Role of Hexosamines in Insulin Resistance and Nutrient Sensing in Human Adipose and Muscle Tissue

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It has been proposed that the hexosamine pathway acts as a nutrient-sensing pathway, protecting the cell against abundant fuel supply, and that accumulation of hexosamines represents a biochemical mechanism by which hyperglycemia and hyperlipidemia induce insulin resistance. We hypothesized that if an increased flux through the hexosamine pathway caused insulin resistance in humans, the hexosamine levels should be increased in adipose and/or muscle tissue in insulin-resistant subjects, such as patients with type 2 diabetes and obese individuals. In addition, we reasoned that if the hexosamine pathway were a nutrient-sensing pathway, hexosamine levels in adipose and skeletal muscle tissue should be correlated with levels of circulating nutrients, such as glucose and free fatty acids (FFAs) and leptin concentrations.

In a human cross-sectional study of 55 patients [20 with type 2 diabetes mellitus (DM) and 21 normal-lean (NL) and 14 normal-obese (NO) subjects] who underwent hip replacement surgery, adipose and muscle tissue biopsies were obtained and analyzed for levels of hexosamines [UDP-N-acetylglucosamine (UDP-GlcNAc) and UDP-N-acetylgalactosamine] and hexoses (UDP-glucose and UDP-galactose). Fasting plasma glucose, glycosylated hemoglobin, serum insulin and homeostasis model assessment calculations, serum lipids, and leptin were measured on the same day.

Hexosamines were not elevated in adipose and muscle tissue of patients with type 2 DM compared with NL and NO subjects (UDP-GlcNac DM vs. NL vs. NO, 3.3 ± 2.3 vs. 2.2 ± 2.1 vs. 3.0 ± 2.0 nmol/g tissue in adipose tissue and 8.1 ± 2.9 vs. 7.8 ± 2.8 vs. 7.6 ± 2.8 nmol/g tissue in muscle tissue, respectively). Hexosamines in adipose tissue were positively correlated with circulating levels of FFA (UDP-GlcNAc, r = 0.33, P < 0.05; UDP-N-acetylgalactosamine, r = 0.41, P < 0.01). Adipose tissue UDP-GlcNAc was correlated with leptin levels (r = 0.38; P < 0.05). No such relationship was identified in muscle tissue.

In conclusion, these findings argue against a pathophysiological role of the hexosamine pathway in insulin resistance in humans but support the hypothesis that the hexosamine pathway in adipose tissue, not in muscle, is a FFA-sensing pathway and could be involved in the regulation of leptin expression. (J Clin Endocrinol Metab 89: 5132–5137, 2004)

THE PATHOGENESIS OF insulin resistance, a central feature of type 2 diabetes mellitus (DM), is complex and incompletely understood, but environmental factors including excess nutrients and obesity play a major role. Once diabetes exists, both chronic hyperglycemia and hyperlipidemia further aggravate the already impaired action and secretion of insulin. These adverse metabolic consequences of chronic hyperglycemia and hyperlipidemia have been conceptualized as glucose toxicity (1), and lipotoxicity (2).

It has been suggested that accumulation of products of the hexosamine biosynthetic pathway represents a biochemical mechanism by which hyperglycemia and hyperlipidemia induce insulin resistance (3, 4). Glutamine fructose-6-phosphate amidotransferase (GFAT) is the key enzyme of this pathway, using the amide group of glutamine to convert fructose 6-phosphate amidotransferase (GFAT) is the key enzyme of this pathway, using the amide group of glutamine to convert fructose 6-phosphate to glucosamine-6-phosphate (5, 6). Glucosamine-6-phosphate is then converted to UDP-hexosamines, i.e. UDP-N-acetylglucosamine (UDP-GlcNAc) and UDP-N-acetylgalactosamine (UDP-GalNAc).

Indeed, animal studies show that hyperglycemia in rats with streptozotocin-induced diabetes (7), hyperglycemia induced by glucose or somatostatin infusion in normal rats (7, 8), prolonged elevations in serum free fatty acids (FFAs) induced by lipid infusion (8), and high-fat feeding in normal rats (9) all result in increased muscle UDP-hexosamines (as a result of an increased flux of fructose-6-phosphate into the hexosamine pathway), as well as in insulin resistance, as demonstrated by euglycemic-hyperinsulinemic clamp studies (8, 9). Furthermore, in normal rats, it has been found that hyperglycemia or hyperlipidemia not only cause increased muscle UDP-GlcNAc levels and insulin resistance (8) but also result in increased leptin mRNA levels in fat and skeletal muscle and in increased serum leptin concentrations (10). Based on these observations, the hexosamine pathway has been proposed as a nutrient-sensing pathway (10).

Human studies focused on the hexosamine pathway are scarce. We hypothesized that if an increased flux through the hexosamine pathway caused insulin resistance in humans, the levels of the stable metabolites of the hexosamine pathway, UDP-GlcNAc and UDP-GalNAc, should be increased in adipose and/or muscle tissue in disease states characterized by insulin resistance; for example, in patients with type 2 DM or in obese subjects. To test this hypothesis, we mea-
sured UDP-GlcNAc and UDP-GalNAc levels in adipose and skeletal muscle tissue of patients with type 2 DM and of lean and obese subjects. In addition, we reasoned that if the hexosamine pathway acts as a nutrient-sensing pathway, levels of UDP-GlcNAc and UDP-GalNAc in adipose and skeletal muscle tissue should be correlated with levels of circulating nutrients, like glucose and FFA, and with serum leptin concentrations.

**Subjects and Methods**

**Subjects**

Fifty-five Caucasian patients (female to male ratio of 38:17) who were referred for elective hip replacement surgery participated in the study. The study was performed in two hospitals (University Medical Center Nijmegen and Sint Maartenskliniek Nijmegen, The Netherlands). Inclusion criteria were an age greater than 30 yr and the ability to give informed consent. Exclusion criteria were evidence for coagulation disorders and clinical evidence for the presence of other metabolic or endocrine disorders. All patients provided written informed consent before participating in the study. The Hospital Ethics Committee approved the experimental protocol.

All patients referred for surgical hip replacement therapy had osteoarthritis. A total of 44 patients had primary osteoarthritis (degenerative joint disease), one of whom had osteoarthritis in combination with polymyalgia rheumatica and one in combination with polymyalgia rheumatica and osteoporosis. Eleven patients had secondary osteoarthritis; in four patients it had been caused by rheumatoid arthritis, in four patients by congenital dislocation of the hip, in two patients by avascular necrosis [one after fracture of the femoral neck and one possibly associated with low-dose prednisone (5 mg) therapy for rheumatoid arthritis], and in one patient by Legg-Calvé-Perthes disease.

In the whole group, four patients used prednisone, 5 mg once daily, because of polymyalgia rheumatica, obstructive lung disease, or rheumatoid arthritis.

After inclusion in the study, 20 patients were classified as diabetic; 12 of these had a medical history of diabetes, and eight had a fasting plasma glucose concentration greater than 126 mg/dl (≥7.0 mmol/liter) (11), but no previous history of diabetes. The other 35 patients were classified as nondiabetic. To adjust for differences in body weight between the diabetic and the nondiabetic patient group, the nondiabetic group was further divided into lean [body mass index (BMI) ≤ 27 kg/m², normal-lean (NL) group, n = 21] and an obese (BMI > 27 kg/m², normal-obese (NO) group, n = 14) groups.

**Protocol**

At the day of hip surgery, fasting blood samples were obtained for measurements of plasma glucose and insulin concentrations, percentage glycosylated hemoglobin (HbA1c), serum lipid analyses (total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triglycerides, and FFAs), and serum leptin levels. Patients underwent regional or general anesthesia; during surgery, they all received iv fluids consisting of 5% glucose and 0.9% saline (500–1000 ml) followed by Ringer’s solution.

After incision, adipose and muscle biopsies were taken from the sc fat at the hip region and from the gluteus maximus muscle, respectively. After biopsy, all samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis.

**Routine laboratory analyses**

Plasma glucose was measured using the glucose oxidation method (GOD-PAP, Hitachi 747; Roche Molecular Biochemicals, Indianapolis, IN). Plasma insulin was determined using an in-house RIA [interassay coefficient of variation (CV), 6%]. Hemoglobin A1c (reference range, 4.8–6.2%) was determined with an HPLC technique (Bio-Rad, Hercules, CA). Triglycerides (Triglycerides GPO-PAP method), total cholesterol (Cholesterol CHOD-PAP method), and high-density lipoprotein-cholesterol (after addition of cholesterol precipitation reagents) were all determined on a Hitachi 747 auto-analyzer, and low-density lipoprotein-cholesterol was calculated. The concentrations of FFA were analyzed using an enzymatic method (acyl-CoA-synthetase-acyl-CoA-oxidase, FFA C-kit, Waco Chemicals, Neuss, Germany). Serum leptin was determined with a commercial human leptin RIA kit (Linco Research Inc., St. Louis, MO).

**Measurement of UDP-hexosamines and UDP-hexoses**

UDP-hexosamines (UDP-GlcNAc and UDP-GalNAc) and UDP-hexoses (UDP-glucose and UDP-galactose) were quantitated using an HPLC-based assay, suitable for application in small samples of human adipose and muscle tissue, as earlier described (12). For UDP-GlcNAc, intra- and interassay CVs were less than 6%. For UDP-GalNAc, the intraassay CV was 5.4% and the interassay CV was less than 13%. For UDP-glucose and UDP-galactose, the intraassay CV were less than 6.5% and the interassay CV was less than 7%. The limit of detection for all metabolites (UDP-GlcNAc, UDP-GalNAc, UDP-glucose, and UDP-galactose) in muscle and in adipose tissue was 0.6 nmol/g tissue (12).

**Calculations**

Insulin resistance was calculated using homeostasis model assessment (HOMA2) [fasting insulin in milliunits per liter × fasting glucose in millimoles/liter]/22.5] (13).

**Data presentation and statistical analysis**

To test differences between two groups, the unpaired Student’s t test was used. Differences between more than two study groups were tested using one-way ANOVA in case of normal distributions and the Kruskall-Wallis test in case of nonnormal distributions. If statistically significant differences were identified, least significant difference post hoc analysis was performed in case of normal distributions. Correlations were calculated by Spearman’s ranks method. For all statistical analyses, the SPSS software package 10.0 (SPSS, Inc., Chicago, IL) was used. P < 0.05 was considered statistically significant. Results are given as mean ± sd.

**Results**

Baseline characteristics of the three groups [DM (n = 20), NL (n = 21), and NO (n = 14)] are described in Table 1. In DM, the 12 patients with a previous history of diabetes were known to have the disease for 12 yr (range, 1–35 yr). They were treated with diet (n = 3), sulfonylureas (n = 3), metformin (n = 1), the combination sulfonylurea and metformin (n = 1), metformin and insulin (n = 1), or with insulin alone (n = 3). Of the eight patients with an abnormal fasting glucose level but no history of diabetes, one used prednisone for obstructive lung disease and one had a medical history of gestational diabetes. Of those classified as nondiabetic, four subjects had a fasting glucose level greater than 110 but less than 126 mg/dl (>6.1 but <7.0 mmol/liter); none of these subjects had a history of diabetes or had symptoms of hyperglycemia, and all had normal HbA1c levels.

Groups differed significantly with respect to plasma glucose and insulin levels, HbA1c, HOMA, BMI, and serum leptin levels (ANOVA, all P < 0.05). Post hoc analyses revealed that the diabetic group had significantly higher plasma glucose and insulin concentrations, higher HOMA values, and higher HbA1c percentages, compared with the NL and NO group (Table 1). The DM group had significantly higher BMI (29 ± 5.2 vs. 25 ± 3.7 kg/m², P < 0.005) and leptin levels (26 ± 22 vs. 11 ± 7 ng/ml, P < 0.05) compared with the NL group; BMI and leptin levels were similar between the DM and NO group (Table 1). Age and lipid levels were not significantly different among the three groups.
Comparison of hexosamines and hexoses in adipose and muscle tissues

In Table 2 hexosamine (UDP-GlcNAc and UDP-GalNAc) and hexose (UDP-glucose and UDP-galactose) levels in both adipose and skeletal muscle tissue are presented. No significant differences in hexosamine levels in either adipose or muscle tissue were observed among the three different study groups. Also, when the DM group was compared with the whole nondiabetic group (NL and NO subjects), UDP-hexosamines were similar [DM group vs. nondiabetic groups: adipose tissue, UDP-GlcNAc, 3.3 ± 2.3 vs. 2.6 ± 2.1; UDP-GalNAc, 0.74 ± 0.71 vs. 0.75 ± 0.70 nmol/g tissue; muscle tissue, UDP-GlcNAc, 8.1 ± 2.9 vs. 7.7 ± 2.8, UDP-GalNAc, 4.7 ± 1.4 vs. 4.6 ± 1.1 nmol/g tissue, P = not significant (NS) for all comparisons]. Finally, when the DM and obese groups were combined and compared with NL subjects, again, similar results were obtained (DM + obese vs. NL, adipose tissue, UDP-GlcNAc, 3.2 ± 2.1 vs. 2.2 ± 2.1; UDP-GalNAc, 0.86 ± 0.74 vs. 0.55 ± 0.53 nmol/g tissue; muscle tissue, UDP-GlcNAc, 7.9 ± 2.8 vs. 7.8 ± 2.8; UDP-GalNAc, 4.7 ± 1.3 vs. 4.6 ± 1.1 nmol/g tissue, P = NS for all comparisons).

No statistically significant differences in hexosine levels in either adipose or muscle tissue were observed among the three different study groups (ANOVA). Again, when the diabetic patient group was compared with the whole nondiabetic group (NL and NO subjects), UDP-glucose levels in muscle were comparable (DM group, 16 ± 11 vs. nondiabetic groups, 20 ± 10 nmol/g tissue, P = NS).

No significant correlations were found between fasting plasma insulin concentrations or HOMA\textsubscript{R} on the one hand and UDP-GlcNAc and UDP-GalNAc concentrations in adipose and muscle tissues on the other hand.

Correlations between UDP-GlcNAc and UDP-GalNAc in adipose and muscle tissue and fasting glucose, FFA, and leptin concentration

No correlations were found between fasting plasma glucose levels and UDP-GlcNAc and UDP-GalNAc concentrations in adipose and muscle tissues, neither in the total group of patients (n = 55) nor in the nondiabetic group (n = 35). There was a positive correlation between adipose tissue UDP-GlcNAc concentration and plasma FFA level (r = 0.33, P < 0.05; n = 35). This correlation was even stronger between adipose tissue UDP-GalNAc concentrations and plasma FFA levels (r = 0.41, P < 0.01; n = 55). If diabetic patients were excluded from this analysis, similar results for the correlation between adipose tissue UDP-GlcNAc concentration and plasma FFA level were obtained (r = 0.44, P < 0.05; n = 35), whereas there was a trend between adipose tissue UDP-GalNAc concentrations and plasma FFA levels (r = 0.37; P < 0.06; n = 35) (Fig. 1). No correlation was found between muscle tissue UDP-GlcNAc concentration and plasma FFA level (r = 0.06; P = NS; n = 55), nor between muscle tissue UDP-GalNAc concentration and plasma FFA levels (r = −0.01; P = NS; n = 55). Plasma leptin levels were positively correlated with adipose tissue UDP-GlcNAc concentrations (r = 0.33; P < 0.05; n = 55), but not with muscle tissue UDP-GalNAc concentrations (r = 0.04; P = NS; n = 55). When diabetic and nondiabetic patients were analyzed separately, the correlation no longer reached statistical significance (diabetic patients, r = −0.05, P = NS, n = 20; nondiabetic subjects, r = 0.29, P = NS, n = 35). Plasma leptin levels did not correlate with adipose or muscle tissue UDP-GalNAc concentrations (r = 0.20 and −0.09, respectively; P = NS; n = 55).

Discussion

Two main conclusions can be drawn from this cross-sectional study. First, levels of stable hexosamine pathway end-products are not increased in adipose and muscle tissue of patients that are characterized by insulin resistance, and there was no correlation between hexosamine levels and parameters associated with insulin resistance. Second, hexosamine levels in adipose tissue, but not in muscle, are cor-
related with circulating FFA and leptin levels. All together, these findings argue against involvement of the hexosamine pathway in insulin resistance in humans but support the concept that the hexosamine pathway in adipose tissue, not in muscle, can act as a FFA-sensing pathway and could be involved in the regulation of leptin expression.

The first conclusion is based on the finding that the tissue concentrations of UDP-GlcNAc and UDP-GalNAc, which represent the stable end products of the hexosamine pathway, were similar in our three different study groups. The diabetic and the nondiabetic obese groups are by definition characterized by insulin resistance, which is also reflected by higher HOMA\textsubscript{R} values. Also, if the diabetic group was compared with the whole nondiabetic group, similar results were found. In addition, no correlation at all was found between parameters of insulin resistance (fasting insulin and HOMA\textsubscript{R} values) on the one hand and tissue hexosamine levels on the other.

Although induction of insulin resistance by activation of the hexosamine pathway is fairly well documented in animals (7–9, 14, 15), studies in humans are limited and results are conflicting with animal studies (16–20). For example, short-term glucosamine infusion, a reproducible method to increase the flux through the hexosamine pathway, resulting in increased hexosamine levels in skeletal muscle tissue in animals (9, 21, 22), did not affect insulin sensitivity in humans (18, 19). In patients with type 2 DM, GFAT activity in skeletal muscle was found to be elevated (17), but inverse correlations were reported between GFAT activity and glucose disposal rate (16, 17), which is in contrast with the reported positive correlation between GFAT activity in adipose (14) and muscle tissue (14, 15) and glucose disposal rate in transgenic mice. We have reported that in type 2 diabetic patients, hexosamine levels did not decrease, but even increased in skeletal muscle, after amelioration of hyperglycemia-induced insulin resistance (20). Including the results from the present study, there is a fair amount of human data now that oppose involvement of (over)activation of the hexosamine pathway in insulin resistance in humans.

**Fig. 1.** Correlations between UDP-GlcNAc (top) and UDP-GalNAc (bottom) in adipose tissue on the one hand and serum FFA concentrations on the other hand in the whole patient group (n = 55). ○, Diabetic patients; ●, nondiabetic subjects.
biochemical pathway in the pathogenesis of insulin resistance at the level of the skeletal muscle.

Our second conclusion is based on the finding that adipose (but not muscle) tissue concentrations of UDP-GlcNAc show a positive correlation with circulating plasma FFA and serum leptin concentrations. Also, there was a positive correlation between adipose UDP-GalNAc concentrations and circulating plasma FFA. These findings are to some extent in agreement with findings in animals, in which an increased fatty acid metabolism results in increased muscle UDP-GlcNAc levels and insulin resistance (8, 9) and increased hexosamine levels in adipose tissue in GFAT transgenic mice result in increased serum leptin levels and induce leptin gene expression in adipose and muscle tissue (9, 23), which would be consistent with a role for the hexosamine pathway as a general nutrient-sensing pathway (9). Our finding that hexosamine levels in human adipose tissue are correlated with serum leptin levels also fits with the previously reported observation that leptin production is regulated by hexosamines in human adipose tissue (24). However, we have not proven per se that hexosamines induce leptin expression because we do not have data on tissue leptin mRNA or protein levels from the same samples.

In the present study, we found a positive correlation between hexosamines in human adipose tissue and serum FFAs as well as serum leptin, but, in contrast with animal studies (10), such a relationship did not exist in muscle tissue (to date, human studies are not reported). As such, our study provides evidence for the hypothesis that in humans the hexosamine pathway may serve as a FFA-sensing pathway in adipocytes and could be involved in the regulation of leptin expression.

Our study has a number of limitations. Inherent to human in vivo studies, this cross-sectional study provides only associative data and does not prove a definite cause and effect relationship. Nevertheless, the convincing absence of any relationship between tissue hexosamine metabolites and measures of insulin resistance, in combination with similar previous findings in humans, renders a role for the hexosamine pathway within the concept of insulin resistance highly unlikely. Within the study population, eight subjects had a fasting glucose concentration of greater than 126 mg/dl (>7.0 mmol/liter) but had no prior history of diabetes and normal HbA1c levels. These patients were classified as having diabetes, but one may question whether a fasting glucose level just before major surgery is really diagnostic for diabetes. If data were reanalyzed after exclusion of these patients, however, the results remained the same (data not shown).

The indications for total hip replacement surgery, comorbidity, and medication used by our subjects might also have influenced the results. The indication for total hip surgery was osteoarthritis, the majority being primary degenerative joint disease. In 11 patients, osteoarthritis was secondary to other diseases, like congenital dislocation, avascular necrosis, or rheumatoid arthritis. Concerning comorbidity, diabetic patients have more comorbidity, especially cardiovascular morbidity and hypertension, and comedication compared with nondiabetic patients, but this is inherent in this population.

Our study approach, i.e. collection of tissue during surgery, has the advantage that a relatively large tissue sample can be obtained. However, it also has some limitations, including the fact that samples were obtained under anesthesia. In addition, the fasting blood samples were drawn at least 30 min before the muscle and adipose tissue samples were taken and before induction of anesthesia was started. It seems unlikely that this has influenced the results, and other studies with a similar study design have been reported (24). Finally, sc fat in the hip region was used to determine hexosamine metabolite levels, which may differ from the levels of hexosamines in visceral fat. Nevertheless, metabolic end-products of the hexosamine pathway have been studied comprehensively in animals, but in humans, the metabolic products of this pathway are mainly investigated in skeletal muscle tissue (16, 17, 20), whereas studies in adipose tissue (24) are scarce. The present study is the first that investigates the relation between nutrients (glucose and FFAs) and hexosamine concentrations in adipose tissue in vivo and that quantifies the products of the hexosamine pathway in both adipose and skeletal muscle tissue simultaneously of the same patients.

In summary, the levels of hexosamines in fat and muscle tissue of human subjects that are characterized by insulin resistance are similar to the levels found in insulin-sensitive subjects. These findings strongly oppose to a pathophysiological role of the hexosamine pathway in insulin resistance in humans. Our finding of a relationship between adipose tissue levels of hexosamines and plasma FFA and serum leptin levels is in agreement with a role of the hexosamine pathway as a FFA-sensing pathway, which could be involved in the regulation of leptin expression. Such a relationship was not identified in muscle tissue. More research is needed to determine in more detail which role the hexosamine pathway plays in intermediate metabolism in humans.

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