The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome

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We examined the hypothesis that insulin resistance in skeletal muscle promotes the development of atherogenic dyslipidemia, associated with the metabolic syndrome, by altering the distribution pattern of postprandial energy storage. Following ingestion of two high carbohydrate mixed meals, net muscle glycogen synthesis was reduced by ≈60% in young, lean, insulin-resistant subjects compared with a similar cohort of age–weight–body mass index–activity-matched, insulin-sensitive, control subjects. In contrast, hepatic *de novo* lipogenesis and hepatic triglyceride synthesis were both increased by >2-fold in the insulin-resistant subjects. These changes were associated with a 60% increase in plasma triglyceride concentrations and an ≈20% reduction in plasma high-density lipoprotein concentrations but no differences in plasma concentrations of TNF-α, IL-6, adiponectin, resistin, retinol binding protein-4, or intraabdominal fat volume. These data demonstrate that insulin resistance in skeletal muscle, due to decreased muscle glycogen synthesis, can promote atherogenic dyslipidemia by changing the pattern of ingested carbohydrate away from skeletal muscle glycogen synthesis into hepatic *de novo* lipogenesis, resulting in an increase in plasma triglyceride concentrations and a reduction in plasma high-density lipoprotein concentrations. Furthermore, insulin resistance in these subjects was independent of changes in the plasma concentrations of TNF-α, IL-6, high-molecular-weight adiponectin, resistin, retinol binding protein-4, or intraabdominal obesity, suggesting that these factors do not play a primary role in causing insulin resistance in the early stages of the metabolic syndrome.

type 2 diabetes | nonalcoholic fatty liver disease | adipocytokines | abdominal obesity | atherogenic dyslipidemia

The metabolic syndrome is characterized by a clustering of risk factors for cardiovascular disease that include insulin resistance, abdominal obesity, atherogenic dyslipidemia, hypertension, hyperuricemia, a prothrombotic state, and a proinflammatory state (1, 2). The metabolic syndrome is estimated to afflict >50 million Americans, and approximately half of all Americans are predisposed to it (2). Individuals with the metabolic syndrome are at increased risk for the development of coronary heart disease and other diseases related to plaque buildup in artery walls, such as stroke and peripheral vascular disease, as well as type 2 diabetes mellitus (T2DM).

Abdominal obesity and insulin resistance have each been hypothesized to be the primary factors underlying the metabolic syndrome; however, the biologic mechanisms linking these and other metabolic risk factors associated with the metabolic syndrome are not fully understood and appear to be complex.

In this study we examined the hypothesis that insulin resistance in skeletal muscle may promote the development of atherogenic dyslipidemia by diverting ingested carbohydrate away from muscle glycogen storage and into hepatic *de novo* lipogenesis, resulting in hypertriglyceridemia. To examine this hypothesis we assessed liver and muscle triglyceride synthesis by ^{1}H magnetic resonance spectroscopy (MRS) and liver and muscle glycogen synthesis by ^{13}C MRS in young, lean, healthy, insulin-resistant subjects and compared them to a group of age–weight–body mass index–activity-matched, insulin-sensitive subjects after ingestion of two high-carbohydrate mixed meals. In addition, hepatic *de novo* lipogenesis was assessed at the same time by monitoring the incorporation of deuterium from deuterium-labeled water into plasma triglycerides (3, 4). Because intraabdominal obesity has been postulated to be at the core of the metabolic derangements and directly responsible for the atherogenic dyslipidemia associated with the metabolic syndrome, we also measured intraabdominal fat content in these two groups of subjects by MRI (5–8).

The advantage of examining this question in healthy, young, lean, insulin-resistant individuals is that they have none of the other confounding factors that are typically associated with obesity and T2DM, which have been postulated to play a major role in causing the metabolic syndrome. Furthermore previous studies have demonstrated that insulin resistance in these subjects can mostly be attributed to defects in insulin-stimulated muscle glycogen synthesis caused by defects in insulin-stimulated glucose transport/phosphorylation activity (9–11). Therefore, the role of skeletal muscle insulin resistance *per se* in the pathogenesis of atherogenic dyslipidemia and the metabolic syndrome can be examined at its earliest stages.

Results

We screened ≈400 young, healthy, lean, sedentary subjects with an oral glucose tolerance test and assessed insulin sensitivity in them by using the insulin sensitivity index (ISI) (12). From this screening we identified 12 insulin-resistant [lowest ISI quartile (13)] subjects and 12 insulin-sensitive [highest ISI quartile (13)] subjects who were willing to undergo additional inpatient MRS/MRI/stable isotope studies to assess liver and muscle glycogen/triglyceride synthesis and hepatic *de novo* lipogenesis.

Abbreviations: HMW, high molecular weight; HDL, high-density lipoprotein; ISI, insulin sensitivity index; LDL, low-density lipoprotein; MRS, magnetic resonance spectroscopy; NAFLD, nonalcoholic fatty liver disease; RBP-4, retinol binding protein-4; T2DM, type 2 diabetes mellitus; VLDL, very-low-density lipoprotein.

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Table 1. Subject characteristics

Subject	ISI	Age, yr	BW, kg	Height, m	BMI, kg/m^2	FM, %	LBM, kg	AFV, ml	SBP, mmHg	DBP, mmHg	Activity, miles/day
Insulin-sensitive	7.87 ± 0.34	28 ± 2	67.1 ± 3.6	1.72 ± 0.03	22.6 ± 0.6	21.8 ± 2.3	52.8 ± 3.6	340 ± 91	113 ± 2	66 ± 2	4.83 ± 0.45
Insulin-resistant	2.80 ± 0.20	23 ± 1	69.6 ± 2.9	1.71 ± 0.03	23.9 ± 0.6	26.6 ± 2.3	51.5 ± 3.3	390 ± 92	110 ± 4	66 ± 2	3.89 ± 0.54
P value	<0.0001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

BW, body weight; BMI, body mass index; FM, fat mass; LBM, lean body mass; AVF, abdominal fat volume; SBP, systolic blood pressure (1 mmHg = 133 Pa); DBP, diastolic blood pressure; NS, not significant.

Despite having large differences in the ISI, the insulin-resistant and insulin-sensitive volunteers were similar in age, body mass index, lean body mass, fat mass, and monitored physical activity (Table 1). There also were no differences in systolic or diastolic blood pressure between the groups.

Fasting plasma glucose, triglyceride, and insulin concentrations were higher in the insulin-resistant subjects compared with the insulin-sensitive subjects (Table 2). After the mixed meals, postprandial plasma glucose concentrations were similar in the two groups (Fig. 1*A*). In contrast, postprandial plasma concentrations of insulin and triglycerides were markedly increased in the insulin-resistant subjects (Fig. 1 *B* and *C*). There were no differences in fasting or postprandial plasma fatty acid concentrations between the two groups, except from 10 to 11 p.m., when plasma fatty acid concentrations were lower in the insulin-resistant subjects compared with the insulin-sensitive subjects (Fig. 1*D*).

Fasting plasma high-density lipoprotein (HDL) cholesterol concentrations were 20% lower in the insulin-resistant subjects (Table 2), but there were no differences in total cholesterol or low-density lipoprotein (LDL) between the two groups. The concentrations of small very-low-density lipoprotein (VLDL) and large LDL particles were decreased by 38% and 30%, respectively, in the insulin resistant subjects (Table 2). Plasma concentrations of uric acid were increased by ≈30% in the insulin-resistant subjects compared with the insulin-sensitive control subjects (Table 2). In contrast, there were no differences in plasma concentrations of high-molecular-weight (HMW) adiponectin, IL-6, resistin, plasminogen activator inhibitor-1, retinol binding protein-4 (RBP-4), or TNF-α between the two groups (Table 3).

Basal concentrations of muscle glycogen content were similar in the insulin-sensitive (87.1 ± 5.7 mmol/liter muscle) and insulin-resistant (84.1 ± 4.6 mmol per liter of muscle, $P = 0.69$) subjects. After the mixed meals, however, net muscle glycogen synthesis was 61% lower in the insulin-resistant subjects compared with the insulin-sensitive subjects (Fig. 2*A*). In contrast, there were no differences between the two groups in basal liver glycogen concentration (insulin-sensitive, 131.6 ± 9.2 mmol per liter of liver, vs. insulin-resistant, 125.0 ± 17.7 mmol per liter of

liver; $P = 0.74$) or net liver glycogen synthesis after ingestion of the meals (Fig. 2*B*).

There were no differences in the basal concentrations of hepatic triglyceride in the two groups (insulin-sensitive, 0.65 ± 0.14%, vs. insulin-resistant, 0.76 ± 0.20%; $P = 0.63$); however, net hepatic triglyceride synthesis was ≈2.5-fold greater in the insulin-resistant subjects than the insulin-sensitive subjects after the carbohydrate meals (Fig. 2*D*). There were no changes in intramyocellular triglyceride content after the meals in either group (Fig. 2*C*). The mean (Table 1) and distribution (Fig. 3*C*) of intraabdominal fat volume assessed by MRI were similar between the two groups, whether or not the single outlier in each group was excluded from analysis.

Postprandial fractional hepatic *de novo* triglyceride lipogenesis, as assessed by the incorporation of deuterated water into plasma triglyceride, was increased by 2.2-fold in the insulin-resistant subjects (15.7 ± 1.5%) compared with the insulin-sensitive subjects (7.2 ± 0.7%, $P = 0.00005$) (Fig. 4).

Discussion

We found that the pattern of stored energy distribution derived from two high-carbohydrate meals was markedly different in young, lean, insulin-resistant individuals compared with young, lean, insulin-sensitive individuals. In contrast to the young, lean, insulin-sensitive subjects, who stored most of their ingested energy in liver and muscle glycogen, the young lean insulin-resistant subjects had a marked defect in muscle glycogen synthesis and diverted much more of their ingested energy into hepatic *de novo* lipogenesis, resulting in increased plasma triglycerides, lower HDL, and increased hepatic triglyceride synthesis (Fig. 5).

These data have several important implications for the role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome and T2DM. First, the data are consistent with the hypothesis that insulin resistance in skeletal muscle promotes atherogenic dyslipidemia by promoting the conversion of energy derived from ingested carbohydrate into hepatic *de novo* lipogenesis and increased VLDL production. This hypothesis is further supported by studies in mice with muscle-specific inactivation of the insulin receptor gene, which have been shown to have increased plasma triglycerides and increased adiposity as a result of muscle-specific insulin resistance (14).

Table 2. Fasting plasma metabolite and hormone concentrations

	Glucose, mg/dl	Insulin, microunits/ml	Triglyceride, mg/dl	Total cholesterol, mg/dl	LDL, mg/dl	HDL, mg/dl	VLDL SP, nmol/liter	LDL LP, nmol/liter	Uric acid, mg/dl	CRP, mg/liter
Insulin-sensitive	84.1 ± 1.7	7.6 ± 0.6	53 ± 7	182 ± 12	93 ± 9	78 ± 5	38.6 ± 4.0	446.4 ± 34.2	3.9 ± 0.3	0.47 ± 0.16
Insulin-resistant	90.6 ± 1.5	12.1 ± 1.2	86 ± 13	157 ± 5	77 ± 6	62 ± 3	23.8 ± 2.9	311.9 ± 46.0	5.2 ± 0.5	1.02 ± 0.24
P value	0.009	0.003	0.03	NS	NS	0.01	0.007	0.03	0.04	0.07

SP, small particles; LP, large particles; CRP, C-reactive protein; NS, not significant.
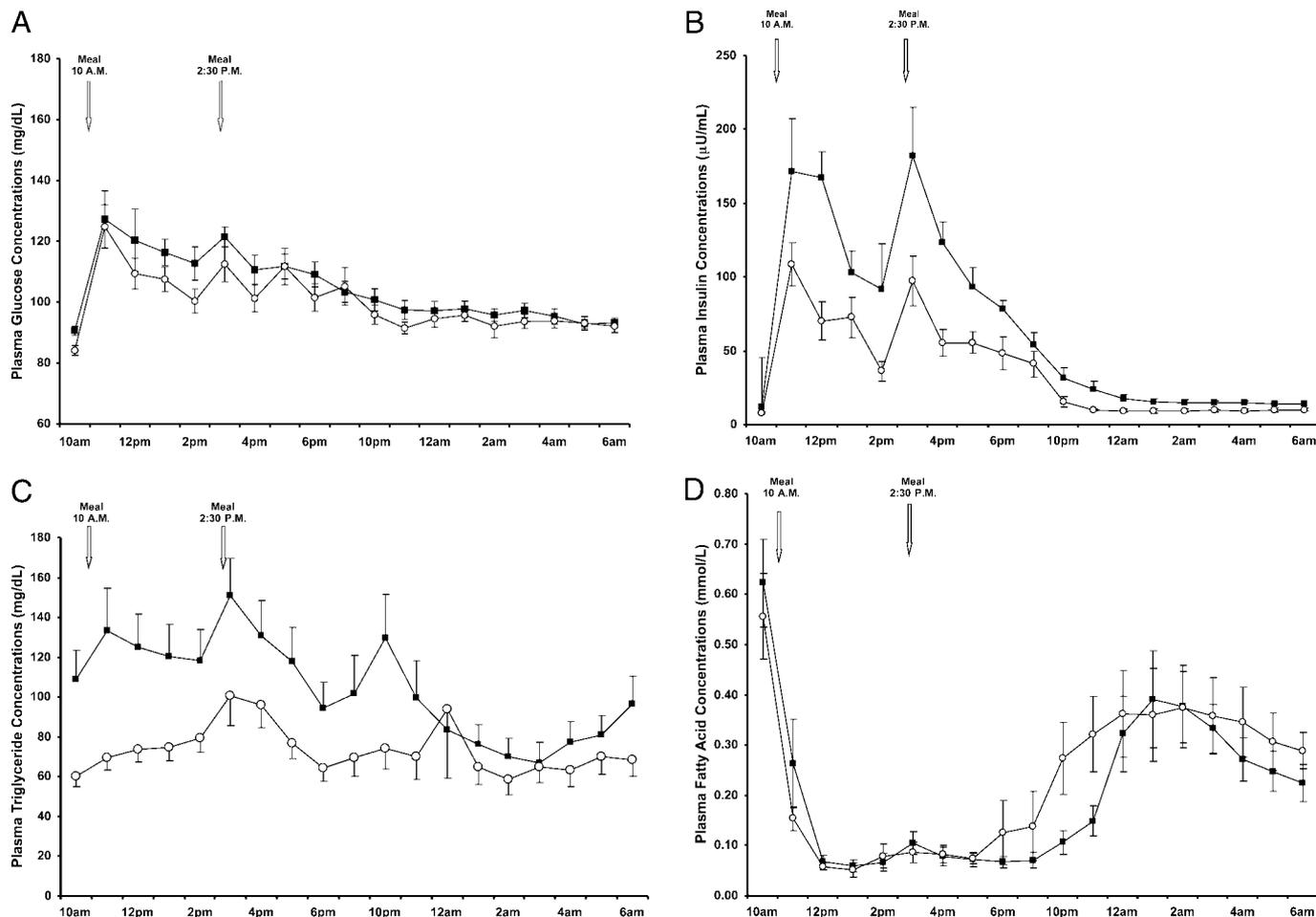
Fig. 1. Plasma concentrations of glucose (*A*), insulin (*B*), triglyceride (*C*), and fatty acids (*D*) in insulin-sensitive (○) and insulin-resistant (■) participants before and after the mixed meals.

Second, increased hepatic *de novo* lipogenesis resulting in hypertriglyceridemia also can explain the lower HDL levels in the insulin-resistant subjects by the following mechanism: In the presence of increased plasma VLDL concentrations and normal activity of cholesteryl ester transfer protein, VLDL triglycerides can be exchanged for HDL cholesterol, where a VLDL particle donates a molecule of triglyceride to an HDL particle in return for one of the cholesteryl ester molecules from HDL. This process leads to a cholesterol-rich VLDL remnant particle that is atherogenic and a triglyceride-rich, cholesterol-depleted HDL particle (15). The triglyceride-rich HDL particle can undergo further modification, including hydrolysis of its triglyceride, which leads to the dissociation of the apo A-I protein. The free apo A-I in plasma is cleared more rapidly than apo A-I associated with HDL particles, leading to reductions in the amount of circulating apo A-I, HDL cholesterol, and the number of HDL particles (15).

These data also demonstrate that skeletal muscle insulin resistance predates hepatic insulin resistance and that hepatic triglyceride synthesis is increased in these insulin-resistant subjects after high-carbohydrate meals, which may predispose them to nonalcoholic fatty liver disease (NAFLD). The absence of hepatic steatosis in this group of insulin-resistant subjects is consistent with recent studies by our group demonstrating the relatively low prevalence of hepatic steatosis in young, lean, healthy subjects of different ethnic backgrounds with the important exception of Asian Indian males who have a marked increase in the prevalence of hepatic steatosis (13). Although it is believed that hepatic reesterification of fatty acids accounts for the majority of newly synthesized triglyceride, these results suggest that increased hepatic *de novo* lipogenesis precede the development of adipose tissue insulin resistance, which subsequently leads to increased flux of fatty acids to the liver (16, 17). This hypothesis is supported by our previous findings of similar

Table 3. Fasting plasma adipocytokine concentrations

	HMW adiponectin, μg/ml	IL-6, pg/ml	Resistin, ng/ml	TNF-α, pg/ml	PAI-1, ng/ml	RBP-4, μg/ml	TTR, μg/ml
Insulin-sensitive	5.8 ± 2.5	0.91 ± 0.20	11.0 ± 1.1	1.49 ± 0.12	13.8 ± 3.9	29.5 ± 3.3	134 ± 8
Insulin-resistant	3.5 ± 0.7	1.31 ± 0.23	12.5 ± 0.9	1.49 ± 0.17	13.2 ± 2.5	28.1 ± 3.1	149 ± 11
P value	NS	NS	NS	NS	NS	NS	NS

PAI-1, plasminogen activator inhibitor-1; TTR, transthyretin; NS, not significant.
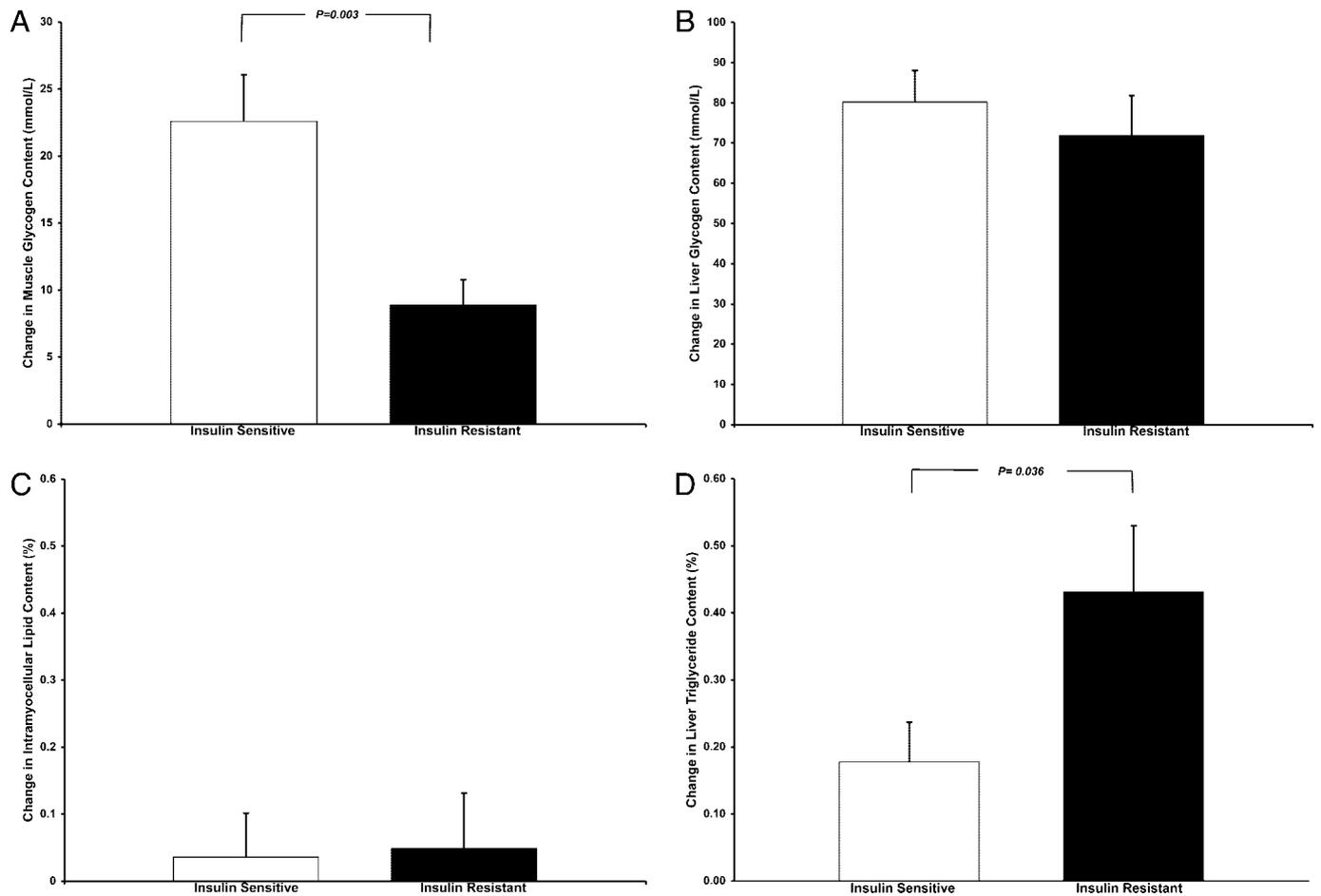
Fig. 2. ¹³C MRS measurements of changes in muscle (*A*) and liver (*B*) glycogen concentrations and ¹H MRS measurements of changes in intramyocellular triglyceride (*C*) and hepatic triglyceride (*D*) content in insulin-sensitive and insulin-resistant subjects after the mixed meals.

basal and insulin suppressed rates of whole body and subcutaneous fat lipolysis in similar groups of insulin-sensitive and insulin-resistant subjects (12). NAFLD is strongly linked to hepatic insulin resistance, and recent studies have demonstrated that NAFLD-induced hepatic insulin resistance is a major factor responsible for the transition from normoglycemia to fasting hyperglycemia and T2DM (18–23). The cellular signal driving this increased hepatic *de novo* lipogenesis can most likely be attributed to hyperinsulinemia promoting increased expression of the sterol regulatory element binding protein-1c in the liver, which coordinately regulates transcription of all of the key enzymes involved in lipogenesis (24). Indeed, assuming a portal vein–artery concentration gradient for insulin of ≈3, portal vein insulin concentrations can be estimated to exceed 500 microunits/ml in the insulin-resistant subjects

Increased export of triglyceride from the liver to the peripheral and visceral adipose tissue in the form of VLDL may also predispose these insulin-resistant individuals to abdominal obesity (25). Although abdominal obesity has been postulated to play the major role in causing the atherogenic dyslipidemia and insulin resistance associated with the metabolic syndrome (5–7), we did not observe any significant differences in the mean volume of intraabdominal fat between these two groups. These data suggest that abdominal obesity develops later in the course of the metabolic syndrome along with NAFLD and that it is likely more a consequence of insulin resistance in skeletal muscle rather than a primary cause of insulin resistance and atherogenic dyslipidemia (5–7). These results are consistent with recent

observations in lipodystrophic patients and mice (20) as well as rodent models of hepatic insulin resistance (18) that have disassociated intraabdominal adiposity from insulin resistance and instead have implicated hepatic steatosis in causing hepatic insulin resistance associated with the metabolic syndrome and T2DM (22, 23, 26).

Finally, alterations in plasma adipocytokine concentrations have also been proposed to play a major role in the development of insulin resistance associated with the metabolic syndrome and T2DM. However, we observed no differences in circulating concentrations of HMW adiponectin, IL-6, resistin, TNF-α, or RBP-4 in the two groups (27–29). Taken together these data suggest that alterations in the concentrations in these plasma adipocytokines do not play a primary role in the early development of insulin resistance and atherogenic dyslipidemia in these young, lean, insulin-resistant subjects and that the observed alterations in the concentrations of these adipocytokines in the metabolic syndrome and T2DM are likely to be secondary to other factors, such as obesity.

In summary, these data support the hypothesis that insulin resistance in skeletal muscle, due to decreased muscle glycogen synthesis, promotes atherogenic dyslipidemia by diverting energy derived from ingested carbohydrate away from muscle glycogen synthesis into increased hepatic *de novo* lipogenesis. These findings have important implications for understanding the mechanism by which insulin resistance in skeletal muscle promotes the development of the metabolic syndrome, NAFLD, T2DM, and the associated cardiovascular disease. These data
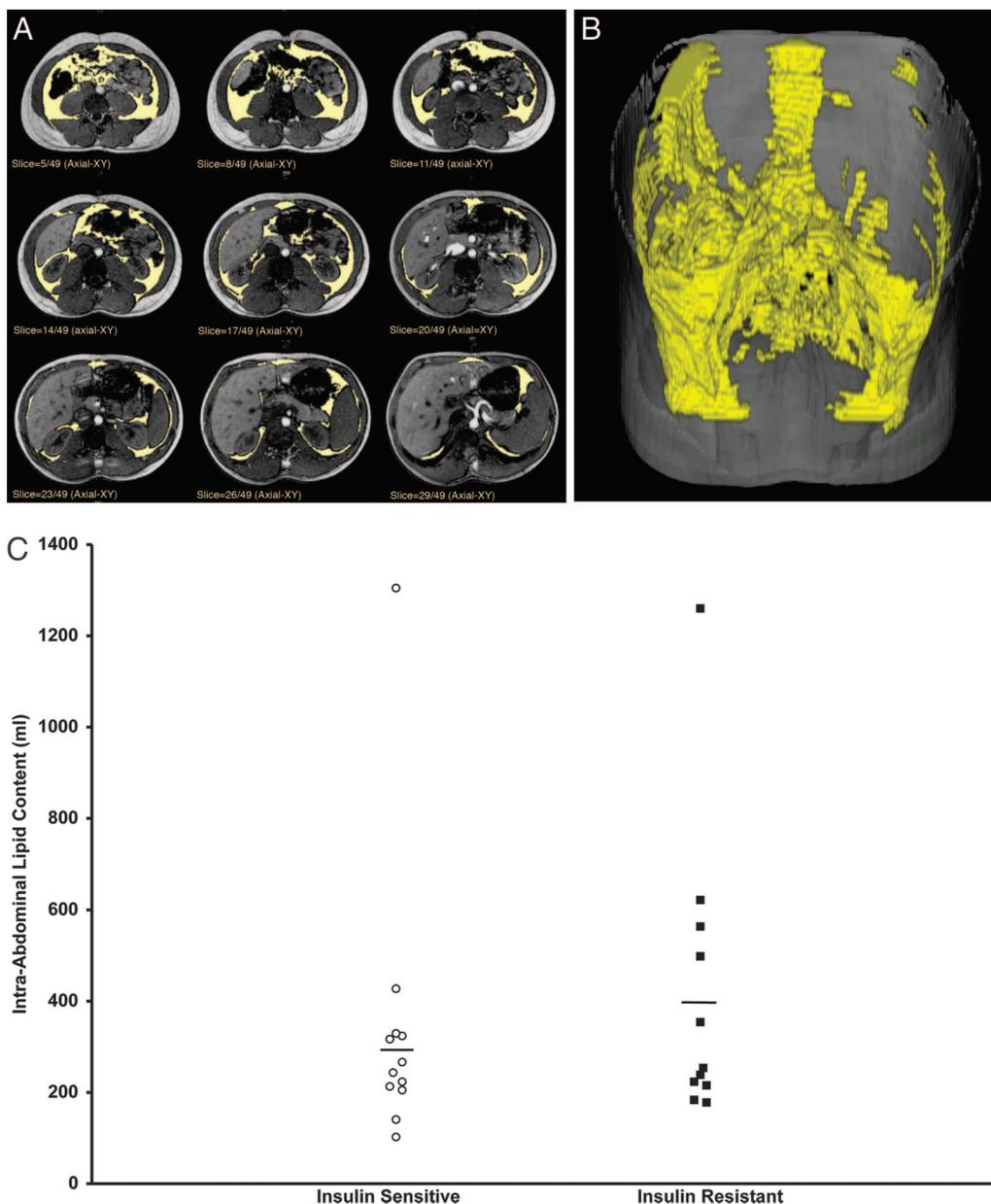
Fig. 3. MRI imaging of intraabdominal fat of several slices through the abdomen in a subject (*A*), three-dimensional reconstruction of intraabdominal fat in the same subject (*B*), and intraabdominal fat volume in the insulin-sensitive and insulin-resistant subjects (*C*).

also suggest that reversing defects in insulin-stimulated glucose transport in skeletal muscle to reverse insulin resistance in this organ might be the best way to prevent the development of the metabolic syndrome at its earliest stages of development.

Materials and Methods

Participants. Between 2005 and 2007 we studied ≈400 young, healthy, lean, nonsmoking, sedentary volunteers from the New Haven community. All subjects were in excellent health, taking no medications, lean, nonsmoking, of normal birth weight, and sedentary as defined by a physical activity questionnaire (30) and subsequently, by 3 days of physical activity monitoring using a pedometer (Pedometer 342; Sportline). Blood pressure was measured in the resting state by a digital 300 Vital Signs Monitor (Welch Allyn, Skaneateles Falls, NY). Subjects were excluded if they had any metal implants, body piercing, or history of claustrophobia.

Yale University Human Investigation Committee approved the protocol, and written consent was obtained from each subject after the purpose, nature, and potential complications of the studies were explained.

ISI. Whole-body insulin sensitivity was assessed with a 3-hr, 75-g oral glucose tolerance test in combination with the ISI (12, 31, 32). Twenty minutes after insertion of an antecubital i.v. line, fasting blood samples were collected for determination of plasma glucose, insulin, fatty acid, C-reactive protein, HMW adiponectin, resistin, IL-6, RBP-4, and TNF-α concentrations. The dextrose drink (Glucola; Curtin Matheson Scientific, Houston, TX) was administered, and blood samples were collected at 10, 20, 30, 60, 90, 120, 150, and 180 min for determination of plasma glucose, fatty acid, and insulin concentrations. The ISI measures the overall effects of insulin to stimulate glucose disposal and inhibit glucose production. From this cohort, 12
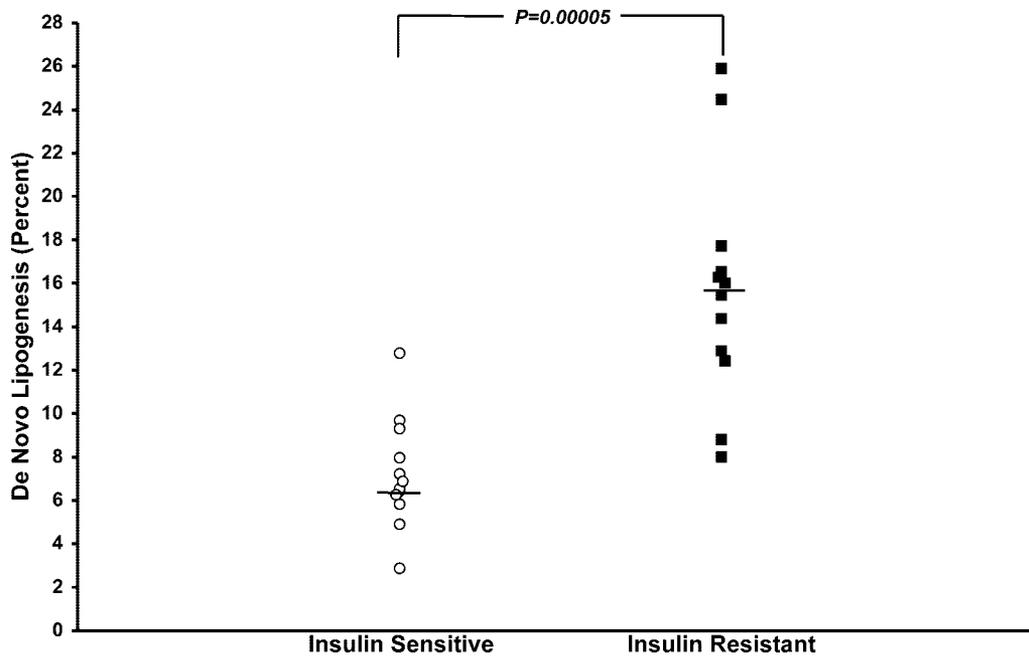
Fig. 4. Fractional *de novo* lipogenesis in insulin-sensitive and insulin-resistant subjects after the two high-carbohydrate mixed meals.

insulin-resistant subjects (four male and eight female; two African American, eight Caucasian, and two Hispanic) as defined by an ISI of $<3.90 \times 10^{-4}$ dl/min per microunit/ml (lower quartile of the ISI distribution) and 12 insulin-sensitive subjects (five male and seven female; one black and 11 Caucasian) as defined as having an ISI of $>6.06 \times 10^{-4}$ dl/min per microunit/ml (top quartile of ISI distribution) volunteered to be studied further as described below (13).

All qualifying subjects subsequently underwent dual-energy x-ray absorptiometry scanning (QDR-4500 W; Hologic, Bedford, MA) to assess lean body mass and fat mass (33), a complete medical history, a physical examination, and blood tests to verify that the following were normal: blood and platelet counts, electrolytes, aspartate amino transferase, alanine amino transferase, blood urea nitrogen, creatinine, prothrombin time, partial prothrombin time, cholesterol, and triglyceride.

Dietary Regimen. To ensure that all participants were weight-stable and following a regular dietary regimen, each participant answered a questionnaire about their usual daily food intake and eating habits over the past 12 months. For 3 days before the

Fig. 5. Schematic of whole-body energy distribution after high-carbohydrate mixed meals in insulin-sensitive and insulin-resistant individuals.

study, the participants received all their meals from the General Clinical Research Center (GCRC) Metabolic Kitchen (34–37). The diet was eucaloric [≈35 kcal (1 cal = 4.18 J) per kilogram of body weight per day] and contained 55% carbohydrate, 10% protein, and 35% fat.

Experimental Protocol. On day 1, the subjects were admitted to the GCRC at 5 p.m. and had dinner at 7 p.m. (33% of their daily caloric requirements). The subjects remained fasting until the first part of the test meal the following day (day 2). Baseline MRS measurements of lipid and glycogen content in muscle and liver were performed on day 2 starting at 6:30 a.m. After completion of these baseline MRS measurements, the subjects were returned to the GCRC for the test meals and determination of *de novo* lipogenesis as described below. At 7 p.m. the postprandial MRS measurements of lipid and glycogen content in muscle and liver were started. To avoid physical activity, the subjects were encouraged to remain in bed throughout the study, and they were brought to and from the Yale Magnetic Resonance Research Center in a wheelchair.

De Novo Lipogenesis. After completion of the baseline MRS measurements, the liquid test meals were served. These meals were prepared by the GCRC Metabolic Kitchen for each subject and contained all of the required daily energy (35 kcal per kilogram of body weight; 55% carbohydrate, 10% protein, and 35% fat) with an additional 25% of the daily energy requirements added in the form of sucrose. The meals were equal in size and first portion was served at 10 a.m., and the second was served at 2:30 p.m. Each meal was consumed within 15–20 min. Between the two test meals at noon and at 2 p.m., the loading doses of deuterium-labeled water (3 ml per kilogram of body water; 99.8%; Cambridge Isotopes, Cambridge, MA) was given in two portions of equal size. To maintain constant deuterium enrichment in plasma water, deuterium-labeled drinking water (0.45% enrichment) was given ad libitum for the remainder of the study. The incorporation of deuterium into lipids during administration of deuterium-labeled water was used to determine the fractional synthetic rate of fatty acids as described previously (3, 4). Blood was collected to assess *de novo* lipogen-

esis before the first dose of deuterium-labeled water and hourly from 10 p.m. until 6 a.m. (4). After centrifugation, plasma was split into two parts, one part was stored at −20°C until analysis, and the other part was processed immediately by ultracentrifugation for purification and removal of chylomicrons and plasma triglyceride extraction (3, 4, 38). Deuterium enrichment in palmitate and plasma water was measured by gas chromatography–mass spectrometry (5971A Mass Selective Detector; Hewlett–Packard, Wilmington, DE) as previously described (3, 4, 38), and fractional rates of *de novo* lipogenesis were calculated as previously described (39).
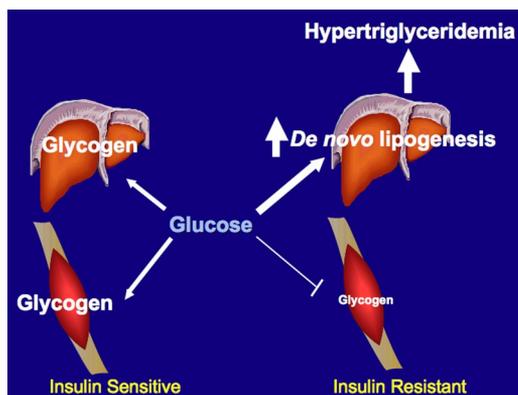
¹H MRS and ¹³C MRS. All MRS measurements were performed on a whole-body, 4.0-T Medspec (Bruker, Billerica, MA) system. Muscle glycogen and lipid content were measured in the calf muscle by using an 8.5-cm-diameter, circular ¹³C surface coil with twin, orthogonal circular 13-cm ¹H quadrature coils. The probe was tuned and matched, and scout images of the lower leg were obtained to ensure correct positioning of the subject and to define an adequate volume for localized shimming using the FASTMAP procedure (40). ¹³C MRS spectra were acquired in a $(60 \times 30 \times 60)$-mm³ volume placed within the gastrocnemius/soleus muscles to measure glycogen content. Protons were decoupled during the 25-ms acquisition time with a WALTZ-4, and localization of the volume was performed with a three-dimensional adiabatic outer-volume suppression.

After the 20-min acquisition for the glycogen spectra, localized ¹H spectra were acquired to assess the muscle lipid content from a $(10 \times 10 \times 10)$-mm³ voxel centered in the soleus muscle using a stimulated echo acquisition mode sequence, with three modules of water suppression with the chemical-shift selective imaging sequence. The total lipid content was estimated from comparison of a water-suppressed lipid spectrum and a lipid-suppressed water spectrum, with the appropriate peak for each spectrum on-resonance. The intramyocellular lipid and water resonances were corrected for T_1 and T_2 relaxation, and intramyocellular lipid content was expressed as a percentage of the water content.

The glycogen and triglyceride content in the liver was measured with a coil assembly composed of a 12-cm, circular ¹³C coil and twin (13×9)-cm elliptical ¹H radio frequency coils arranged in quadrature. The probe was secured on the abdomen over the liver with Velcro straps, and a nonmagnetic pneumatic expansion bellows were used for gating the MRS acquisition to the respiratory movements. After imaging the liver and localized shimming with the FASTMAP method with respiration gating (40), ¹³C MRS for liver glycogen content was acquired with an adiabatic half passage pulse and with WALTZ-4 decoupling during the acquisition time.

The liver triglyceride content was measured by ¹H respiratory-gated stimulated echo acquisition mode sequence spectroscopy in a $(15 \times 15 \times 15)$-mm³ voxel (41–43). Acquisition was synchronized to the respiratory cycle and triggered at the end of expiration. A water-suppressed lipid spectrum and a lipid-suppressed water spectrum were acquired, and a minimum of two lipid spectra and two water spectra were time-averaged to minimize variations due to chest movements. This sequence was repeated in a second location of the liver to account for liver inhomogeneity. A minimum of eight spectra was acquired for each subject and the total lipid content was averaged and calculated as previously described (44).

Abdominal Fat by MRI. A MRI of the trunk was acquired in each subject by using a Siemens Sonata 1.5-T Instrument (multi-breathold T1-weighted acquisition; field of view, 38.0 × 38.0 cm; matrix, 256 × 256; in-plane resolution, 1.48 mm; 50 contiguous slices; slice thickness, 5-mm). After slice intensity homogeneity correction, intraabdominal fat was interactively segmented in each slice by using the Yale BioImage Arbor Suite software package (www.bioimagesuite.org) and the segmentation map was summed for the entire trunk to generate volume measurements (33).

Analytical Methods. Plasma glucose concentrations were measured by using a YSI STAT 2700 Analyzer (Yellow Springs Instrument Co., Yellow Springs, CA). Plasma concentrations of insulin, resistin, and HMW adiponectin were measured with double-antibody RIA kits (Linco, St. Louis, MO), and plasma concentrations of IL-6 and TNF-α were measured with Quantine High-Sensitivity kits (R&D Systems, Minneapolis, MN). Plasma fatty acid and triglyceride concentrations were determined by using a microfluorimetric method (45). RBP4 and transthyretin (TTR) were measured by quantitative Western blotting (46) with protein standards prepared with purified full-length human recombinant RBP4 and purified human plasma TTR (Sigma, St. Louis, MO). Immunodetection was performed with polyclonal antibodies to human RBP4 (DakoCytomation, Glostrup, Denmark) and TTR (DakoCytomation). Plasma VLDL and LDL particle concentrations were determined by using MRS (LipoScience, Raleigh, NC).

Statistical Analysis. Statistical analyses were performed by using the StatView package (Abacus Concepts, Berkeley, CA). Wilcoxon Rank Sum test or unpaired Student's *t* tests were performed where appropriate to detect statistical differences between insulin-resistant and insulin-sensitive subjects. Paired *t* tests were performed to detect statistical differences within individuals. All data are expressed as mean ± SEM.

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