Acute Hyperinsulinism Modulates Plasma Apolipoprotein B-48 Triglyceride–Rich Lipoproteins in Healthy Subjects During the Postprandial Period

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The role of postprandial insulin in the regulation of postprandial lipid metabolism is still poorly understood. The roles of hyperinsulinemia and insulin resistance in the alteration of postprandial lipid metabolism are not clear either. To improve knowledge in this area, we submitted healthy men to acute hyperinsulinemia in two different ways. In the first study, we compared in 10 men the effects of four isolipidic test meals that induce different degrees of hyperinsulinemia on postprandial lipid metabolism. Three different carbohydrate sources were compared according to their glycemic indexes (GIs; 35, 75, and 100 for white kidney bean, spaghetti, and white bread test meals, respectively); the fourth test meal did not contain any carbohydrates. Postprandial plasma insulin levels were proportional to the GIs (maximal plasma insulin concentrations: 113 ± 16 to 266 ± 36 pmol/l). We found a strong positive correlation during the 6-h postprandial period between apolipoprotein (apo) B-48 plasma concentration and insulin plasma concentration (r^2 = 0.70; P = 0.0001). In a second study, 5 of the 10 subjects again ingested the carbohydrate-free meal, but during a 3-h hyperinsulinemic- (550 ± 145 pmol/l plasma insulin) euglycemic (5.5 ± 0.8 mmol/l plasma glucose) clamp. A biphasic response was observed with markedly reduced levels of plasma apoB-48 during insulin infusion, followed by a late accumulation of plasma apoB-48 and triglycerides. Overall, the data obtained showed that portal and peripheral hyperinsulinism delays and exacerbates postprandial accumulation of intestinally derived chylomicrons in plasma and thus is involved in the regulation of apoB-48-triglyceride–rich lipoprotein metabolism, in the absence of insulin-resistance syndrome. *Diabetes* 50:462–469, 2001

From INSERM (National Institute of Health and Medical Research) (A.H., C.D., C.J., M.S., D.L.), Marseille; the Service de Nutrition (H.N., C.A.-G., B.V.), Hôpital Sainte-Marguerite, Marseille; UPRES 2193 (H.N., C.A.-G., B.V.), Université de la Méditerranée, Marseille; Bledina sa (C.L.), Villefranche-sur-Saône; Service d'Endocrinologie (B.D.), Centre Hospitalier du Pays d'Aix, Aix-en Provence; Laboratoire Central de Biochimie-Hématologie (H.P.), Hôpital Sainte-Marguerite, Marseille; and Laboratoire de Chimie Analytique (C.D.), Faculté de Pharmacie, Marseille, France.

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Received for publication 12 June 2000 and accepted in revised form 23 October 2000.

apo, apolipoprotein; AUC, area under the curve; B, white bread; CF, carbohydrate-free; EIC, euglycemic insulin clamp; ELISA, enzyme-linked immunosorbent assay; GI, glycemic index; HL, hepatic lipase; KB, white kidney bean; LPL, lipoprotein lipase; S, spaghetti; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

The role of insulin in the regulation of postprandial lipemia still remains misunderstood. However, abnormalities in postprandial lipid and lipoprotein metabolism are increasingly involved in the etiology of cardiovascular diseases (1,2) and in hypertriglyceridemic patients (3–5), type 2 diabetic patients (6–10), and patients affected by polymetabolic syndrome (5, 11,12). In the latter two situations particularly, an abnormal postprandial lipid metabolism is observed and acknowledged to favor atherogenesis (13). Because these patients display both hyperinsulinism and insulin resistance, it is difficult to evaluate the respective role of hyperinsulinism and insulin resistance in the abnormalities of the postprandial lipemia observed. Several studies have shown that the observed accumulation of exogenous triglyceride-rich lipoproteins (TRLs) after a fat-containing test meal positively correlates with both fasting and postprandial plasma insulin levels in normal or obese subjects (5,12,14,15). Some reports, on the basis of indirect arguments, have suggested that insulin resistance is the main factor in this phenomenon (5,12,14,15), and that the amplitude of postprandial lipemia correlates with the severity of insulin resistance (14). Thus, even if a clear consensus can be derived from these studies that hyperinsulinemia and insulin resistance are associated with exacerbated postprandial lipid accumulation, the causal relationship is not yet established.

It is widely accepted that the hypertriglyceridemic state found in the postprandial period results from accumulation of both hepatically derived triglyceride-rich lipoproteins (VLDL and LDL) and intestinally derived ones (chylomicrons and remnants) (5,16), although the relative importance and fate of the two kinds of lipoprotein particles is still being debated.

The aim of this study was to determine the role of postprandial hyperinsulinemia per se on postprandial lipemia and, especially, on TRLs from both origins. To achieve this goal, healthy normal-weight subjects with normal fasting insulin and lipid levels were recruited. In the nutritional study, they were given four regular fat-containing mixed test meals with different carbohydrate sources selected according to their glycemic/insulinemic indexes (17,18) to induce graded physiological insulin responses. In a complementary mechanistic approach, five subjects were submitted or not to a hyperinsulinemic-euglycemic clamp after the ingestion of a carbohydrate-free fat-containing meal during the first part (0–3 h) of the postprandial phase, to model the transient

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hyperinsulinism observed under high-glycemic–index meals. Under the two hyperinsulinemic states, the accumulation of apolipoprotein (apo) B-48–intestinally derived and apoB-100–hepatically derived TRLs in plasma was determined. The data obtained indicate that acute hyperinsulinism (in the absence of insulin-resistance syndrome) modulates the concentration of intestinally derived TRLs in plasma.

RESEARCH DESIGN AND METHODS

Study 1: Postprandial lipemia responses to test meals with different glycemic indexes.

Subjects. The 10 adult male volunteers in this study, average age 24 years (range 21–30), participated after giving written informed consent according to a protocol approved by the local medical ethics committee (Comite Consultatif pour la Protection des Personnes se Prêtant à des Recherches Bio-Médicales, Marseille I). No volunteer suffered from any digestive or metabolic disease, as confirmed by medical history and fasting blood parameters, nor was any subject on any drug treatment that could influence carbohydrate or lipid metabolism. Fasting blood concentrations were in the normal range, with the following overall mean ± SE values: serum triacylglycerol, 0.84 ± 0.07 mmol/l; total cholesterol, 4.6 ± 0.20 mmol/l; glucose, 5.20 ± 0.9 mmol/l; and insulin, 54.90 ± 5.64 pmol/l. No volunteer was obese (mean BMI, 22.8 ± 1.4 kg/m^2). All subjects had E_3/E_3 apoE genotypes. The subjects relied on a typical Western diet, with a moderate energy intake (means: 10,120 kJ/d; 2,421 kcal/d), with protein, carbohydrate, and fat accounting for 17, 44, and 39% energy, respectively. They were instructed to avoid alcohol consumption and intensive exercise for 36 h before being tested, and to reduce carbohydrate consumption the day before the study. The subjects were asked to have a light standard dinner before 9:00 P.M. the evening before the experiment. Each subject ingested the four test meals at a 1-week interval in a random order.

Test meals. Four different test meals were designed. Three carbohydrate components (90 g) were chosen according to their glycemic indexes (GIs) (18): white bread (GI 100; B test meal), spaghetti (GI 70; S test meal), or white kidney beans (GI 35; KB test meal). The fourth test meal was free of carbohydrates, containing only lipids and proteins (CF test meal). The four meals were isolipidic (40 g) and isoproteic (33 g) and were composed of commercially available food. The fat was provided as olive oil incorporated into tomato sauce. Protein was provided as cooked egg whites to adjust the protein level.

After an overnight fast, an antecubital vein was catheterized with intravenous cannulas equipped with disposable obturators (Jelco-Critikon, Chatenay-Malabry, France). A baseline fasting blood sample was collected at 0 h. The subjects then ingested the test meal within 20 min. The countdown was fixed at the middle of food intake. Blood samples were collected every half hour during the first 2 h and then every hour until 6 h after the meal.

Analytical determinations. Plasma and serum were immediately separated from whole blood by centrifugation (10°C, 910*g* for 10 min). Plasma glucose and triglyceride (TG) concentrations were determined by enzymatic procedure with commercial kits (Boehringer Mannheim, Meylan, France). Insulin was determined by an immunoenzymatic method with commercial kits (Boehringer Mannheim).

The chylomicron fraction (Sf >400) was isolated from 1.5 ml plasma layered under 1.5 ml 0.9% NaCl by ultracentrifugation at 15°C (33,810*g* for 6 min) using a Beckman TLX100 ultracentrifuge and a 100.3 rotor, as previously described (19). The VLDL fraction (Sf = 20–400) was isolated by sequential ultracentrifugation (after recovery of the chylomicron fraction) under 1.5 ml KBr (d = 1.006) at 15°C (540,960*g* for 2 h 40 min). TGs were assayed in lipoprotein fractions by enzymatic procedure with commercial kits, as described above.

ApoB-48 assayed by enzyme-linked immunosorbent assay. Assessment of apoB-48 was by competitive enzyme-linked immunosorbent assay (ELISA) with a specific apoB-48 antibody obtained in rabbits according to the procedure of Peel et al. (20). The assays were performed after modification of the method of Lovegrove et al. (21), according to Lorec et al. (22). A 96-well microtiter plate (Nunc Maxisorp, Polylabo, France) was coated with a C-terminal apoB-48–specific heptapeptide. Plasma samples were incubated with Triton X-100 to allow competition between immobilized antigen and plasma apoB-48. ApoB-48 containing chylomicrons was used as the standard. Peroxidase-labeled anti-rabbit immunoglobulins and their substrate allowed color development. The absorbance was read at 450 nm. Intra- and interassay coefficients of variation were 5.4 and 5.5%, respectively (22).

Assessment of apoB-100 in VLDL by noncompetitive sandwich ELISA. Briefly, a 96-well microtiter plate (Nunc Maxisorp) was coated with 2G8 monoclonal anti–apoB-100 antibody (Mona, Moscow, Russia), which does not cross-react with apoB-48 (23). VLDL samples were added, and the apoB-100–containing VLDLs that reacted with the monoclonal antibody were quantified after addition of an anti–apoB-100 polyclonal antibody (Calbiochem, Meudon, France) and then peroxidase-labeled immunoglobulins. The color was developed and measured as described above. ApoB-100 containing LDLs was used as the standard.

After 6-h postprandial blood sampling, each subject was injected with 80 U/kg heparin (Choay, Gentilly, France); a blood sample was drawn 10 min after heparin administration for subsequent determination of lipoprotein lipase (LPL) and hepatic lipase (HL) activities, according to the method of Krauss et al. (24).

Study 2: Postprandial lipemia responses to a CF test meal during a euglycemic insulin clamp

Euglycemic insulin clamp. Five subjects from the first study were included in this study and instructed to maintain their regular food habits. The goal of the euglycemic insulin clamp, as previously described by de Fronzo et al. (25), was to acutely raise plasma insulin concentrations to a new plateau and to maintain them at that level to determine the role of hyperinsulinemia in the changes observed during the processing of a test meal. Each subject received a continuous intravenous infusion of insulin (40 mU/ml; NovoNordisk) via an electric syringe to obtain insulin concentrations in the range 460–620 pmol/l over 3 h. To avoid hypoglycemia, the plasma glucose concentration was maintained at its euglycemic level with a 20% glucose solution infused in the same way with variable rate. Venous blood samples were obtained from similar catheters inserted in the contralateral antecubital vein kept open with 0.9% NaCl infusion.

Test meal. Subjects ingested the CF test meal 20 min after beginning insulin infusion, as described above, (at time 0 h) within 20 min (euglycemic insulin clamp [EIC] test meal). The countdown was fixed at the middle of food intake. Blood samples were collected every half hour during the first 3 h (–30 min to 3 h) and then every hour until 6 h after the meal.

Analytical determinations. Plasma glucose, insulin, lipids, apoB-48, and apoB-100 concentrations were determined as described above for the first study. In the same way, lipoprotein fractions were isolated as described above.

Statistical analysis. In this randomized study, each subject experimented with the four (or five) test meals and served as his own control. Results are given as means ± SE. Absolute postprandial changes are given as concentration values. The 0- to 6-, 0- to 3-, and 3- to 6-h areas under the curve (AUCs) were calculated with the incremental postprandial variations (postprandial values minus baseline) by the trapezoidal method. Statistical significance (P < 0.05) of differences observed among test meals was assessed by analysis of variance for repeated values and Fisher's test.

RESULTS

Study 1: Postprandial lipemia responses to test meals with different GIs. Ingestion of the three carbohydrate-containing meals resulted in a significant increase in glycemia (incremental 0- to 6-h AUC: 3.8 ± 0.94 [B] vs. 2.52 ± 0.4 [S] vs. 0.68 ± 0.57 [KB] vs. –1.03 ± 0.38 mmol · h^{-1} · l^{-1} [CF]) and insulinemia, which lasted the first hour after the S and KB test meals and 2 h after the B test meal. The CF test meal resulted in a slight increase in insulinemia. Figure 1 shows the incremental insulin postprandial responses during the first 3 and 6 h after the four test meals. The 0- to 3-h postprandial insulinemia was higher (P < 0.05) after the B meal than after the S and KB meals, which had comparable values; values were lower after the CF meal than after the other three meals. The 0- to 6-h postprandial insulinemia ranked in a comparable order.

Postprandial serum TGs rose significantly over baseline after the four test meals, with no significant differences observed among the four test meals (Fig. 2). Incremental 0- to 3-, 3- to 6-, and 0- to 6-h AUCs were not different overall.

Absolute postprandial changes in plasma apoB-48 concentrations (µg/ml) are shown in Fig. 3. The four test meals resulted in a significant increase in plasma apoB-48 concentration over baseline. The apoB-48 peaked 4 h after the two high-GI test meals (B and S test meals) and peaked 3 h after the KB and CF test meals, as shown in Fig. 3. The 4-h

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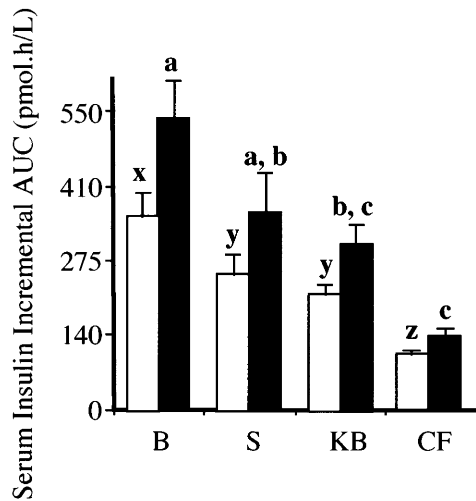

FIG. 1. Incremental 0- to 3-h (□) and overall 0- to 6-h (■) AUCs for serum insulin after ingestion of B, S, KB, or CF test meal. Data represent means ± SE; $n = 10$. Means not sharing a common letter designation (a, b, c or x, y, z) differed from each other at $P < 0.05$.

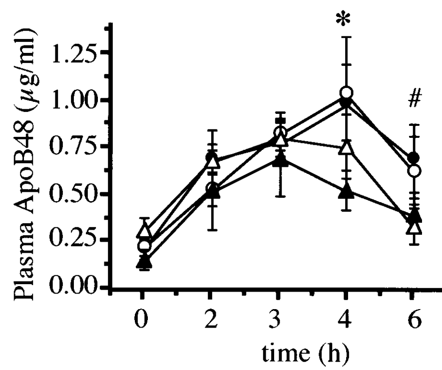

FIG. 3. Plasma apoB-48 concentrations for 6 h after ingestion of B (●), S (○), KB (▲), or CF (△) test meal. *B and S significantly higher than KB ($P < 0.05$); #B and S significantly higher than CF ($P < 0.05$). Data represent means ± SE; $n = 10$.

apoB-48 concentrations were significantly higher ($P < 0.05$) after the B and S test meals than after the KB test meal, and the 6-h apoB-48 concentrations were significantly higher ($P < 0.05$) after the B and S test meals than after the CF test meal. No differences were found among the incremental apoB-48 0- to 3-h AUCs after the four test meals (Fig. 4*A*). Conversely, incremental apoB-48 3- to 6-h AUCs after the KB test meal, and more markedly after the CF test meal, were lower than after the B and S test meals (Fig. 4*B*). Although no significant differences were observed for the incremental apoB-48 0- to 6-h AUCs after the four test meals, a strong positive correlation was found ($r^2 = 0.70$; $P = 0.0001$) between incremental 0- to 6-h plasma apoB-48 and insulin postprandial responses (Fig. 5). Conversely, as shown in Fig. 6, VLDL apoB-100 did not change noticeably during the four postprandial periods, and thus no relationship was found with insulin postprandial responses.

TG concentrations in chylomicron fractions transiently and significantly ($P < 0.05$) rose over baseline after the four test meals (3-h peak) and returned to baseline after 6 h. The amplitudes of the chylomicron TG responses were not markedly different after the four test meals (incremental 0- to 6-h TG AUC range: 1.26–1.90 mmol · h^{-1} · l^{-1}), nor were any differences found for 0- to 3-h or 3- to 6-h AUCs for chylomicron TGs. The VLDL TG concentrations were comparable after the four test meals (incremental 0- to 6-h TG AUC range: 0.30–0.59 mmol · h^{-1} · l^{-1}).

LPL and HL activities determined on postheparin plasma 6 h after food intake did not show marked differences after the four test meals (3.8–5.2 vs. 4.6–5.1 μmol · h^{-1} · ml^{-1} for LPL and HL activity, respectively).

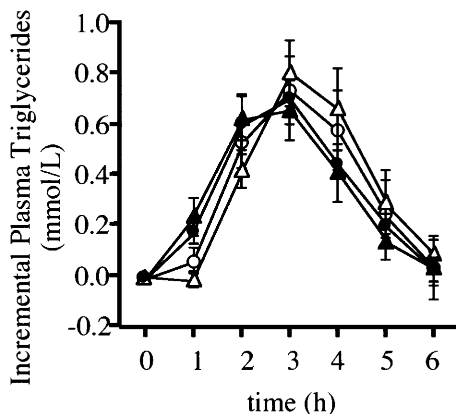

FIG. 2. Incremental serum TG responses for 6 h after ingestion of B (●), S (○), KB (▲), or CF (△) test meal. Data represent means ± SE; $n = 10$.

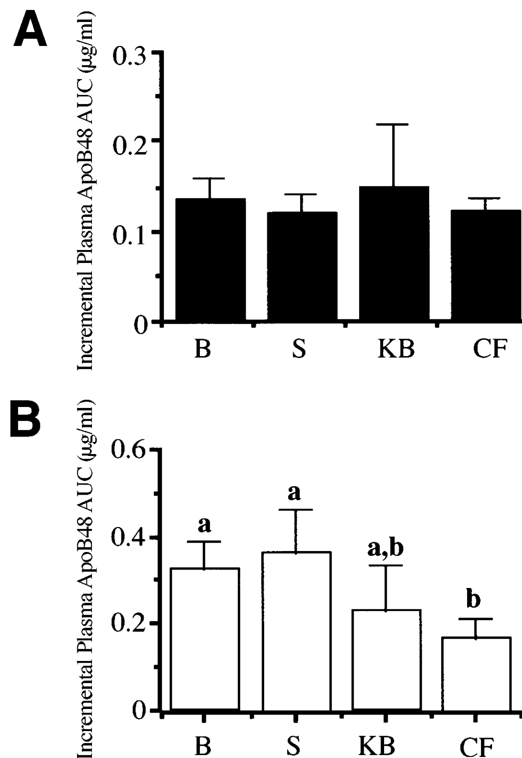

FIG. 4. Incremental 0- to 3-h (*A*) and 3- to 6-h (*B*) AUCs for plasma apoB-48 after ingestion of B, S, KB, or CF test meal. Means not sharing a common letter designation (a, b, c) differed from each other at $P < 0.05$. Data represent means ± SE; $n = 10$.

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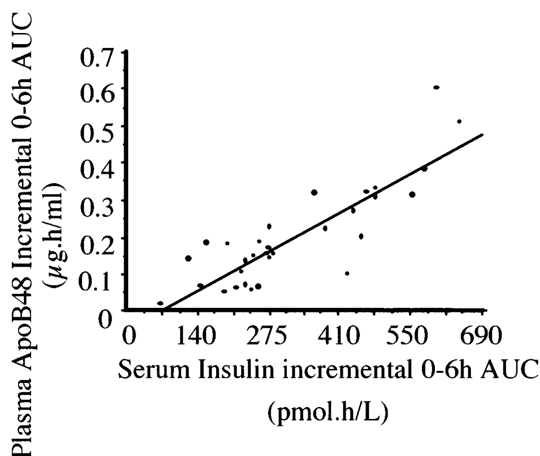

FIG. 5. Regression analysis for plasma apoB-48 for 0- to 6-h incremental AUCs and plasma insulin 0- to 6-h incremental AUCs. P = 0.0001; r^2 = 0.70.

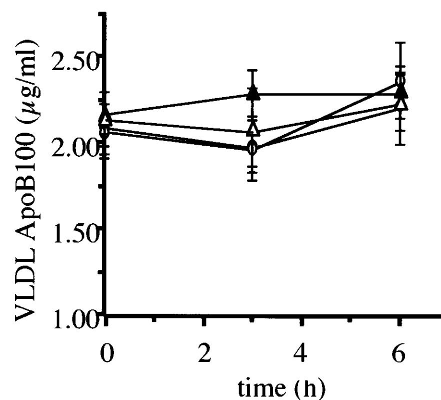

FIG 6. Plasma apoB-100 concentrations for 6 h after ingestion of B (●), S (○), KB (▲), or CF (△) test meal. Data represent means ± SE; n = 10.

Study 2: Postprandial lipemia responses to CF test meal during a euglycemic insulin clamp. The test procedures were well tolerated by the subjects. Glycemia was maintained at fasting level. As expected, plasma insulin increased to 460–620 pmol/l (550 ± 145) 20 min after intravenous injection of insulin until the end of the infusion (Fig. 7*A*). At the time of food intake (30 min after beginning insulin infusion), insulinemia had a mean value of 491 ± 61 pmol/l. The stop in insulin infusion at time 2 h 30 min resulted in a drop in insulinemia to values close to fasting values at 3–6 h.

The postprandial changes in serum TG concentrations elicited by intake of the CF test meal without or with insulin (EIC test meal) are shown in Fig. 7*B*. Serum TGs peaked 4 h after the EIC test meal, whereas they peaked 3 h after the CF test meal. The serum TG responses (incremental 3- to 6-h or 0- to 6-h AUCs) after the EIC test meal were significantly higher than after the CF test meal. Conversely, no differences were found among serum TG incremental 0- to 3-h AUCs (Table 1).

Postprandial changes in plasma apoB-48 concentrations are shown in Fig. 7*C*. ApoB-48 peaked 4 h after the EIC test meal but 3 h after the CF test meal. Furthermore, the apoB-48 level 2 h after the EIC test meal was significantly lower than the corresponding value after the CF test meal. The incremental 0- to 3-h AUCs after the EIC test meal were significantly lower than after the CF test meal (Table 1), whereas no differences were found for the incremental 3- to 6-h AUC values.

Postprandial VLDL apoB-100 concentration variations (μg/ml) are shown in Fig. 7*D*. Postprandial values were not significantly different from baseline during the postprandial period after the CF and EIC test meals. There were no differences in apoB-100 levels under both conditions.

Figure 8 shows the postprandial changes in TG concentrations in chylomicrons (Fig. 8*A*) and VLDL (Fig. 8*B*). As for plasma TGs and apoB-48, TGs in chylomicrons peaked 4 h after the EIC test meal and 3 h after the CF test meal. TGs in VLDL rose moderately 5 h after the EIC test meal and 4 h after the CF test meal. For both plasma and chylomicrons after the EIC test meal, TG rises tended to be lower (Table 1) during insulin infusion (–30 min to 2 h 30 min) and peaked after the stop in insulin infusion. As shown in Table 1, the TG incremental 3- to 6-h AUCs were significantly higher for chylomicron TGs after the EIC test meal than after the CF test meal, whereas VLDL fraction TGs showed a transient and moderate elevation during the same period.

DISCUSSION

In the vast majority of studies already conducted in the field of postprandial lipid metabolism, both insulin resistance and related hyperinsulinism took part in the abnormal metabolic syndrome exhibited by the subjects (5,12,14,15). Thus, although key data showing a positive association between insulin resistance/hyperinsulinism and exaggerated postprandial lipemia have been obtained, it has not yet been possible to discriminate between the respective roles of insulin resistance or hyperinsulinism on postprandial lipid metabolism. The present work was designed specifically to test the role of hyperinsulinism. Particular emphasis was given to the changes in apoB-48 TG-rich lipoproteins from intestinal origin (chylomicrons) and apoB-100–containing TRLs (VLDL) secreted by the liver, as both kinds of particles are involved in postprandial hyperlipemia (5,12,16) as well as atherogenesis (1,26–28).

In the nutritional approach, we observed graded physiological insulin responses in the increasing order predicted by the GIs of carbohydrates used and not used (29,30), whereas postprandial plasma TG responses were not significantly different after the four meals. This first finding indicates that, in healthy subjects, physiological ranges of postprandial hyperinsulinemia as generated by starchy foods do not induce any noticeable alterations in the overall postprandial TG response. No marked changes in hepatically derived apoB-100–containing VLDL were observed either. Variable effects of dietary carbohydrates have already been reported, with the addition of 50 and 100 g glucose (31) or dietary fiber sources (32) reducing postprandial triglyceridemia generated by a fat meal, and the addition of sucrose (33) or fructose (34) increasing postprandial triglyceridemia. In contrast, we observed two different patterns of postprandial responses in plasma apoB-48 (a specific component of intestinally derived TRLs) after meals. With the lowest GI meals (KB and CF), apoB-48 peaked 1 h earlier than with the two highest GI meals (B and S), meaning that postprandial intestinal TRL accumulated later after high-GI meals than after low-GI

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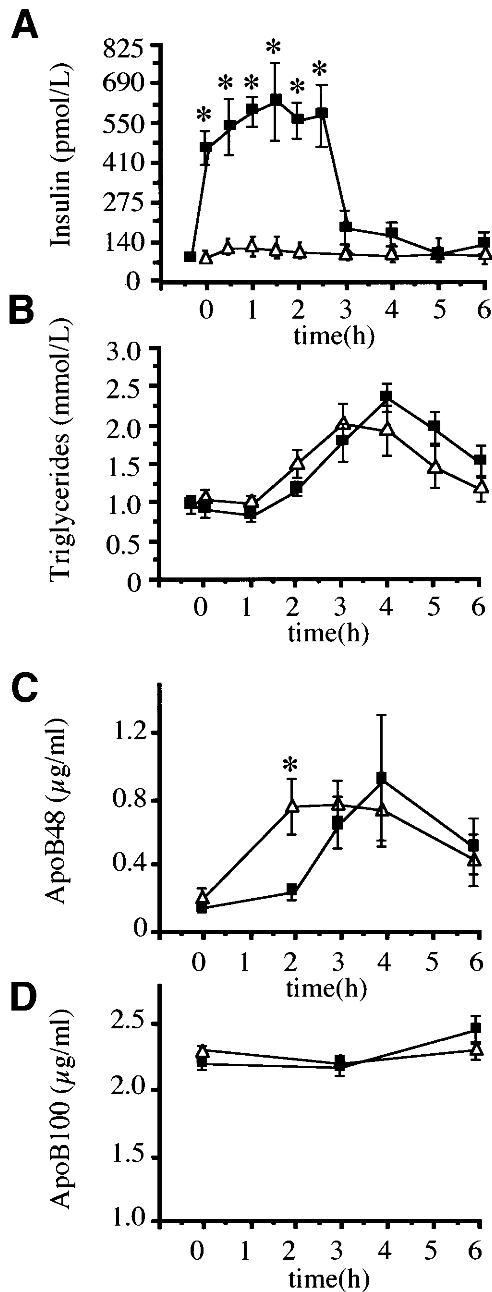

FIG. 7. Plasma insulin (*A*) , TGs (*B*), apoB-48 (*C*), and apoB-100 (*D*) concentrations for 6 h after the CF △) or EIC test meal (■). *P < 0.05 between meals. Data represent means ± SE; n = 10.

meals. Indeed, the apoB-48 3- to 6-h AUCs were two times higher after high-GI meals than after the CF meal. Some timing differences between carbohydrate-rich and -poor meals (31) or diets (35) have already been observed for plasma TGs. These different responses lead to the observation of a strong positive correlation between the 0- to 6-h plasma apoB-48 levels and the 0- to 6-h insulin response. This relationship has been suggested by other studies during which insulin resistance and hyperinsulinemia were found to be associated with elevated intestinal TRL or large TRL levels (5,9,12,15,36). Because an accumulation of intestinal-TRL remnants in the circulation is associated with the progression of coronary heart disease (37), low-GI foods and meals could be recommended to patients at cardiovascular risk.

In the second approach we aimed to directly test the hypothesis that hyperinsulinemia, independent from insulin resistance and hyperglycemia, is involved in the observed alteration in postprandial lipid metabolism. Thus the response to the carbohydrate-free, fat-containing meal (CF test meal) was compared to the one generated by the same meal superimposed with a 0- to 3-h hyperinsulinemic euglycemic clamp (EIC test meal). Hyperinsulinemia was maintained through the clamp for 3 h to mimic the insulin kinetics observed under high-GI meals. This experimental protocol, however, differed from high-GI meals by a higher insulin level and route of insulin delivery (systemic versus portal). Nevertheless, the need for portal insulin delivery to achieve normal lipoprotein clearance has been widely discussed in the literature as well as in reports of transplantation data on insulin infusions in animals and patients (38).

The clamped hyperinsulinemic state and CF meal induced a 1-h delay in the occurrence of the postprandial plasma TG peak as compared with the CF meal alone, which maintained baseline insulinemia. During the 0- to 3-h period under insulin infusion, plasma apoB-48 concentrations remained paradoxically much lower than after the CF meal alone. Conversely, plasma apoB-100 levels did not change noticeably. This observation suggests that postprandial hyperinsulinemia acutely and specifically slows down the accumulation of apoB-48–containing intestinally derived TRLs. This effect most likely is not attributable to an alteration in LPL activity, as VLDL TGs and apoB-100 levels were not affected during this period. A somewhat reduced apoB-48 rise has already been observed during the postprandial period in type 2 diabetic patients, who exhibit a known postprandial hyperinsulinism (39).

In contrast, stopping the superimposed hyperinsulinemic clamp after 3 h led to a fast return to baseline insulin levels and generated exacerbated 3- to 6-h TRL responses as compared with the control CF meal without any previous clamp. Indeed, significantly higher responses for plasma TGs, chy-

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TABLE 1
Metabolic variables (AUC) after EIC and CF test meals

	Change at 0- to 3-h AUCs		Change at 3- to 6-h AUCs	
	EIC	CF	EIC	CF
Plasma TG	0.61 ± 0.14	0.89 ± 0.19	3.18 ± 0.3*	1.85 ± 0.61
Plasma apoB-48	0.05 ± 0.01*	0.10 ± 0.02	0.23 ± 0.12	0.19 ± 0.08
Chylomicron TG	0.51 ± 0.17	0.694 ± 0.09	2.12 ± 0.28*	0.99 ± 0.15
VLDL TG	0.18 ± 0.09	0.10 ± 0.17	1.19 ± 0.19*	0.39 ± 0.3

Data are means ± SE; n = 5. *P < 0.05 between meals.

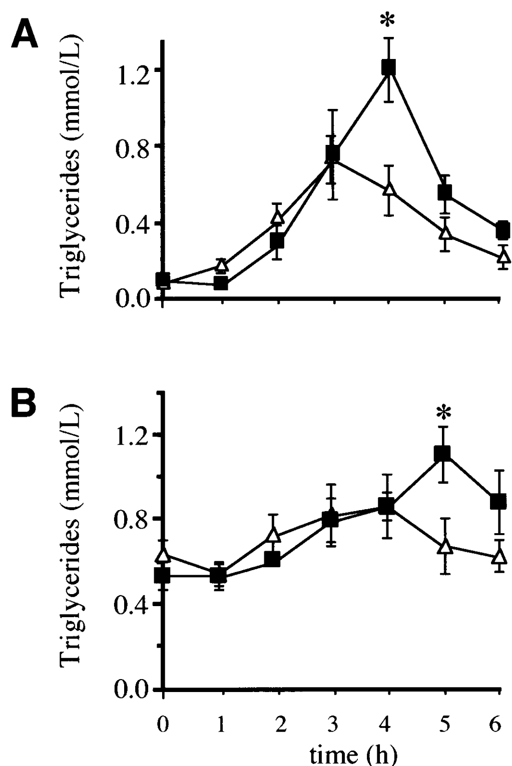

FIG. 8. TG concentrations in large TRL (Sf > 400) (*A*) and small TRL fractions (Sf 20–400) (*B*) in response to CF (△) or EIC test meal (■). * $P < 0.05$ between meals.

lomicron TGs, and, to a lesser extent, VLDL TGs were observed during the 3- to 6-h postprandial period. The fact that the increase in apoB-48 was only moderate could have resulted from the dramatic drop observed just before the end of the clamp. Such a late accumulation in plasma TGs postprandially has been observed during several studies in subjects with insulin resistance and hyperinsulinism (5,16,39–41). Another new finding is that transient hyperinsulinemia is responsible for this late accumulation in plasma of TG-rich lipoproteins of intestinal origin and, to a lesser extent, hepatic origin. This concept fits well with the relationship already observed between fasting insulinemia (and thus related postprandial hyperinsulinemia) and postprandial TG response (5,14,15). It is also noteworthy to emphasize that low-fat, highly digestible carbohydrate diets are known to induce hyperinsulinism as well as fasting and postprandial hyperlipidemia (41,42). It has been confirmed that this kind of diet induces a significant decrease in TRL clearance in normal subjects, with the presence of apoB-48–containing TRLs in the fasting state illustrating a delay in the clearance of these particles (43), in line with other data (5,9). Taken together, these data and the present ones suggest that exogenous TRLs are particularly susceptible to a hyperinsulinism-induced clearance delay.

Insulin resistance and hyperinsulinism are associated with fasting and postprandial hypertriglyceridemia, resulting on the one hand from an increase in hepatic VLDL secretion (44) and on the other hand from a decrease in LPL-mediated clearance of TG-rich lipoproteins. Nevertheless, as previously suggested in healthy (45) or type 2 diabetic patients (46), hyperinsulinism induces a decrease in hepatic VLDL production whereas insulin resistance induces an increase in VLDL production (44). In the nutritional part of our study, VLDL apoB-100 levels were not markedly affected by carbohydrates, a finding in line with the observation in healthy subjects that apoB-100 and VLDL TG synthesis are not affected by a low-fat, high-carbohydrate diet (43). The absence of a decrease in apoB-100 levels during the EIC test meal in the present study differs from the lowering of VLDL TGs observed in clamp studies performed on fasting patients (45,46). This difference is likely attributable to the existence of the fat assimilation process at the time of acute hyperinsulinism. On the whole, these data suggest that insulin resistance, more than hyperinsulinism, is involved in alterations of apoB-100 TG-rich lipoproteins postprandially.

Regarding postheparin lipase activities, we observed no noticeable differences after the four test meals in these subjects and no noticeable trait of insulin resistance. This indicated that the transient and moderate hyperinsulinemia elicited by these meals did not noticeably modify the overall level of LPL and HL activities. Also, blockade of insulin secretion by octreotide, a somatostatin analogue, was shown to be unable to suppress postheparin LPL activity in plasma (47). During clamps, despite the fact that postheparin LPL activity was not measured, the absence of a decrease in VLDL apoB-100 made drastic improvement in LPL activity unlikely. However, insulin is known to acutely upregulate adipose tissue LPL (48,49) and have the opposite effect on muscle (49,50). Accordingly, we cannot exclude any subtle tissue-specific alterations of LPL activity under high-insulin levels.

Observations obtained after test meals and hyperinsulinemic clamp also showed the existence of a late accumulation of apoB-48 TRLs as a consequence of transient hyperinsulinism, suggesting that insulin delays the secretion of apoB-48–containing TRLs by the small intestine during the postprandial period. Such a mechanism has already been proposed as a regulatory pathway in the liver (51), and an abnormality in intestinal secretion has been previously suggested in diabetic patients (40). Although very scarce, some experimental evidence for this possible mechanism has been obtained from cultured fetal small intestine (52) and using cultured differentiated human CaCo2 intestinal cells (A.H., D.L., unpublished data). This leads us to suggest that marked insulin elevation would depress chylomicron secretion from enterocytes into the lymph, despite efficient apical uptake of fatty acids and insulin-activated lipogenesis (53). The return to basal insulin levels observed in this study under both experimental conditions would then permit maximum chylomicron secretion, late occurrence, and accumulation in the circulation. It is not known whether insulin and/or glucose alter the process of lipid absorption by the intestine mucosa.

In conclusion, elevated postprandial insulin levels as observed in healthy subjects without insulin-resistance syndrome could alter some postprandial lipid parameters, especially intestinally derived TG-rich lipoproteins. This may indicate that hyperinsulinemia, at the time of fat assimilation, is involved more than insulin resistance in the alteration of postprandial intestinal TRL metabolism, whereas insulin resistance could be involved more than hyperinsulinism in the alteration of hepatic TRL metabolism. These two joined phenomena can induce fasting- and postprandial-exacerbated hypertriglyceridemia as observed in patients affected

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by the polymetabolic syndrome. High-GI food ingested as part of a mixed meal with fats can generate, via induced insulin secretion, an accumulation of intestinal apoB-48 TRLs in plasma at the last stage of the postprandial phase. Although further experimental evidence should be given, our results support the close link between meals and diet composition, insulin response, postprandial lipoprotein metabolism, and potential arteriosclerosis risk.

ACKNOWLEDGMENTS

This study received a research grant from the DANONE Group, the French Research Ministry (Aliment Demain), and the Provence-Alpes-Côte-d'Azur Council.

We greatly thank M.F. Allibert, L. Caniparoli, and Y. Le Carrour for their medical assistance.

REFERENCES

1. Zilversmit DB: Atherogenesis: a postprandial phenomenon. *Circulation* 60: 473–485, 1979
2. Karpe F, Hamsten A: Postpranial lipoprotein metabolism and atherosclerosis. *Curr Opin Lipidol* 6:123–129, 1995
3. Weintraub MS, Eisenberg S, Breslow JL: Different patterns of postprandial lipoprotein metabolism in normal, type IIa, type III, and type IV hyperlipoproteinemic individuals: effect of treatment with cholestyramine and gemfibrozil. *J Clin Invest* 79:1110–1119, 1987
4. Castro Cabezas M, de Bruin TWA, de Valk HW, Shoulders CC, Jansen H, Erkelens DW: Impaired fatty acid metabolism in familial combined hyperlipidemia: a mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. *J Clin Invest* 92:160–168, 1993
5. Mekki N, Chritophilis MA, Charbonnier M, Atlan-Gepner C, Defoort C, Juhel C, Borel P, Portugal H, Pauli AM, Vialettes B, Lairon D: Influence of obesity and body fat distribution on postprandial lipemia and triglyceride-rich lipoproteins in adult women. *J Clin Endocrinol Metab* 84:184–191, 1999
6. Reaven GM: Role of insulin resistance in human disease. *Diabetes* 37:1595–1607, 1988
7. Lewis GF, O'Meara NM, Soltys PA, Blackman JD, Iverieus PH, Pugh WL, Getz GS, Polonsky KS: Fasting hypertriglyceridemia in non-insulin-dependent diabetes mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. *J Clin Endocrinol Metab* 72:934–944, 1991
8. Taskinen MR: Insulin resistance and lipoprotein metabolism. *Curr Opin Lipidol* 6:153–160, 1995
9. Curtin A, Deegan P, Owens D, Collins P, Johnson A, Tomkin GH: Elevated triglyceride-rich-lipoproteins in diabetes: a study of apolipoprotein B-48. *Acta Diabetol* 33:205–210, 1996
10. De Man FH, Cabezas MC, Van Barlingen HH, Erkelens DW, de Bruin TW: Triglyceride-rich lipoproteins in non-insulin-dependent diabetes mellitus: post-prandial metabolism and relation to premature atherosclerosis. *Eur J Clin Invest* 26:89–108, 1996
11. Potts JL, Coppack SW, Fisher PM, Humphreys SM, Gibbons GF, Frayn KN: Impaired postprandial clearance of triacylglycerol-rich lipoproteins in adipose tissue in obese subjects. *Am J Physiol* 268:E588–E594, 1995
12. Couillard C, Bergeron N, Prud'homme D, Bergeron J, Tremblay A, Bouchard C, Mauriège P, Desprès JP: Postprandial triglyceride response in visceral obesity in men. *Diabetes* 47:953–960, 1998
13. Frayn KN: Insulin resistance and lipid metabolism. *Curr Opin Lipidol* 4:197–204, 1993
14. Jeppesen J, Hollenbeck C, Zhou M-Y, Coulston A, Jones C, Chen Y-DI, Reaven GM: Relation between insulin resistance, hyperinsulinism, postheparin plasma lipoprotein lipase activity and postprandial lipemia. *Arterioscler Thromb Vasc Biol* 15:320–324, 1995
15. Boquist S, Hamsten A, Karpe F, Ruotolo G: Insulin and non-esterified fatty acid relations to alimentary lipaemia and plasma concentrations of postprandial triglyceride-rich lipoproteins in healthy middle-aged men. *Diabetologia* 43:185–193, 2000
16. Cohn JS, Johnson EJ, Millar JS, Cohn SD, Milne RW, Marcel YL, Russell RM, Schaefer EJ: Contribution of apoB-48 and apoB-100 triglyceride-rich lipoprotein (TRL) to postprandial increases in plasma concentration of TRL triglycerides and retinyl esters. *J Lipid Res* 34:2033–2039, 1993
14. Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV: Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 34:362–366, 1981
18. Foster-Powell K, Brand Miller J: International tables of glycemic index. *Am J Clin Nutr* 62:871S–893S, 1995
19. Brousseau T, Clavey V, Bard JM, Fruchart JC: Sequential ultracentrifugation micromethod for separation of serum lipoproteins and assays of lipids, apolipoproteins, and lipoprotein particles. *Clin Chem* 39:960–964, 1993
20. Peel A, Zampelas A, Williams CM, Gould BJ: A novel antiserum specific to apolipoprotein B-48: application in the investigation of postprandial lipidaemia in humans. *Clin Sci (Colch)* 85:521–524, 1993
21. Lovegrove JA, Isherwood SG, Jackson KG, Williams CM, Gould BJ: Quantitation of apolipoprotein B-48 in triacylglycerol-rich lipoproteins specific enzyme-linked immunosorbent assay. *Biochim Biophys Acta* 1301:221–229, 1996
22. Lorec AM, Juhel C, Pafumi Y, Portugal H, Pauli AM, Lairon D, Defoort C: Determination of apolipoprotein B-48 in plasma by a competitive ELISA. *Clin Chem* 46:1638–1642, 2000
23. Kosykh VA, Novikov DK, Trakht IN, Podrez EA, Victorov AV, Repin VS, Smirnov VN: Effect of chylomicron remnants on cholesterol metabolism in cultured rabbit hepatocytes: very low density lipoprotein and bile acid production. *Lipids* 26:799–805, 1991
24. Krauss RM, Levy RI, Frederickson DS: Selective measurements of two lipase activities in postheparin plasma from normal subjects and patients with hyperlipoproteinemia. *J Clin Invest* 54:1107–1124, 1974
25. de Fronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
26. Patsch JR, Miesenböck G, Hopferwieser T, Muhlberger V, Knapp E, Dunn JK, Gotto AM Jr, Patch W: Relation of triglyceride metabolism and coronary artery disease: studies in the postprandial state. *Arterioscler Thromb Vasc Biol* 12:1336–1345, 1992
27. Havel R: Triglyceride-rich lipoproteins and atherosclerosis: new perspectives. *Am J Clin Nutr* 59:795–799, 1994
28. Juhel C, Dubois C, Senft M, Levy E, Lafont H, Lairon D: Postprandial lipaemia is exacerbated in fat-cholesterol-fed rabbits: relationship to atheroma deposition. *Br J Nutr* 78:301–311, 1997
29. Brand Miller J, Pang E, Bramall L: Rice: a high or low glycemic index food? *Am J Clin Nutr* 56:1034–1036, 1992
30. Holt S, Brand J, Soveny C, Hansky J: Relationship of satiety to postprandial glycaemic, insulin and cholescystokinin responses. *Appetite* 18:129–141, 1992
31. Cohen JC, Berger GM: Effect of glucose ingestion on postprandial lipemia and triglyceride clearance in humans. *J Lipid Res* 31:597–602, 1990
32. Cara L, Dubois C, Borel P, Armand M, Senft M, Portugal H, Pauli AM, Bernard PM, Lairon D: Effects of oat bran, rice, wheat fiber, and wheat germ on postprandial lipemia in healthy adults. *Am J Clin Nutr* 55:81–88, 1992
33. Grant KI, Marais MP, Dhansay MA: Sucrose in a lipid-rich meal amplifies the postprandial excursion of serum and lipoprotein triglyceride and cholesterol concentrations by decreasing triglyceride clearance. *Am J Clin Nutr* 59:853–860, 1994
34. Jeppesen J, Chen Y-DI, Zhou M-Y, Schaaf P, Coulston A, Reaven GM: Postprandial triglyceride and retinyl ester responses to oral fat: effects of fructose. *Am J Clin Nutr* 61:787–791, 1994
35. Chen Y-DI, Swami S, Skowronski R, Coulston A, Reaven GM: Effect of variations in dietary fat and carbohydrate intake on postprandial lipemia in patients with noninsulin dependent diabetes mellitus. *J Clin Endocrinol Metab* 76:347–351, 1993
36. Chen Y-DI, Swami S, Skowronski R, Coulston A, Reaven GM: Differences in postprandial lipemia between patients with normal glucose tolerance and non-insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 76:172–177, 1993
37. Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A: Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* 106:83–97, 1994
38. Canavan JP, Flecknell PA, New JP, Alberti KB, Home PD: The effect of portal and peripheral insulin delivery on carbohydrate and lipid metabolism in a miniature pig model of human IDDM. *Diabetologia* 40:1125–1134, 1997
39. Curtin A, Deegan P, Owens D, Collins P, Johnson A, Tomkin GH: Alterations in apolipoprotein B-48 in the postprandial state in NIDDM. *Diabetologia* 37:1259–1264, 1994
40. Curtin A, Deegan P, Owens D, Collins P, Johnson A, Tomkin GH: Elevated triglyceride-rich lipoproteins in diabetes: a study of apolipoprotein B-48. *Acta Diabetol* 33:205–210, 1996
41. Lithell H, Jacobs I, Vessby B, Hellsing K, Karlsson J: Decrease of lipoprotein lipase activity in skeletal muscle in man during a short-term carbohydrate-rich dietary regime: with special reference to HDL-cholesterol, apolipoprotein and insulin concentrations. *Metabolism* 31:994–998, 1982
42. Chen YD, Coulston AM, Zhou MY, Hollenbeck CB, Reaven GM: Why do low-fat high-carbohydrate diets accentuate postprandial lipemia in patients with NIDDM? *Diabetes Care* 18:10–16, 1995
43. Parks ES, Krauss RM, Christiansen MP, Neese RA, Hellerstein MK: Effect of low-fat, high-carbohydrate diet on VLDL-triglyceride assembly, production, and clearance. *J Clin Invest* 104:1087–1096, 1999

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44. Cummings MH, Watts GF, Umpleby AM, Hennessy TR, Naoumova R, Slavin BM, Thompson GR, Sönksen PH: Increased hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in NIDDM. *Diabetologia* 38:959–967, 1995
45. Malmstrom R, Packard CJ, Watson TD, Rannikko S, Caslake M, Bedford D, Stewart P, Yki-Jarvinen H, Shepherd J, Taskinen MR: Metabolic basis of hypotriglyceridemic effects of insulin in normal men. *Arterioscler Thromb Vasc Biol* 17:1454–1464, 1997
46. Cummings MH, Watts GF, Umpleby AM, Hennessy TR, Kelly JM, Jackson NC, Sönksen PH: Acute hyperinsulinemia decreases the hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in NIDDM. *Diabetes* 44: 1059–1065, 1995
47. Ranganath LP, Beety JM, Morgan LM: Inhibition of insulin, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) secretion by octreotide has no effect on post-heparin plasma lipoprotein lipase activity. *Horm Metab Res* 31:262–266, 1999
48. Kraemer FB, Takeda D, Natu V, Sztalryd C: Insulin regulates lipoprotein lipase activity in rat adipose cells via wortmannin- and rapamycin-sesitive pathways. *Metabolism* 47:555–559, 1998
49. Picard F, Naimi N, Richard D, Deshaies Y: Response of adipose tissue lipoprotein lipase to the cephalic phase of insulin secretion. *Diabetes* 48:452–459, 1999
50. Laville M, Aubœuf D, Khalfallah Y, Vega N, Riou JP, Vidal H: Acute regulation by insulin of phosphatidylinositol-3-kinase, Rad, Glut 4, and lipoprotein lipase mRNA levels in human muscle. *J Clin Invest* 98:43–49, 1996
51. Sparks JD, Sparks CE: Insulin regulation of triacylglycerol-rich lipoprotein synthesis and secretion (Review). *Biochim Biophys Acta* 1215:9–32, 1994
52. Levy E, Sinnett D, Thibault L, Nguyen TD, Delvin E, Ménard D: Insulin modulation of newly synthesized apolipoproteins B-100 and B-48 in human fetal intestine: gene expression and mRNA editing are not involved. *FEBS Lett* 393:253–258, 1996
53. Hulstijn M, Lopez-Soriano J, Lopez-Soriano FJ, Argiles JM: Lipogenesis in rat tissues following carbohydrate refeeding: spleen lipogenesis is modulated by insulin. *Mol Cell Biochem* 175:149–152, 1997

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