Effect of insulin resistance on postprandial elevations of remnant lipoprotein concentrations in postmenopausal women[1–3]

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ABSTRACT

Background: Questions remain as to why postmenopausal women are at a higher risk of coronary artery disease (CAD) than are premenopausal women. Studies have shown that plasma concentrations of remnant lipoproteins (RLPs) are elevated in patients with CAD and that increases in plasma RLP concentrations may be related to variations in insulin-mediated glucose disposal.

Objective: We sought to evaluate the possibility that postprandial accumulation of plasma RLPs will be accentuated in insulin-resistant, postmenopausal women.

Design: Postmenopausal women were divided into insulin-sensitive (n = 15) and insulin-resistant (n = 15) groups according to their steady state plasma glucose concentrations in response to a 180-min infusion of octreotide, insulin, and glucose. Plasma insulin, triacylglycerol, and RLP-cholesterol concentrations were measured either hourly (insulin) or every 2 h (triacylglycerol and RLP cholesterol) for 8 h, before and after breakfast (0800) and lunch (1200).

Results: By selection, insulin-resistant women had higher steady state plasma glucose concentrations than did insulin-sensitive women (10.8 ± 0.5 compared with 4.1 ± 5 mmol/L, respectively; $P < 0.001$), associated with higher fasting triacylglycerol (1.58 ± 0.04 compared with 1.00 ± 0.03 mmol/L; $P = 0.01$) and lower HDL-cholesterol (1.06 ± 0.08 compared with 1.34 ± 0.05; $P = 0.01$) concentrations. In addition, measurements of daylong concentrations of insulin, triacylglycerol, and RLP cholesterol were also significantly greater in insulin-resistant than in insulin-sensitive women ($P < 0.001$).

Conclusions: Postprandial accumulation of RLPs is accentuated in insulin-resistant, postmenopausal women. This may contribute to the increased risk of CAD in these individuals. *Am J Clin Nutr* 2001;74:592–5.

KEY WORDS Postmenopausal women, remnant lipoproteins, postprandial lipemia, coronary artery disease, CAD, insulin resistance, insulin sensitivity, insulin

INTRODUCTION

It has been >20 y since Zilversmit (1) first introduced the notion that atherogenesis is a postprandial phenomenon and that the lipoprotein particles formed during the catabolism of triacylglycerol-rich lipoproteins are highly effective cholesterol-ester donors. Although a considerable period of time elapsed before much attention was given to the role of postprandial lipemia as a coronary artery disease (CAD) risk factor, articles published within the past decade have provided considerable support for the Zilversmit hypothesis (2–6). In a somewhat parallel fashion, our research group over the past few years has been exploring possible mechanisms to account for differences in the magnitude of postprandial lipemia. A relation between fasting plasma triacylglycerol concentrations and postprandial lipemia has been known for some time (7, 8), but we have more recent evidence suggesting that resistance to insulin-mediated glucose disposal or compensatory hyperinsulinemia enhances the postprandial accumulation of triacylglycerol-rich lipoproteins of both endogenous and exogenous origin in patients with type 2 diabetes (9), in postmenopausal women (10), and in healthy volunteers (11).

The ability to study the relation between postprandial lipemia and various clinical syndromes and physiologic states has been significantly aided by the introduction of an assay method (12, 13) for quantifying apolipoprotein (apo) E–rich lipoproteins (d < 1.006 g/mL) by using an immunoaffinity gel mixture of antibodies to apo B-100 and apo A-1 coupled to Sepharose 4B (JIMRO, Tokyo). The characterization of the unbound lipoproteins isolated in this manner indicated that they represent chylomicron and VLDL remnants, collectively called remnant lipoprotein particles (RLPs). By using this technique, evidence was published showing that RLP concentrations were significantly higher in subjects with type 2 diabetes and impaired glucose tolerance (14) and in insulin resistant individuals with normal glucose tolerance (15) after they had fasted overnight. In addition, we also showed that postprandial concentrations of RLPs increased throughout the day in response to carbohydrate-enriched diets (16). The present study was conducted to compare daylong changes in these atherogenic lipoproteins in postmenopausal women, who were divided into

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TABLE 1
Baseline characteristics of insulin-sensitive and insulin-resistant postmenopausal women[1]

Characteristics	Insulin-sensitive	Insulin-resistant	*P*
SSPG (mmol/L)	4.1 ± 0.3	10.8 ± 0.5	<0.001
SSPI (pmol/L)	3540 ± 360	3660 ± 420	NS
Age (y)	58 ± 2	58 ± 2	NS
BMI (kg/m^2)	24.8 ± 0.5	26.6 ± 0.6	<0.05
Insulin (pmol/L)	54 ± 6	68 ± 7	NS
Cholesterol (mmol/L)	4.37 ± 0.21	4.55 ± 0.21	NS
LDL cholesterol (mmol/L)	2.71 ± 0.18	2.84 ± 0.18	NS
HDL cholesterol (mmol/L)	1.34 ± 0.05	1.06 ± 0.08	<0.01
Triacylglycerol (mmol/L)	1.00 ± 0.03	1.58 ± 0.04	<0.01

[1] $\bar{x}$ ± SE. SSPG, steady state plasma glucose; SSPI, steady state plasma insulin.

insulin-resistant and insulin-sensitive subgroups, the former group being at highest risk of CAD.

SUBJECTS AND METHODS

The present study was approved by the Stanford Institutional Review Board and each volunteer gave written, informed consent before entering the study. Nondiabetic (17), postmenopausal women with a body mass index (in kg/m^2) between 19 and 33 who were not taking any medication known to affect lipid metabolism were screened for enrollment in the study after they responded to an advertisement in a local newspaper. To qualify for further evaluation, volunteers were required to have completed a normal menopause ≥12 mo previously. The number of participants receiving hormonal replacement therapy was comparable in the insulin-sensitive (n = 9/15) and insulin-resistant (n = 11/15) groups. In addition, results of a physical examination, hemogram, urinalysis, and routine biochemical tests had to be within the normal range. Subjects taking hormone replacement therapy continued to do so throughout the study.

Volunteers who satisfied these initial criteria were admitted to the General Clinical Research Center at Stanford Medical Center, and insulin-mediated glucose disposal was measured by the insulin-suppression test (18, 19). After subjects had fasted overnight, intravenous catheters were placed in each arm. Blood was sampled from one arm for measurement of plasma glucose and insulin concentrations and from the contralateral arm for administration of test substances. Octreotide (Novartis, East Hannover, NJ) was administered at a rate of 0.27 $\mu g \cdot m^{-2} \cdot min^{-1}$ to suppress endogenous insulin secretion. Simultaneously, insulin and glucose were infused at 32 $mU \cdot m^{-2} \cdot min^{-1}$ and 217 $mg \cdot m^{-2} \cdot min^{-1}$, respectively. Blood was sampled for measurement of plasma glucose and insulin concentrations every 30 min for 150 min and then every 10 min until 180 min had elapsed. Insulin concentrations typically plateaued by 60 min, whereas glucose concentrations plateaued after 120 min. The 4 values obtained from 150 and 180 min were averaged and considered to represent the steady state plasma glucose (SSPG) and steady state plasma insulin (SSPI) concentrations achieved during the infusion. Because SSPI concentrations are similar in all individuals (qualitatively and quantitatively) and the glucose infusion rate was identical, the magnitude of the resultant SSPG concentration provides an accurate estimate of how effective insulin is in disposal of the infused glucose load, ie, the higher the SSPG, the more insulin resistant an individual. On the basis of the results of the insulin-suppression test, 15 insulin-sensitive women (SSPG concentrations <5.6 mmol/L) and 15 insulin-resistant women (SSPG >8.9 mmol/L) were enrolled in the present study. Volunteers who did not meet the requirements for either of these categories were not included.

After selection for inclusion in the study, all 30 subjects were admitted to the General Clinical Research Center of Stanford Medical Center. Blood was drawn at 0800 after subjects had fasted for 12 h overnight for measurement of plasma glucose, insulin, cholesterol, LDL-cholesterol, HDL-cholesterol, total cholesterol, and triacylglycerol concentrations. In addition, blood was drawn hourly for measurement of plasma glucose and insulin concentrations and every 2 h for measurement of plasma triacylglycerol and RLP-cholesterol concentrations from 0800 until 1600, after the subjects ingested a standard test breakfast (0800) and lunch (1200). The test meals contained (as percentage of total energy) 20% protein, 50% carbohydrate, and 30% fat. The energy intake of the test meals for each individual was calculated by using the Harris-Benedict equation (20), and the subjects received 20% of daily energy at breakfast and 40% at lunch.

Plasma glucose, insulin, total cholesterol, and triacylglycerol concentrations were measured as described previously (9–11). HDL was isolated by precipitation (21) and LDL cholesterol was calculated by the Friedewald equation (22). An immunosuppression assay was used to measure cholesterol in RLP cholesterol, as described previously (12–16). This method uses monoclonal antibodies to human apo B-100 and apo A-1 to remove most of the apo B– and A-1–containing particles (LDL, nascent VLDL, chylomicrons, and HDL), leaving behind chylomicrons and very low density RLPs that are enriched with apo E. Briefly, plasma (5 μL) was added to an immunoaffinity gel suspension containing the 2 monoclonol antibodies, the reaction mixture incubated at room temperature for 2 h on an RLP mixer, and aliquots of the supernatant fluid drawn for measurement of RLP-cholesterol concentrations.

Data are expressed as means ± SEs. The significance of the difference between baseline characteristics was evaluated by Student's nonpaired t test and the changes from 0800 to 1600 were determined by two-way analysis of variance, with the insulin group and time as the 2 variables. There was no significant group-by-time interaction and the calculated P values represent the daylong difference between the 2 groups (main effect of group).

RESULTS

Baseline characteristics of the insulin-resistant and insulin-sensitive groups are shown in **Table 1**. By selection, SSPG concentrations were almost 3-fold higher in the insulin-resistant women. Although the ages of the 2 groups were not significantly different, body mass index was higher in the insulin resistant women. In addition, triacylglycerol concentrations were higher and HDL-cholesterol concentrations lower in the insulin-resistant group. However, total and LDL-cholesterol concentrations were not significantly different between the 2 groups.

Daylong concentrations of plasma glucose and insulin are shown in **Figure 1**. Hourly plasma glucose concentrations were somewhat higher throughout the day in the insulin-resistant group and this difference was significant by two-way analysis of variance. The overall difference between the daylong insulin responses of the 2 groups was quantitatively much greater than was the difference in the glucose response and was also significant.

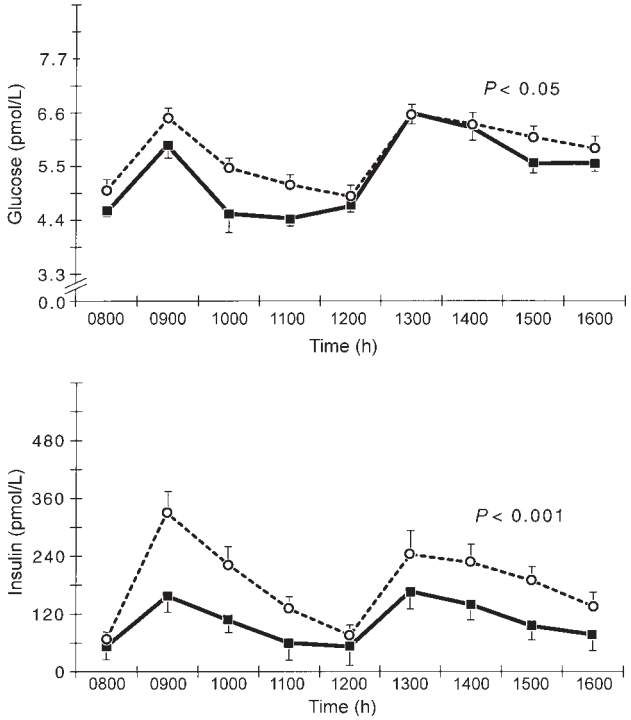

FIGURE 1. Mean (± SE) plasma glucose and insulin concentrations of insulin-resistant (○) and insulin-sensitive (■) women measured every hour from 0800 to 1600. Breakfast was served at 0800 and lunch at 1200.

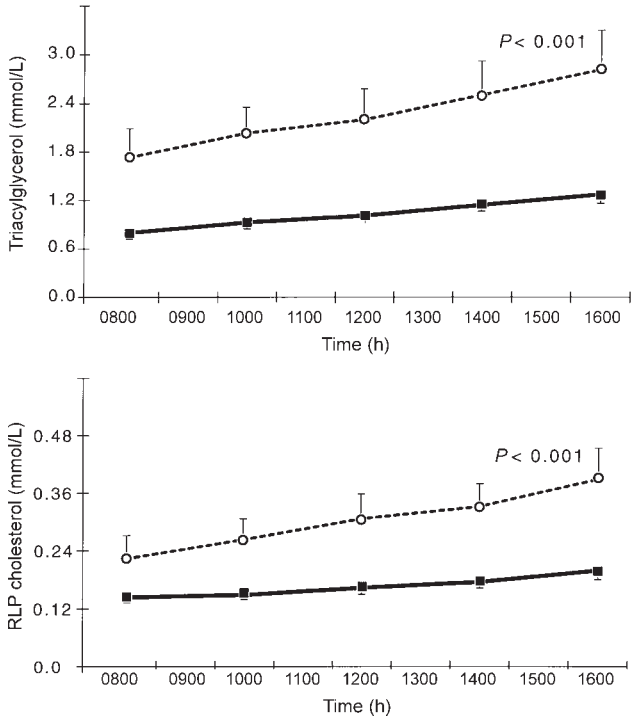

FIGURE 2. Mean (±SE) plasma triacylglycerol and remnant lipoprotein (RLP)-cholesterol concentrations of insulin-resistant (○) and insulin-sensitive (■) women measured every 2 h from 0800 to 1600. Breakfast was served at 0800 and lunch at 1200.

Plasma triacylglycerol and RLP-cholesterol concentration responses to breakfast and lunch in the 2 groups are shown in **Figure 2**. Fasting values of both variables were higher in the insulin-resistant than in insulin-sensitive women before breakfast and remained so throughout the 8-h period of observation.

DISCUSSION

In a series of prospective studies in nondiabetic individuals, insulin resistance or hyperinsulinemia has been identified as increasing the risk of CAD (23–26). The role played by these abnormalities of insulin metabolism in the genesis of CAD is confounded by the fact that insulin-resistant or hyperinsulinemic individuals are often hypertriglyceridemic, with low HDL-cholesterol concentrations, smaller and denser LDL particles, and increased plasma concentrations of plasminogen activator inhibitor 1 (27–31). Because all of these associated changes have been identified as CAD risk factors (32–35), it is difficult to decide whether the increased CAD risk is due to insulin resistance per se; the compensatory hyperinsulinemia that characterizes insulin-resistant, nondiabetic subjects; or any of the cluster of abnormalities associated with insulin resistance or hyperinsulinemia. The results of the present study further complicate this issue by showing that daylong concentrations of RLPs are significantly greater in insulin-resistant than in insulin-sensitive individuals.

Given the evidence in support of a causal relation between postprandial lipemia and CAD (1–6), the present results showing the dramatic difference in daylong concentrations of RLPs between insulin-resistant and insulin-sensitive individuals is of substantial clinical relevance. For example, using a different technique, we previously showed that the more insulin resistant an individual and the higher the plasma insulin concentration, the greater the postprandial accumulation of triacylglycerol-rich lipoproteins (11). Furthermore, the low-fat, high-carbohydrate diets that are currently recommended to lower the risk of CAD (36) increase daylong plasma concentrations of insulin, triacylglycerol, and RLP concentrations more than do diets in which saturated fat is replaced with unsaturated fat (16). In light of the evidence that insulin resistance or hyperinsulinemia increases the risk of CAD (23–26) and the strong association between insulin resistance and RLP concentrations shown in this study, it seems reasonable to suggest that therapeutic interventions aimed at decreasing CAD in postmenopausal women take into account the possible effects of these recommendations on insulin action, daylong plasma insulin concentrations, and degree of postprandial lipemia.

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