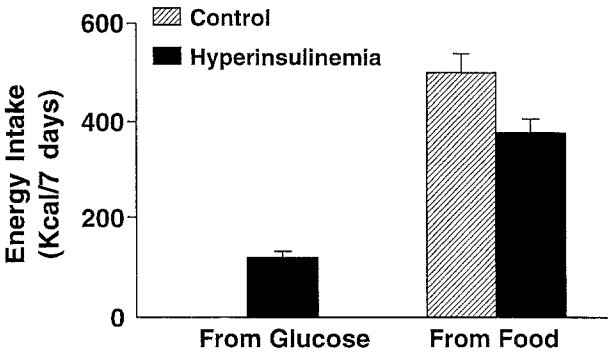

Fig 3. Cumulative (7-day) energy intake in control and chronically hyperinsulinemic rats.

hours of fasting, we know that basal glucose turnover and glycolysis are on the order of 8 to 10 mg/kg · min. Compared with vehicle-infused rats, 7 days of sustained physiologic hyperinsulinemia resulted in an 18% reduction of whole-body glucose uptake but a normal suppression of HGP. Glycolysis was similar in both groups, but glucose storage was decreased by 51% in insulin-infused rats and accounted for all of the reduction in insulin-mediated glucose uptake. The impairment

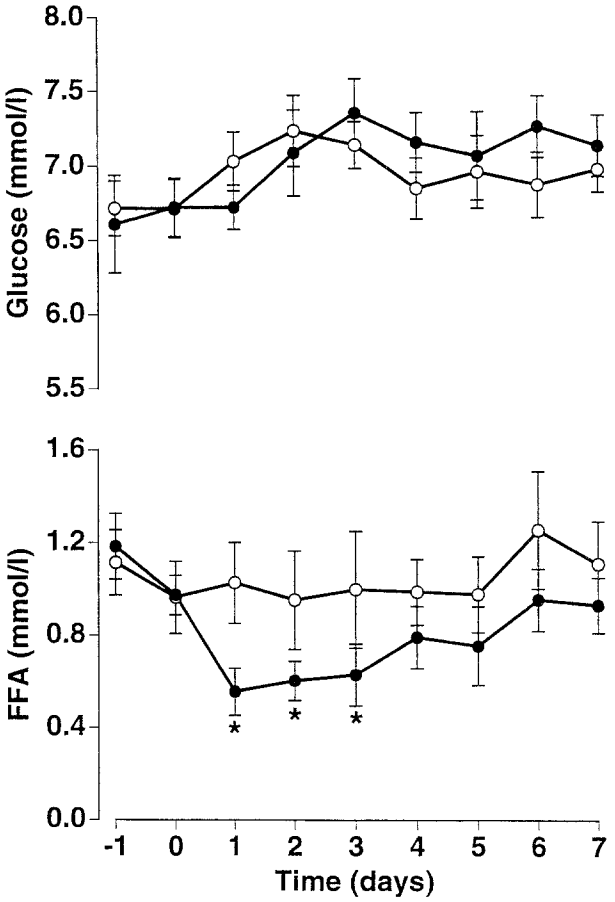

Fig 4. Plasma glucose and FFA concentrations in rats receiving continuous insulin infusion while maintaining euglycemia (●) and in control rats receiving vehicle infusion (○). *$P < .05$ *v* days −1, 0, 6, and 7; *$P < .05$ for day 1 hyperinsulinemic rats *v* control rats.

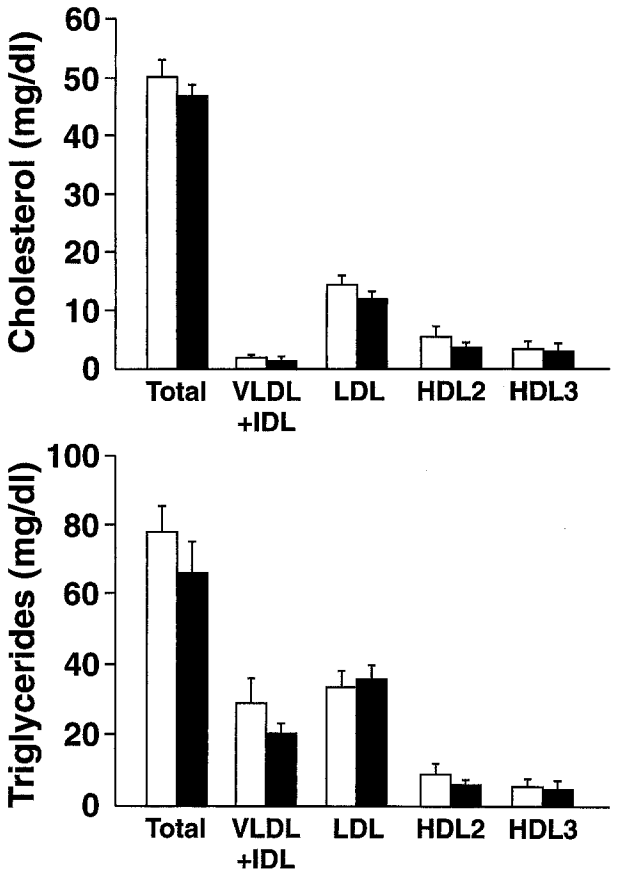

Fig 5. Total and subfraction plasma triglyceride and cholesterol lipoprotein concentrations in control (□) and chronically hyperinsulinemic (■) rats after 7 days of vehicle or insulin infusion.

in insulin action was observed despite steady-state plasma insulin levels during the hyperinsulinemic-euglycemic clamp that were 32% higher in chronically insulin-infused rats compared with vehicle-infused rats. When insulin-mediated whole-body glucose disposal is expressed as the insulin sensitivity index (amount of glucose metabolized per unit of insulin), whole-body insulin-mediated glucose uptake was reduced by 38% (from 19.3 ± 14 to 12.0 ± 6 mg/kg · min per mU/L), glycolysis by 28% (from 14.1 ± 1.4 to 10.1 ± 0.7 mg/kg · min per mU/L), and glucose storage by 63% (from 5.1 ± 0.4 to 1.9 ± 0.5 mg/kg · min per mU/L). Insulin-mediated suppression of HGP was similar in insulin-infused and vehicle-infused rats (5.6 ± 1.5 *v* 5.7 ± 1.3 mg/kg · min per mU/L). Plasma glucose and lactate levels were identical during insulin clamp studies in both groups. The ability of insulin to suppress plasma FFA levels was significantly impaired in the chronically hyperinsulinemic rats (0.91 ± 0.11 mmol/L) compared with controls (0.66 ± 0.06 mmol/L, $P < .05$) (Table 2).

Insulin-stimulated rates of tissue glycogenesis and lipogenesis are shown in Table 3. Chronically hyperinsulinemic rats showed a reduction in tissue glycogen synthesis (~30%) and an increase in tissue de novo lipogenesis (~twofold) compared with control rats. The observed decrease in the rate of glycogenesis occurred in the face of a 32% higher plasma insulin level in chronically hyperinsulinemic versus control rats (91 ± 8 *v* 69 ± 5 mU/L; Table 2). The concentration of glycogen in skeletal muscle (6.4 ± 0.3 *v* 7.2 ± 0.7 mg glycogen/g tissue, respectively) and liver (19.3 ± 3.2 *v* 18.3 ± 2.6, respectively) was not different in chronically hyperinsulinemic versus control rats.

DISCUSSION

The present results demonstrate that chronic hyperinsulinemia while maintaining euglycemia results in an impairment of insulin's ability to suppress plasma FFA levels. This indicates the development of insulin-induced insulin resistance for antilipolysis and/or a reduction in tissue FFA uptake. The former speculation is supported by previous in vitro[26] and in vivo[27] studies, which demonstrate that chronic continuous exposure of rat adipocytes to insulin enhances lipolysis, activates hormone-sensitive lipase, and causes an impairment in the antilipolytic action of insulin. The latter speculation is based on the Randle cycle,[28] which predicts that chronic stimulation of glucose

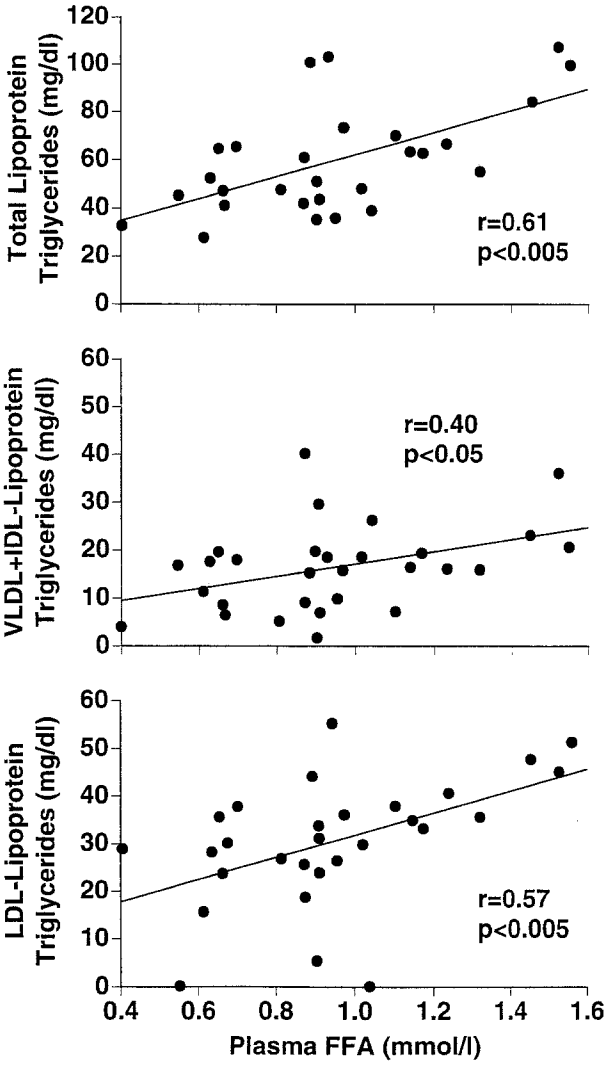

Fig 6. Relationship between plasma FFA concentration and plasma total, VLDL+ IDL, and LDL lipoprotein triglyceride concentrations in control and hyperinsulinemic rats (n = 29).

Table 2. Insulin-Mediated Glucose Metabolism and FFA Levels in Control and Chronically Hyperinsulinemic Rats, as Measured During a Euglycemic-Hyperinsulinemic (insulin infusion rate, 1 mU/min) Clamp on Day 7 of the Study

Parameter	Control Rats Acutely (2 h) Exposed to Hyperinsulinemia	Hyperinsulinemic Rats Chronically (7 d) Exposed to Hyperinsulinemia
Plasma insulin (mU/L)	69 ± 5	91 ± 8*
Plasma glucose (mmol/L)	6.9 ± 0.2	7.1 ± 0.1
Plasma lactate (mmol/L)	1.4 ± 0.1	1.6 ± 0.2
Plasma FFA (mmol/L)	0.66 ± 0.06	0.91 ± 0.11*
Whole-body glucose uptake (mg/kg · min)	19.3 ± 1.4	15.8 ± 0.8†
Whole-body glycolysis (mg/kg · min)	14.1 ± 1.4	13.3 ± 0.9
Whole-body glucose storage (mg/kg · min)	5.1 ± 0.4	2.5 ± 0.6†
HGP (mg/kg · min)	5.6 ± 1.5	4.3 ± 1.0

*$P < .05$, †$P < .01$ *v* control.

uptake and oxidation by insulin ultimately will lead to a reduction in FFA uptake and oxidation (ie, the concept of substrate competition). The metabolic constellation of chronic hyperinsulinemia, impaired muscle glycogen synthesis, impaired suppression of plasma FFA levels, and increased tissue lipogenesis clearly mimics the situation observed in type 2 diabetic patients.[2] Adipose tissue in type 2 diabetic individuals is resistant to the antilipolytic action of insulin, resulting in increased lipolysis.[29] At the same time, most type 2 diabetic patients are obese, suggesting that lipogenesis also is enhanced. This metabolic situation is mimicked in the present study, in which primary hyperinsulinemia caused a twofold increase in lipogenesis and an impaired ability of insulin to suppress plasma FFA levels. Therefore, the hyperinsulinemia present in type 2 diabetic patients may contribute to the observed disturbances in fat metabolism. In addition, primary hyperinsulinemia also induces insulin resistance for the stimulation of skeletal muscle glycogen synthesis, a defect that is characteristic of type 2 diabetes mellitus (Table 2). Thus, chronic hyperinsulinemia is capable of inducing and/or perpetuating abnormalities in both glucose and lipid metabolism.

Table 3. Insulin-Stimulated Tissue-Specific Glycogen Synthesis and De Novo Lipogenesis in Control and Chronically Hyperinsulinemic Rats, as Measured During a 2-Hour Hyperinsulinemic (insulin infusion rate, 1 mU/min)-Euglycemic Clamp Performed With [3-^{3}H]-Glucose on Day 7 of the Study

Parameter	Control Rats Acutely (2 h) Exposed to Hyperinsulinemia	Hyperinsulinemic Rats Chronically (7 d) Exposed to Hyperinsulinemia
Glycogen synthesis (mg/kg · min)		
Muscle	0.49 ± 0.09	0.31 ± 0.04*
Liver	0.35 ± 0.08	0.30 ± 0.04
Adipocyte	0.06 ± 0.03	0.02 ± 0.01*
Total	0.90 ± 0.18	0.63 ± 0.05
De novo lipogenesis (mg/kg · min)		
Muscle	0.20 ± 0.06	0.25 ± 0.04
Liver	0.11 ± 0.02	0.15 ± 0.03
Adipocyte	0.13 ± 0.04	0.50 ± 0.09†
Total	0.44 ± 0.08	0.90 ± 0.10†

*$P < .05$, †$P < .005$ *v* control.

Insulin resistance with regard to the suppression of plasma FFA developed gradually, beginning several days after the initiation of chronic hyperinsulinemia. This is in contrast to hyperinsulinemia-induced insulin resistance for muscle glycogen synthesis, which develops within 12 hours.[24] This further underscores the independent action of insulin on different pathways of glucose and fat metabolism, and clearly demonstrates that insulin-induced resistance is not a universal phenomenon that involves all aspects of insulin action.[30] As an extension of this concept, the present study shows that insulin resistance for tissue lipogenesis does not develop after 1 week of hyperinsulinemia. On the contrary, particularly in adipose tissue, chronic hyperinsulinemia leads to a fourfold increase in de novo lipogenesis. Similar results have been reported previously by others in rats.[31-33]

The results of the present study also show that chronic physiologic hyperinsulinemia induces insulin resistance for whole-body insulin-mediated glucose uptake but does not lead to abnormalities in plasma lipoprotein levels. The ability of hyperinsulinemia to induce insulin resistance for glucose metabolism also has been observed in man.[34,35] The rat model we used to create a state of chronic primary hyperinsulinemia is characterized by a fourfold elevation in plasma insulin levels, development of insulin resistance for whole-body glucose uptake, normal suppression of HGP by insulin, normal basal plasma glucose and FFA concentrations, and normal plasma triglyceride and cholesterol lipoprotein concentrations. In a previous study,[27] we have shown that prandial and postprandial plasma insulin levels are in the range of 50 to 150 mU/L. Thus, the level of chronic hyperinsulinemia in the present study is well within the physiological range of plasma insulin levels in rats.

Although chronic physiologic hyperinsulinemia causes moderate to severe insulin resistance with regard to peripheral glucose disposal and an impaired ability of insulin to suppress plasma FFA levels, it was not associated with the development of dyslipidemia in the present study. By examining the effect of hyperinsulinemia under conditions of euglycemia and unchanged basal plasma FFA levels, we were able to distinguish between the effects of insulin per se versus the substrate-driven (by mass action) effects of glucose and FFA on plasma lipoprotein concentrations. It is well established that an increased availability of FFA results in increased hepatic triglyceride production and subsequent stimulation of hepatic VLDL secretion.[14,36,37] Moreover, in both obese nondiabetic subjects and obese type 2 diabetic individuals, the antilipolytic effect of insulin is impaired,[38] resulting in elevated plasma levels and increased flux of FFA to the liver. Since type 2 diabetic and obese subjects also are hyperglycemic, both of the major substrates, ie, FFA and glucose, for hepatic triglyceride production are present in excess and contribute to the development of hypertriglyceridemia. Indeed, our study shows a positive correlation between plasma FFA levels and plasma levels of total, VLDL + IDL, and LDL triglycerides, suggesting that the plasma FFA concentration may be a more important determinant of dyslipidemia than insulin. In contrast, neither plasma insulin nor glucose concentrations show any correlation with

plasma lipoprotein levels. Most studies have shown an inhibitory effect of insulin on VLDL secretion. This would oppose the stimulatory effect of elevated plasma FFA on VLDL production. For instance, studies in isolated hepatocytes have shown that insulin inhibits VLDL secretion, and intensive insulin therapy in diabetic patients leads to a reduction in plasma triglyceride levels[39] and a reduction in cholesterol synthesis,[40] although the interpretation of the latter two in vivo studies is clouded by the concomitant decline in plasma glucose and FFA levels. Insulin also stimulates adipose tissue lipoprotein lipase,[41,42] and this results in increased uptake of VLDL and reduced plasma VLDL concentrations.[39]

Since we found a close correlation between the plasma FFA concentration and plasma triglyceride lipoprotein levels, it is possible that primary hyperinsulinemia may (after months to years) lead to the emergence of dyslipidemia. According to this scenario, a more prolonged hyperinsulinemia could lead to a further worsening of the ability of insulin to suppress plasma FFA levels and a further enhancement of de novo lipogenesis. As this process continues, an elevation in the plasma triglyceride lipoprotein concentration develops. In addition, an increase in plasma FFA levels could induce hepatic insulin resistance.[43] This would interfere with the insulin-mediated suppression of VLDL-triglyceride secretion, further contributing to the development of hypertriglyceridemia.

The observation that primary hyperinsulinemia can induce individual components of the insulin resistance syndrome without producing others (ie, the absence of dyslipidemia) within the time frame of this study indicates the presence of a complex interplay between the individual components of the insulin resistance syndrome. This is consistent with rat studies showing that diet-induced hypertriglyceridemia can develop without hyperinsulinemia or impaired insulin-mediated glucose disposal and vice versa.[44] Similarly, correction of dyslipidemia with fish oil has no effect on plasma insulin levels in rats.[30]

In summary, chronic primary hyperinsulinemia induces whole-body insulin resistance primarily involving the glycogen synthetic pathway, increases lipogenesis, and impairs the suppression of FFA by insulin, but does not lead to dyslipidemia within the time frame of the present experimental design. If defects in lipogenesis and suppression of FFA by insulin continue to worsen with time, a substrate-driven induction of dyslipidemia may occur.

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REFERENCES

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

2. DeFronzo RA, Ferrannini E: Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991

3. DeFronzo RA: Insulin resistance, hyperinsulinemia, and coronary artery disease: A complex metabolic web. Coron Artery Dis 3:11-25, 1992

4. Reaven GM: Insulin resistance, hyperinsulinemia, hypertriglyceridemia and hypertension. Parallels between human disease and rodent models. Diabetes Care 14:195-202, 1991

5. Kasiske BL, O'Donnell MP, Keane WF: The Zucker rat model of obesity, insulin resistance, hyperlipidemia, and renal injury. Hypertension 19:110-115, 1992

6. Reaven GM, Twersky J, Chang H: Abnormalities of carbohydrate and lipid metabolism in Dahl rats. Hypertension 18:630-635, 1991

7. Stolba P, Dobesova Z, Husek P, et al: The hypertriglyceridemic rat as a genetic model of hypertension and diabetes. Life Sci 51:733-740, 1992

8. Kazumi T, Hirano T, Odaka H, et al: VLDL triglyceride kinetics in Wistar fatty rats, an animal model of NIDDM. Diabetes 45:806-811, 1996

9. Ferrannini E, Haffner SM, Mitchell BD, et al: Hyperinsulinemia: The key feature of a cardiovascular and metabolic syndrome. Diabetologia 34:416-422, 1991

10. Mitchell BD, Haffner SM, Hazuda HP, et al: The relation between serum insulin levels and 8-year changes in lipid, lipoprotein, and blood pressure levels. Am J Epidemiol 136:12-22, 1992

11. Cummings MH, Watts GF, Umpleby AM, et al: Acute hyperinsulinemia decreases the hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in NIDDM. Diabetes 44:1059-1065, 1995

12. Steiner G, Haynes FJ, Yoshino G, et al: Hyperinsulinemia and in vivo very-low-density lipoprotein-triglyceride kinetics. Am J Physiol 246:E187-E192, 1984

13. Bourgeois CS, Wiggins D, Gibbons GF: Chronic exogenous hyperinsulinaemia does not modify the acute inhibitory effect of insulin on the secretion of very-low-density lipoprotein triacylglycerol and apolipoprotein B in primary cultures of rat hepatocytes. Biochem J 314:103-108, 1996

14. Lewis GF, Uffelman KD, Szeto LW, et al: Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. J Clin Invest 95:158-166, 1995

15. O'Brien T, Young WF, Palumbo PJ, et al: Hypertension and dyslipidemia in patients with insulinoma. Mayo Clin Proc 68:141-146, 1993

16. Leonetti F, Lozzo P, Giaccari A, et al: Absence of clinically overt atherosclerotic vascular disease and adverse changes in cardiovascular risk factors in 70 patients with insulinoma. J Endocrinol Invest 16:875-880, 1993

17. McGuinness OP, Friedman A, Cherrington AD: Intraportal hyperinsulinemia decreases insulin-stimulated glucose uptake in the dog. Metabolism 39:127-132, 1990

18. Rosetti L, Giaccari A, DeFronzo RA: Glucose toxicity. Diabetes Care 13:610-630, 1990

19. Smith D, Rossetti L, Ferrannini E, et al: In vivo glucose metabolism in the awake rat. Tracer and insulin clamp studies. Metabolism 36:1167-1174, 1987

20. Koopmans SJ, de Boer SF, Sips HCM, et al: Whole body and hepatic insulin action in normal, starved and diabetic rats. Am J Physiol 260:E825-E832, 1991

21. Koopmans SJ, Maassen JA, Radder JK, et al: In vivo insulin responsiveness for glucose uptake at eu- and hyperglycemic levels in normal and diabetic rats. Biochim Biophys Acta 1115:230-238, 1992

22. McGill HC, McMahan CA, Kushwaha RS, et al: Dietary effects on serum lipoproteins of dyslipoproteinemic baboons with high HDL. Arteriosclerosis 6:651-663, 1986

23. Rossetti L, Giaccari A: Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. J Clin Invest 85:1785-1792, 1990

24. Koopmans SJ, Mandarino L, DeFronzo RA: Time course of insulin action on tissue-specific intracellular glucose metabolism in conscious normal rats. Am J Physiol 274:E642-E650, 1998

25. Ferrannini E, Del Prato S, DeFronzo RA: Glucose kinetics: Tracer methods, in Larner J, Pohl S (eds): Methods in Diabetes Research, vol 2. New York, NY, Wiley, 1985, pp 62-84

26. Kang ES, Betts D, Fain JN, et al: Chronic exposure of rat fat cells to insulin enhances lipolysis and activation of partially purified hormone-sensitive lipase. Diabetes 42:1415-1424, 1993

27. Koopmans SJ, Sips HCM, Krans HMJ, et al: Pulsatile intravenous insulin replacement in streptozotocin-diabetic rats is more efficient than continuous delivery: Effects on glycaemic control, insulin-mediated glucose metabolism and lipolysis. Diabetologia 39:391-400, 1996

28. Randle PJ, Garland PB, Hales CN, et al: The glucose-fatty-acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963

29. Nurjhan N, Consoli A, Gerich J: Increased lipolysis and its consequences on gluconeogenesis in non–insulin-dependent diabetes mellitus. J Clin Invest 89:169-175, 1992

30. Lombardo YB, Chicco A, D'Alessandro ME, et al: Dietary fish oil normalizes dyslipidemia and glucose intolerance with unchanged insulin levels in rats fed a high sucrose diet. Biochim Biophys Acta 1299:175-182, 1996

31. Koopmans SJ, Sips HCM, Bosman J, et al: Antilipolytic action of insulin in adipocytes from starved and diabetic rats during adenosine-controlled incubations. Endocrinology 125:3044-3050, 1989

32. Cusin I, Terrettaz J, Rohner-Jeanrenaud F, et al: Metabolic consequences of hyperinsulinemia imposed on normal rats on glucose handling by white adipose tissue, muscles and liver. Biochem J 267:99-103, 1990

33. Wardzala LJ, Hirshman M, Pofcher E, et al: Regulation of glucose utilization in adipose cells and muscle after long-term experimental hyperinsulinemia in rats. J Clin Invest 76:460-469, 1985

34. Rizza RA, Mandarino LJ, Genest J, et al: Production of insulin resistance by hyperinsulinemia in man. Diabetologia 28:70-75, 1985

35. Del Prato S, Leonetti F, Simonson DC, et al: Effect of sustained physiologic hyperinsulinemia and hyperglycemia on insulin secretion and insulin sensitivity in man. Diabetologia 37:1025-1035, 1994

36. Azain MJ, Fukuda N, Chao FF, 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

37. Byrne CD, Wareham NJ, Brown DC, et al: Hypertriglyceridaemia in subjects with normal and abnormal glucose tolerance: Relative contributions of insulin secretion, insulin resistance and suppression of plasma non-esterified fatty acids. Diabetologia 37:889-896, 1994

38. Groop L, Bonadonna RC, DelPrato S, et al: Effect of insulin on oxidative and non-oxidative pathways of glucose and FFA metabolism in NIDDM. J Clin Invest 84:205-213, 1989

39. Howard BV, Schneiderman N, Falkner B, et al: Insulin, health behaviors, and lipid metabolism. Metabolism 42:25-35, 1993 (suppl 1)

40. Scoppola A, Testa G, Frontoni S, et al: Effects of insulin on cholesterol synthesis in type II diabetes patients. Diabetes Care 18:1363-1369, 1995

41. Tavanger K, Murata Y, Pedersen ME, et al: Regulation of lipoprotein lipase in the diabetic rat. J Clin Invest 90:1672-1678, 1992

42. Terrettaz J, Cusin I, Etienne J, et al: In vivo regulation of adipose tissue lipoprotein lipase in normal rats made hyperinsulinemic and in hyperinsulinemic genetically-obese (fa/fa) rats. Int J Obes 18:9-15, 1994

43. Reaven GM, Chen IDI: Role of abnormal free fatty acid metabolism in the development of non–insulin-dependent diabetes mellitus. Am J Med 85:106-112, 1988

44. Boivin A, Deshaies Y: Dietary rat models in which the development of hypertriglyceridemia and that of insulin resistance are dissociated. Metabolism 44:1540-1547, 1995