Differential Responses of Visceral and Subcutaneous Fat Depots to Nutrients

Francine H. Einstein,[1] Gil Atzmon,[2] Xiao-man Yang,[2] Xiao-Hui Ma,[2] Marielisa Rincon,[3] Eric Rudin,[2] Radhika Muzumdar,[2,3] and Nir Barzilai[2]

Increased visceral adiposity is a pivotal component of the metabolic syndrome. Differential gene expression patterns of fat-derived peptides (FDPs) in visceral fat and subcutaneous fat have been characterized in the fasting state. Here we examined whether delivery of nutrients differentially affects the expression of FDPs in visceral fat versus subcutaneous fat (in the fed state). We increased the rate of glucose flux into adipose tissue of normal rats ($n = 16$) by hyperglycemia or hyperinsulinemia using the clamp technique. Glucose uptake was associated with increased expression of FDPs, including resistin (~5-fold), adiponectin (~2-fold), leptin (~15-fold), plasminogen activating inhibitor-1 (~10-fold), and angiotensinogen (~4-fold) in visceral fat, but markedly less in subcutaneous fat. Cytokine expression de-rived mainly from vascular/stromal/macrophage components of adipose tissue was less dramatically increased. Infusion of glucosamine amplified the results obtained by increasing glucose uptake into adipose tissue, suggesting that flux through the hexosamine biosynthetic pathway may serve as a mechanism for "nutrient sensing." Nutrient-dependent expression of FDPs in visceral fat was also associated with increased plasma levels of several FDPs. Because a biologic sensing pathway can dynamically couple daily food intake to abnormal plasma levels of important FDPs, we challenge the practice of obtaining plasma levels after fasting to assess risk factors for metabolic syndrome. *Diabetes* 54:672–678, 2005

Increased adiposity is a risk factor for insulin resistance, type 2 diabetes, and coronary artery disease (1–3). Increased visceral fat is associated with and may account for the changes in peripheral and hepatic insulin sensitivity seen with obesity (4–6). In rodent and human experiments, removal of subcutaneous fat did not result in improvement of insulin action, supporting the hypothesis that visceral fat may be metabolically more active (7,8). We previously demonstrated a causal relationship between visceral fat and insulin resistance by surgical removal of visceral fat from both old and young rats and further demonstrated an improvement in both peripheral and hepatic insulin resistance as well as an improvement in expression of several fat-derived peptides (FDPs) (9,10).

Previous studies have attempted to explain the physiologic differences between visceral fat and subcutaneous fat in humans by examining variability in gene expression in simultaneously obtained adipose tissue biopsies in the fasting state (11,12). We previously demonstrated these differences using expression array showing that ~20% of the genes expressed differed by at least twofold in one depot relative to the other (13).

In this experiment, we evaluated gene expression and plasma levels of FDPs in response to nutrients. Although changes in glucoregulatory hormones have been documented after glucose tolerance tests and food boluses, nutrients may have a more complex and prolonged action affecting gene transcription, secretion, and ultimately plasma levels. For example, plasma levels of the FDP leptin are increased throughout the day, and its levels are maximal at ~2 A.M. (14), an effect that is linked to food intake and not a diurnal variation (15). This line of investigation is important because plasma levels of these surrogate markers of risk are proportional to fat depot size. In the central obesity typical of the metabolic syndrome, visceral fat accounts for up to 27% of total fat volume in obese humans (16), making an understanding of its regulation significant.

The hexosamine biosynthetic pathway (HBP) has a major role in directing hexose phosphates and glutamine to glycosylation and synthesis glycoproteins. Because the HBP accounts for only a small percentage (1–3%) of the glucose metabolized in myocytes, adipocytes, and other cells, it is hypothesized to be an ideal candidate for a cellular nutrient sensor, responding to energy availability and mediating different metabolic effects (17). Hyperglycemia or hyperinsulinemia, by increasing the flux through the HBP, can lead to insulin resistance and glycosylation of transcription factors such as Sp1 (17–19). Sp1 is an important transcription factor that can modulate expression of FDPs, particularly those involved in insulin action and the metabolic syndrome (20). Leptin gene expression in adipose and muscle tissues is increased during en-

From the [1]Department of Obstetrics and Gynecology and Women's Health, Albert Einstein College of Medicine, Bronx, New York; the [2]Department of Medicine, Diabetes Research and Training Center, Institute for Aging Research, Albert Einstein College of Medicine, Bronx, New York; and the [3]Department of Pediatrics, Albert Einstein College of Medicine, Bronx, New York.

Address correspondence and reprint requests to Nir Barzilai, MD, Institute for Aging Research, Belfer Bldg. #701, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. E-mail: barzilai@aecom.yu.edu.

Received for publication 5 August 2004 and accepted in revised form 18 November 2004.

F.H.E. and G.A. contributed equally to this work.

FDP, fat-derived peptide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HBP, hexosamine biosynthetic pathway; IL, interleukin; PAI-1, plasminogen activating inhibitor-1; TNF-α, tumor necrosis factor-α.

© 2005 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/54/3/672/380812/zdb00305000672.pdf by guest on 13 June 2024

hanced in vivo flux through HBP (21). Hyperglycemia and infusions of glucosamine increased levels of both plasminogen activating inhibitor-1 (PAI-1) (22) and angiotensinogen (23) in subcutaneous fat after 3 h, supporting the link between HBP and the induction of FDP.

To demonstrate nutritional regulation of FDPs, we aimed to increase glucose uptake in adipose tissue by either hyperglycemia or hyperinsulinemia for several hours and examined how the expression patterns of FDPs were altered. Glucosamine was also infused to determine whether changes in FDPs could be induced by a specific nutrient-sensing pathway. Finally, differences in gene expression between subcutaneous fat and visceral fat depots were compared to demonstrate a possible deleterious effect of the visceral fat depot.

RESEARCH DESIGN AND METHODS

Young adult F344 × BN rats (Harlan Worldwide, Somerville, NJ) were housed in individual cages and subjected to standard light (6:00 A.M. to 6:00 P.M.) and dark (6:00 P.M. to 6:00 A.M.) cycles. They were fed ad libitum using regular rat chow that consisted of 64% carbohydrate, 30% protein, and 6% fat with a physiological fuel value of 3.3 kcal/g chow. Rats were studied during young adulthood (3 months of age and ~300 g body wt). One week before the in vivo study, rats were anesthetized by inhalation of methoxyflurane, and indwelling catheters were inserted in the right internal jugular vein and in the left carotid artery (10,24–27). Recovery was continued until body weight was within 3% of the preoperative weight (~5–6 days). Chronically catheterized rats were studied ~16–24 h after their last feeding, while awake and unstressed.

In vivo studies. To mimic components of the in vivo physiologic postmeal conditions, we designed the following four protocols ($n = 4$ in each group):

1. Control study: saline was infused for 5 h.
2. Hyperglycemic clamp study: briefly, 25% dextrose was infused intravenously to raise plasma glucose concentration acutely to ~18 mmol/l. Somatostatin (1.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused to prevent endogenous insulin secretion. This plasma glucose concentration was maintained throughout the study using a variable infusion of glucose, periodically adjusted according to plasma glucose levels (22–24).
3. Hyperinsulinemic-euglycemic clamp study: this protocol was intended to increase and approximately match rates of glucose uptake in adipose depots to those obtained in the hyperglycemic clamp study. A primed continuous insulin infusion (3 $mU \cdot kg^{-1} \cdot min^{-1}$) and a variable infusion of 25% dextrose was periodically adjusted to clamp the plasma glucose concentration at basal level (~7 mmol/l) for 5 h (26,27). Somatostatin (1.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused to prevent endogenous insulin secretion.
4. Glucosamine study: because glucosamine enters the hexosamine pathway directly by being phosphorylated to glucosamine-6-phosphate, its intravenous infusion (30 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) for 5 h (25) was used as a tool to directly increase glucosamine flux through the pathway without changing glucose flux. Somatostatin (1.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused to prevent exogenous insulin secretion. Insulin infusion (3 $mU \cdot kg^{-1} \cdot min^{-1}$) was also used because glucosamine uptake is mediated by insulin-responsive glucose transporters. Infusion of 25% dextrose was titrated to maintain plasma blood glucose at a basal level (~7 mmol/l) for 5 h.

Thus, the hyperglycemia studies increased plasma glucose levels to allow for glucose flux largely through non–insulin-mediated glucose uptake. Although glucose levels were not physiologic, the glucose uptake into fat was. The hyperinsulinemia study attempted to approximately match glucose uptake to the hyperglycemia study. The glucosamine study attempted to increase glucosamine flux without changing glucose flux. The prolonged time course of 5 h was necessary to ensure that a significant increase in the plasma levels of FDPs could be detected after initiation of expression.

All rats received a primed continuous (15–40 µCi/min) infusion of high-performance liquid chromatography–purified [3-^{3}H]glucose (New England Nuclear, Boston, MA) throughout the study to determine glucose fluxes. For determining glucose uptake in adipose tissue, a bolus of 2-[U-^{14}C]deoxyglucose (20 µCi) was administered over 30 min before the end of each study, and plasma samples for determination of plasma 2-[U-^{14}C]deoxyglucose specific activity were obtained at short intervals during the rest of the clamp studies (22,23). At the end of the clamp study, rats were killed using 60 mg/kg body wt pentobarbital sodium intravenously through catheters that were previously implanted. The abdomen was quickly opened, and visceral fat and subcutaneous fat tissue were freeze-clamped in situ with aluminum tongs precooled in liquid nitrogen (28). The study protocol was reviewed and approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine.

In vitro analysis

RNA extraction and labeling. Total RNA was isolated from both visceral and subcutaneous fat depots obtained from individual rats by direct homogenization in 1.0 ml of Trizol reagent (Life Technologies, Grand Island, NY). Total RNA from each depot from each rat in the group was obtained. The RNA was analyzed by 1% agarose gel that contained 2.2 mol/l formaldehyde before use. Further steps in the isolation process were performed according to the manufacturer's recommended protocol. Subsequent purification and size selection of total RNA using RNeasy columns (Qiagen, Valencia, CA) were also done as suggested by the manufacturer.

Quantification of expression by real-time PCR. Real-time quantitative RT-PCR (qRT-PCR) was performed for transcript confirmation using a hot-start technique (Superscript II RT + *Taq* polymerase; Life Technologies, Gaithersburg, MD), with 2 µg of total RNA from individual rats as starting material and an annealing temperature of 58–60°C for 40–45 cycles, depending on the individual transcript being amplified. Resistin, tumor necrosis factor-α (TNF-α), leptin, adiponectin, interleukin (IL)-6, IL-10, PAI-1, angiotensinogen, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and 18S were assessed and quantified by qRT-PCR. For qRT-PCR, the first-strand cDNA was synthesized from 2 µg of total RNA in 20 µl of final incubation volumes by using SuperScript Preamplification System for First Strand cDNA Synthesis (Life Technologies) with random primer. The qRT-PCR was carried out in a 20-µl reaction mixture that contained 2 µl of the above first-strand cDNA, using Light Cycler DNA Master SYBR green kit (Roche). The upstream and downstream primer sequences along with expected qRT-PCR product for β-actin, angiotensinogen, resistin, GAPDH, leptin, and PAI-1 have been described elsewhere (13). For TNF-α, the sequence of the upstream primer was TCGAGTGACAAGCCCGTAG and for the downstream primer was GCTCTTGATGGCAGAGAGGA, and the expected qRT-PCR product was 273 bp. For adiponectin, the sequence of the upstream primer was GTCACTGTCCCCAATGTTCC and for the downstream primer was GTCCCCTTCCCCATACACTT, and the expected qRT-PCR product was 294 bp. For IL-6, the sequence of the upstream primer was GTCAACTCCTGCCCTTC and for the downstream primer was ACTGGTCTGTTGTGGGTGGT, and the expected qRT-PCR product was 164 bp. For IL-10, the sequence of the upstream primer was TCCTTGGGAAGCAACTGAAA and for the downstream primer was AGAGTGATGGCAGGGAGATG, and the expected qRT-PCR product was 293 bp. For 18S, the sequence of upstream primer was AGGGTTCGATTCCGGAGAGGG and for the downstream primer was CCAATAGCGTATAAAGTTG, and the expected RT-PCR product was 242 bp. The conditions for PCR were 94°C for 10 min, cycles of 94°C for 0 s, then annealing temperature that differed between the substrates for 7 s and then 72°C for 12 s using LightCycler system (Roche). The annealing temperature of PAI-1 was 60°C, resistin was 60°C, IL-6 was 60°C, IL-10 was 60°C, leptin was 60°C, angiotensinogen was 60°C, adiponectin was 58°C, TNF-α was 60°C, GAPDH was 60°C, and 18S was 60°C. Quantification of these peptides and their signals was normalized for GAPDH and the 18S signal to correct for loading irregularities. GAPDH expression during the experimental protocols was more consistent compared with saline controls, whereas the percentage changes seen in 18S and β-actin were less acceptable. Therefore, the data presented are those normalized by GAPDH.

Sites of FDP production. In addition to vascular and stromal sources of FDP, it has recently become apparent that adipose tissue macrophages may be numerous (29,30). Adipose tissue was obtained as described above and immediately washed at least three times with saline to remove contaminating blood. The cells were then digested with collagenase type 2, 1 g/30 ml of Hanks' balanced salt solution with 4% BSA (Worthington Biochemical, Lakewood, NJ) for 60 min at 37°C with intermittent shaking. The adipocytes were separated from the stromal-vascular fraction by centrifugation at 3,000 rpm for 10 min. CD14$^+$ macrophages were separated from the stromal-vascular fraction by Dynabeads (Dynal Biotech, Lake Success, NY) as per the manufacturer's recommended method. The separated adipocytes and macrophages were washed by PBS and stored in Trizol for RNA extraction and analyzed by RT-PCR.

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Palo Alto, CA). Plasma insulin was measured by radioimmunoassay, using rat and porcine insulin standards. Plasma [^{3}H]glucose radioactivity was measured in duplicate in the supernatants of $Ba(OH)_2$ and $ZnSO_4$ precipitates of plasma samples (20 µl) after evaporation to dryness to eliminate tritiated water. For measuring plasma [U-^{14}C]2-deoxyglucose, samples were deproteinized, and an aliquot of the supernatant was counted in a double-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/54/3/672/380812/zdb00305000672.pdf by guest on 13 June 2024

TABLE 1
Body composition, metabolic characteristics, and glucose fluxes

	Saline	Glc	Ins	GlcN + Ins
Body weight (g)	285 ± 6	281 ± 8	283 ± 4	289 ± 5
Total visceral fat (g)	5.5 ± 0.5	5.8 ± 0.7	5.3 ± 0.6	5.6 ± 0.5
Perinephric visceral fat (g)	1.8 ± 0.4	1.7 ± 0.3	1.5 ± 0.2	1.7 ± 0.2
Glucose (mmol/l)	7.0 ± 0.4	17.6 ± 0.3*	6.9 ± 0.1	6.8 ± 0.1
Insulin (μU/ml)	11 ± 3	13 ± 4	58 ± 4†	63 ± 7†
Free fatty acids (mEq/l)	0.72 ± 0.09	0.62 ± 0.07	0.53 ± 0.08	0.46 ± 0.06‖
TGU ($mg \cdot kg^{-1} \cdot min^{-1}$)	8.5 ± 1.1	28.0 ± 3.9‡	22.1 ± 3‡	13.1 ± 1.0*
SCGU ($mg \cdot kg\ tissue^{-1} \cdot min^{-1}$)	11.8 ± 1.3§	24.5 ± 1.6‡	16.7 ± 0.6‡§	9.5 ± 1.4§
VFGU ($mg \cdot kg\ tissue^{-1} \cdot min^{-1}$)	3.5 ± 0.1	14.9 ± 1.5‡	9.1 ± 1.5‡	4.5 ± 0.4

Data are means ± SE. Measurements obtained in four groups of rats ($n = 4$ in each group) infused with saline (time control; $n = 4$), hyperglycemia (Glc), hyperinsulinemia (Ins), or glucosamine during hyperinsulinemia (GlcN + Ins). Body weight, weight of visceral fat (total is for the sum of perinephric, epididymal, and mesenteric fat), plasma glucose, insulin, total glucose uptake (TGU), subcutaneous fat tissue glucose uptake (SCGU), and visceral fat tissue glucose uptake (VFGU) during the last 30 min of the clamp (see RESEARCH DESIGN AND METHODS) are presented. *$P < 0.01$ vs. others; †$P < 0.001$ vs. saline and glucose; ‡$P < 0.01$ vs. saline and GlcN + In; §$P < 0.01$ vs. VFGU; ‖$P = 0.03$ vs. saline.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/54/3/672/380812/zdb00305000672.pdf by guest on 13 June 2024

channel β-counter after addition of 500 μl of water and 5 ml of liquid scintillation mixture. For measuring subcutaneous fat [U-^{14}C]2-deoxyglucose, frozen tissue samples were weighed and dissolved in 0.5 ml of 1 mol/l NaOH and kept in a shaking water bath at 60°C for 1 h. After neutralization with 0.5 ml of 1 mol/l of HCl, two aliquots were taken. One was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and the other with 6% $HClO_4$. The $HClO_4$ supernatant contains both phosphorylated and unphosphorylated 2-deoxyglucose, whereas the $Ba(OH)_2$ and $ZnSO_4$ supernatants contain only the unphosphorylated form. The difference in disintegrations per minute between the two supernatants measures the fat content of 2-deoxyglucose phosphate, thus determining the rate of glucose uptake. Plasma levels of IL-6 (Rat IL-6 ELISA kit; Pierce Endogene) and adiponectin (Mouse Adiponectin ELISA kit; Linco Research) were measured by ELISA. Leptin was measured by radioimmunoassay (RAI; Linco Research), and PAI-1 activity was measured by chromogenic assay kit (Spectrolyse/pL PAI, Biopool International). Free fatty acids were measured using Waco NEFA C Kit (Waco Chemicals).

Statistical analysis. The significance of the results of qRT-PCR (fold expression of any gene by visceral fat/GAPDH over subcutaneous fat/GAPDH) was evaluated by the two-sample Student's *t* test. Pearson correlation coefficients were calculated to estimate the linear relationship between variables. All values are presented as means ± SD. $P < 0.05$ was considered significant.

RESULTS

Basal metabolic characteristics and glucose fluxes during saline, glucose, insulin, and glucosamine infusions. Animals were matched in body weight, weight of perinephric fat pads, and other visceral fat pads (data not shown). During hyperglycemic clamps, glucose levels were maintained at target levels (~18 mmol/l), inducing a significant increase in total glucose uptake, subcutaneous fat glucose uptake, and visceral fat glucose uptake. In these studies, total glucose uptake was greater in subcutaneous fat compared with that in visceral fat, but a greater increase in glucose uptake was noted in visceral fat (~4-fold) compared with subcutaneous fat (~2-fold) over saline controls. Physiologic hyperinsulinemia (~60 μU/ml) induced an ~3-fold increase in total glucose uptake, and as seen with hyperglycemia, glucose uptake increased more dramatically in visceral fat (~3-fold) than in subcutaneous fat (~1.5-fold). Although glucosamine was infused during hyperinsulinemic conditions, total glucose uptake was significantly lower, possibly because glucosamine infusion induces insulin resistance beginning 2–3 h after the initiation of its infusion (25,31). We previously reported that glucose uptake was similar to that observed with hyperglycemia or hyperinsulinemia at 2–3 h (22,23). Similarly, glucose uptake into adipose tissue was not increased at the conclusion of the clamp, suggesting some resistance to insulin action in adipose tissue at the end of 5 h (Table 1).

Selected basal FDP gene expression and the cellular compartment source. Expression of resistin was ~10-fold greater in visceral fat than in subcutaneous fat. Adiponectin and IL-10 expression were also significantly greater in visceral fat, making these genes good markers of visceral fat. Leptin expression was significantly lower in visceral fat, whereas no marked differences were found in PAI-1, TNF-α, IL-6, or angiotensinogen expression in subcutaneous fat compared with visceral fat during saline infusion (Table 2).

Because expression of these peptides originates from different cellular components of adipose tissue (adipocytes, vascular/stromal, and macrophages), we assessed the relative contribution of each of these components to the expression of specific peptide. As indicated in Table 2, leptin, adiponectin, resistin, and angiotensinogen are expressed mainly in adipocytes. The cellular source of PAI-1 is mixed, whereas other peptides, including cytokines, are expressed more in nonadipocyte cells. When assessed per total mRNA obtained from per gram of adipose tissue, macrophages may contribute a small percentage even in visceral fat.

Expression of FDPs during hyperglycemia and hyperinsulinemia. Increased glucose uptake, whether by glucose or insulin, seemed to increase expression of FDPs significantly and more dramatically in visceral fat than

TABLE 2
Gene expression (relative to GAPDH) separately obtained from adipocytes, vascular/stromal cells, and macrophages of visceral fat

	Adipocytes	Vascular	Macrophage
Angiotensinogen	22.6	0.2	0.9
Adiponectin	19.2	1.6	0.6
Resistin	4.30	0.08	0.04
Leptin	2	0.1	0.06
PAI-1	3	16	8
TNF-α	0.001	0.6	0.1
IL-10	1	30	4
IL-6	1	60	40

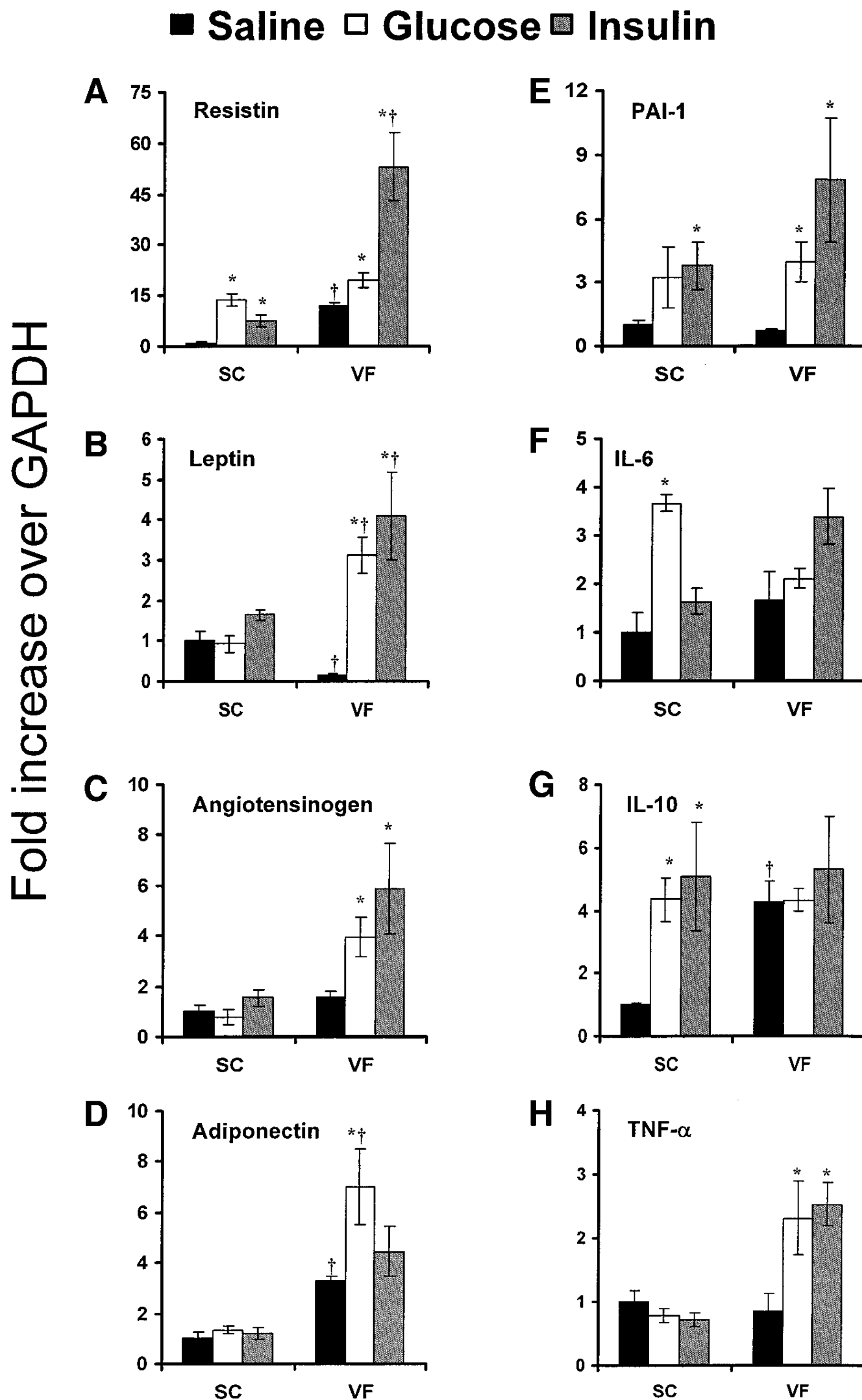

FIG. 1. Selected gene expression of adipokines during hyperglycemia and hyperinsulinemia in subcutaneous fat and visceral fat. Acute increase (5 h) in glucose and insulin infusion was achieved as described in RESEARCH DESIGN AND METHODS. Four rats from each group were studied, and subcutaneous fat and visceral fat of each rat were studied separately. *Significant difference of at least $P < 0.05$ vs. saline; †significant difference of at least $P < 0.05$ vs. same study in subcutaneous fat.

in subcutaneous fat. For example, resistin expression was modulated in both visceral fat and subcutaneous fat by both insulin and glucose, but both absolute and relative expression was greater in visceral fat. Absolute leptin expression was ~10-fold greater in visceral fat. Angiotensinogen, adiponectin, and PAI-1 expression were also more dramatic in visceral fat than in subcutaneous fat. IL-6 and TNF-α expression were not significantly modulated with insulin or glucose. IL-10 expression increased more in subcutaneous fat than in visceral fat (Fig. 1).

Expression of FDPs during glucosamine administration. The expression of resistin, leptin, angiotensinogen, adiponectin, PAI-1, IL-6, and IL-10 increased with glucosamine in both fat depots, but the change was more dramatic in visceral fat. TNF-α expression is also modulated by glucosamine but to a lesser extent than the other FDPs presented (Fig. 2).

Plasma levels of FDPs. Plasma leptin levels increased by twofold with hyperglycemia (0.87 ± 0.16 and 2.67 ± 0.53 ng/ml at 0 and 5 h, respectively) and with hyperinsulinemia (0.95 ± 0.09 and 1.81 ± 0.29 ng/ml at 0 and 5 h,

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/54/3/672/380812/zdb00305000672.pdf by guest on 13 June 2024

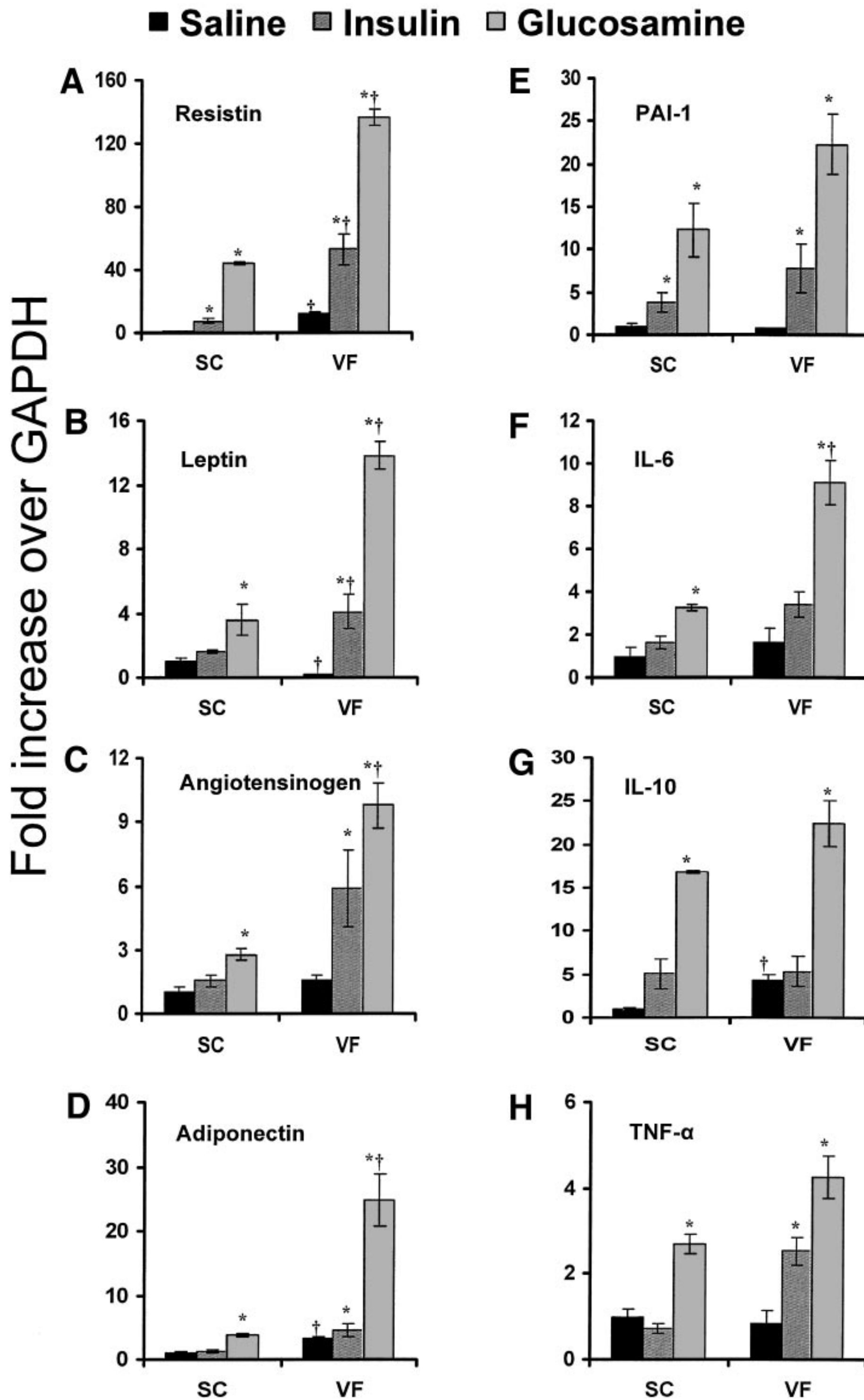

FIG. 2. Selected gene expression of cytokines during hyperinsulinemia and glucosamine infusion in subcutaneous fat and visceral fat (see RESEARCH DESIGN AND METHODS and Fig. 1).

respectively) and by threefold with glucosamine infusion (0.89 ± 0.13 and 3.71 ± 0.22 ng/ml at 0 and 5 h, respectively). Levels during saline infusion were unchanged (0.90 ± 0.09 and 0.85 ± 0.11 ng/ml at 0 and 5 h, respectively). PAI-1 activity in plasma increased dramatically with hyperglycemia (7.9 ± 0.7 and 24.9 ± 1.5 units/ml at 0 and 5 h, respectively) and hyperinsulinemia (7.2 ± 0.5 and 35.6 ± 4.2 units/ml at 0 and 5 h, respectively) and was similar to saline control (7.2 ± 0.8 and 7.8 ± 0.6 units/ml at 0 and 5 h, respectively). IL-6 levels were doubled with hyperinsulinemia (38.3 ± 4.7 and 75.0 ± 10.5 pg/ml at 0 and 5 h, respectively) and tripled with glucosamine (43.2 ± 113.2 ± 14.9 pg/ml at 0 and 5 h, respectively), whereas levels were unchanged during saline infusion (37.3 ± 7.7 and 35.6 ± 6.2 pg/ml at 0 and 5 h, respectively). It is interesting that within this time course, plasma levels of adiponectin did not increase with hyperglycemia (0.58 ± 0.09 and 0.54 ± 0.12 μg/ml at 0 and 5 h, respectively), hyperinsulinemia (0.52 ± 0.11 and 0.54 ± 0.11 μg/ml at 0 and 5 h, respectively), or glucosamine (0.55 ± 0.08 and 0.55 ± 0.12 μg/ml at 0 and 5 h, respectively) and was similar to saline control (0.52 ± 0.09

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/54/3/672/380812/zdb00305000672.pdf by guest on 13 June 2024

and 0.53 ± 0.10 μg/ml at 0 and 5 h). Levels of angiotensinogen, resistin, IL-6, IL-10, and TNF-α were not measured.

DISCUSSION

We have demonstrated that acute increases in glucose uptake into adipose tissue by either hyperglycemia or hyperinsulinemia increased the expression of several FDPs in visceral fat to a greater degree than in subcutaneous fat. Some of these FDPs may be involved in modulating insulin action or are components of the metabolic syndrome. The basal ("static") expression of FDPs in visceral fat and subcutaneous fat were not predictive of the changes in FDP expression seen when exposed to delivery of a nutrient ("dynamic"). In addition, we have shown that increasing flux through the HBP increased the expression of FDPs, mimicking many of the effects exerted by hyperinsulinemia and hyperglycemia and providing evidence for its role as a "nutrient sensor." Finally, we have demonstrated significant increases in plasma levels of FDPs within the time course of the experiment.

Although the expression of many genes is commonly modulated by hyperglycemia, hyperinsulinemia, and glucosamine, other factors need to be considered to appreciate its physiologic significance. Although the absolute changes in leptin were more impressive than the fold increases, we present the remainder of the results as fold changes to emphasize the magnitude of change seen with nutrient delivery. Each gene has its own time course and magnitude of expression. In addition, each peptide may be translated, "packaged," and secreted by the components of adipose tissue, and each peptide has its own half-life in the circulation. Leptin exemplifies this concept. Leptin expression has been coupled to nutrient availability (21,32); it is secreted in a rapid time course and reaches maximal levels after midnight (15). We have measured significantly higher leptin and PAI-1 activity after 5 h. In contrast, although adiponectin expression increased significantly, plasma levels were not changed by any infusion. This may indicate that the time course has not been sufficient for significant increases to be seen, adiponectin clearance is increased in parallel, or the regulation of adiponectin secretion is uncoupled to its expression. Ours is not the first study to show a discrepancy between adiponectin expression and levels (33), suggesting complex regulation between adiponectin expression and secretion. Alternatively, although plasma levels of IL-6 were increased by insulin and glucose, IL-6 gene expression was not increased markedly. This may indicate that small changes in gene expression (as seen with glucosamine) can induce significant secretion of the protein, that nutrients can release stored IL-6, that regulation of IL-6 levels by nutrients is dependent on alterations in clearance, or that the source of IL-6 derives from another tissue. Because many FDPs do not have similar time courses, their plasma levels should be measured in humans in relationship to nutrient intake to establish the exact magnitude of this effect and the associated risk.

Perinephric fat was chosen to represent visceral fat because, whereas humans have a significant amount of omental fat and rodents have significant amount of gonadal fat, perinephric fat is common to both species. Although it has been suggested that visceral fat induces hepatic insulin resistance via a portal effect, there is significant evidence that other factors such as secretion of FDPs contribute independent of direct portal blood flow. In fact, removal of perinephric and epididymal fat pads in our rodent models, neither of which drain directly into the portal circulation, has been shown to improve hepatic and peripheral insulin resistance and modify FDP gene expression (7). This improvement in insulin sensitivity was seen despite the presence of mesenteric/omental fat. In addition, portal levels of free fatty acids did not differ between groups.

We have demonstrated that leptin, resistin, adiponectin, and angiotensinogen are derived mostly from adipocytes, whereas other FDPs, such as PAI-1 and TNF-α, originate in the vascular and/or stromal cell and macrophage compartments. Although macrophages express several cytokines, their number in adipose tissue is proportionally small compared with other cell types and may not indicate a major overall contribution. These different components of fat tissue may have specific regulation by factors other than those that modulate expression in adipocytes. For example, although the HBP exists in all of the cellular compartments of adipose tissue, their response to insulin may be limited. Moreover, some peptides, such as PAI-1 or angiotensinogen, are expressed in liver, which may be a major source contributing to their plasma levels. We previously suggested that angiotensinogen contribution from fat tissue in obesity exceeds the contribution from liver (23). Importantly, it is the expression contributed per adipose tissue mass that is the most physiologically relevant, independent of the actual cellular source.

Because glucosamine also activates specific genes that are activated by glucose or insulin, we suggest that HBP may be a major candidate for nutrient sensing for many FDPs. Similarly, increasing availability of free fatty acids may inhibit glycolytic enzymes and increase fructose-6-phosphate levels and the flux through the HBP, as we have demonstrated in muscle (34).

We have shown that the expression of FDPs after stimulation by nutrients markedly increased in visceral fat compared with subcutaneous fat. Several possible explanations for these differences exist. Because changes in gene expression during hyperglycemia and glucosamine studies were similar, we propose that the HBP may be at least partially responsible for this enhanced expression of FDPs in visceral fat. Nutrient-stimulated glucose uptake increased more in visceral fat than in subcutaneous fat and may activate HBP more significantly. It is also possible that the HBP is intrinsically more active in visceral fat possibly because increased free fatty acid flux in this tissue may shunt more fructose-6-phosphate through the HBP pathway. Differences in the number of vascular cells or macrophages relative to adipocytes may also contribute to the unique expression of visceral fat. We suggest that the "riskiest" visceral fat in humans may be determined by assessing FDP expression, potentially after nutrient stimulation. Furthermore, although the rodents studied are genetically homogeneous, individual sensitivity to nutrients may explain why some humans with abdominal obesity are at more risk than others.

In conclusion, we have shown that adipose tissue, in particular visceral fat, is not biologically static and is

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/54/3/672/380812/zdb00305000672.pdf by guest on 13 June 2024

probably regulated by nutrients. We focused on several FDPs that are relevant for the metabolic syndrome and associated their increased expression with a novel biologic sensor. Unlike previous in vivo and epidemiologic studies, which are standardized for fasting, our study demonstrates that the cumulative effects of nutrient-coupled increases in gene expression may be significantly modulated throughout the day.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (P0-1 AG021654 and RO1-AG 18381 to N.B) and the American Diabetes Association and by the Core Laboratories of the Albert Einstein Diabetes Research and Training Center (DK 20541).

We thank Dr. H. Shamoon, Dr. P. Scherer, and Dr. M. Hawkins for reviewing this manuscript and Rebecca Fabbro and Adam Matos for assistance.

REFERENCES

1. Kissebah AH: Insulin resistance in visceral obesity. *Int J Obes* 15 (Suppl. 2):109–115, 1991
2. Rexrode KM, Carey VJ, Hennekens CH, Walters EE, Colditz GA, Stampfer MJ, Willett WC, Manson JE: Abdominal adiposity and coronary heart disease in women. *JAMA* 280:1843–1848, 1998
3. Bjorntorp P: Metabolic implications of body fat distribution. *Diabetes Care* 14:1132–1143, 1991
4. Gastaldelli A, Miyazaki Y, Pettiti M, Matsuda M, Mahankali S, Santini E, DeFronzo RA, Ferrannini E: Metabolic effects of visceral fat accumulation in type 2 diabetes. *J Clin Endocrinol Metab* 87:5098–5103, 2002
5. Coon PJ, Rogus EM, Drinkwater D, Muller DC, Goldberg AP: Role of body fat distribution in the decline in insulin sensitivity and glucose tolerance with age. *J Clin Endocrinol Metab* 75:1125–1132, 1992
6. Carey DG, Jenkins AB, Campbell LV, Freund J, Chisholm DJ: Abdominal fat and insulin resistance in normal and overweight women: direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes* 45:633–638, 1996
7. Kelley DE: Thermodynamics, liposuction, and metabolism. *N Engl J Med* 350:2542–2544, 2004
8. Klein S, Fontana L, Young VL, Coggan AR, Kilo C, Patterson BW, Mohammed BS: Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. *N Engl J Med* 350:2549–2557, 2004
9. Gabriely I, Ma XH, Yang XM, Atzmon G, Rajala MW, Berg AH, Scherer P, Rossetti L, Barzilai N: Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? *Diabetes* 51:2951–2958, 2002
10. Barzilai N, She L, Liu BQ, Vuguin P, Cohen P, Wang J, Rossetti L: Surgical removal of visceral fat reverses hepatic insulin resistance. *Diabetes* 48:94–98, 1999
11. Montague CT, Prins JB, Sanders L, Zhang J, Sewter CP, Digby J, Byrne CD, O'Rahilly S: Depot-related gene expression in human subcutaneous and omental adipocytes. *Diabetes* 47:1384–1391, 1998
12. Lefebvre AM, Laville M, Vega N, Riou JP, van Gaal L, Auwerx J, Vidal H: Depot-specific differences in adipose tissue gene expression in lean and obese subjects. *Diabetes* 47:98–103, 1998
13. Atzmon G, Yang XM, Muzumdar R, Ma XH, Gabriely I, Barzilai N: Differential gene expression between visceral and subcutaneous fat depots. *Horm Metab Res* 34:622–628, 2002
14. Sinha MK, Ohannesian JP, Heiman ML, Kriauciunas A, Stephens TW, Magosin S, Marco C, Caro JF: Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. *J Clin Invest* 97:1344–1347, 1996
15. Schoeller DA, Cella LK, Sinha MK, Caro JF: Entrainment of the diurnal rhythm of plasma leptin to meal timing. *J Clin Invest* 100:1882–1887, 1997
16. Rattarasarn C, Leelawattana R, Soonthornpun S, Setasuban W, Thamprasit A, Lim A, Chayanunnukul W, Thamkumpee N: Relationships of body fat distribution, insulin sensitivity and cardiovascular risk factors in lean, healthy non-diabetic Thai men and women. *Diabetes Res Clin Pract* 60:87–94, 2003
17. Rossetti L: Perspective: hexosamines and nutrient sensing. *Endocrinology* 141:1922–1925, 2000
18. Goldberg HJ, Scholey J, Fantus IG: Glucosamine activates the plasminogen activator inhibitor 1 gene promoter through Sp1 DNA binding sites in glomerular mesangial cells. *Diabetes* 49:863–871, 2000
19. Massillon D, Barzilai N, Hawkins M, Prus-Wertheimer D, Rossetti L: Induction of hepatic glucose-6-phosphatase gene expression by lipid infusion. *Diabetes* 46:153–157, 1997
20. Goldberg HJ, Whiteside CI, Fantus IG: The hexosamine pathway regulates the plasminogen activator inhibitor-1 gene promoter and Sp1 transcriptional activation through protein kinase C-beta I and -delta. *J Biol Chem* 277:33833–33841, 2002
21. Wang J, Liu R, Liu L, Chowdhury R, Barzilai N, Tan J, Rossetti L: The effect of leptin on Lep expression is tissue-specific and nutritionally regulated. *Nat Med* 5:895–899, 1999
22. Gabriely I, Yang XM, Cases JA, Ma XH, Rossetti L, Barzilai N: Hyperglycemia induces PAI-1 gene expression in adipose tissue by activation of the hexosamine biosynthetic pathway. *Atherosclerosis* 160:115–122, 2002
23. Gabriely I, Yang XM, Cases JA, Ma XH, Rossetti L, Barzilai N: Hyperglycemia modulates angiotensinogen gene expression. *Am J Physiol Regul Integr Comp Physiol* 281:R795–R802, 2001
24. Barzilai N, Hawkins M, Angelov I, Hu M, Rossetti L: Glucosamine-induced inhibition of liver glucokinase impairs the ability of hyperglycemia to suppress endogenous glucose production. *Diabetes* 45:1329–1335, 1996
25. Hawkins M, Barzilai N, Liu R, Hu M, Chen W, Rossetti L: Role of the glucosamine pathway in fat-induced insulin resistance. *J Clin Invest* 99:2173–2182, 1997
26. Gupta G, She L, Ma XH, Yang XM, Hu M, Cases JA, Vuguin P, Rossetti L, Barzilai N: Aging does not contribute to the decline in insulin action on storage of muscle glycogen in rats. *Am J Physiol Regul Integr Comp Physiol* 278:R111–R117, 2000
27. Gupta G, Cases JA, She L, Ma XH, Yang XM, Hu M, Wu J, Rossetti L, Barzilai N: Ability of insulin to modulate hepatic glucose production in aging rats is impaired by fat accumulation. *Am J Physiol Endocrinol Metab* 278:E985–E991, 2000
28. Colberg SR, Simoneau JA, Thaete FL, Kelley DE: Skeletal muscle utilization of free fatty acids in women with visceral obesity. *J Clin Invest* 95:1846–1853, 1995
29. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112:1821–1830, 2003
30. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr: Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796–1808, 2003
31. Rossetti L, Hawkins M, Chen W, Gindi J, Barzilai N: In vivo glucosamine infusion induces insulin resistance in normoglycemic but not in hyperglycemic conscious rats. *J Clin Invest* 96:132–140, 1995
32. Vulin AI, Stanley FM: A forkhead/winged helix-related transcription factor mediates insulin-increased plasminogen activator inhibitor-1 gene transcription. *J Biol Chem* 277:20169–20176, 2002
33. Hazel M, Cooksey RC, Jones D, Parker G, Neidigh JL, Witherbee B, Gulve EA, McClain DA: Activation of the hexosamine signaling pathway in adipose tissue results in decreased serum adiponectin and skeletal muscle insulin resistance. *Endocrinology* 145:2118–2128, 2004
34. Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* 93:2438–2446, 1994

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/54/3/672/380812/zdb00305000672.pdf by guest on 13 June 2024