In Vitro Reversal of Hyperglycemia Normalizes Insulin Action in Fat Cells From Type 2 Diabetes Patients: Is Cellular Insulin Resistance Caused by Glucotoxicity In Vivo?

Jonas Burén, Stina Lindmark, Frida Renström, and Jan W. Eriksson

Chronic hyperglycemia promotes the development of insulin resistance. The aim of this study was to investigate whether cellular insulin resistance is secondary to the diabetic state in human type 2 diabetes. Subcutaneous fat biopsies were taken from 3 age-, sex-, and body mass index (BMI)-matched groups with 10 subjects in each group: type 2 diabetes patients with either good (hemoglobin A_{1c} [HbA_{1c}] < 7%, G) or poor (HbA_{1c} > 7.5%, P) metabolic control and healthy control subjects (C). Insulin action in vitro was studied by measurements of glucose uptake both directly after cell isolation and following a 24-hour incubation at a physiological glucose level (6 mmol/L). The relationship with insulin action in vivo was addressed by employing the euglycemic clamp technique. Freshly isolated fat cells from type 2 diabetes patients with poor metabolic control had ~55% lower maximal insulin response (1,000 μU/mL) on glucose uptake ($P < .05$) compared to C. Cells from P were more insulin-resistant ($P < .05$) than cells from G at a low (5 μU/mL) but not at a high (1,000 μU/mL) insulin concentration, suggesting insulin insensitivity. However, following 24 hours of incubation at physiological glucose levels, insulin resistance was completely reversed in the diabetes cells and no differences in insulin-stimulated glucose uptake were found among the 3 groups. Insulin sensitivity in vivo assessed with hyperinsulinemic, euglycemic clamp (M-value) was significantly associated with insulin action on glucose uptake in fresh adipocytes in vitro ($r = 0.50$, $P < .01$). Fasting blood glucose at the time of biopsy and HbA_{1c}, but not serum insulin, were negatively correlated to insulin's effect to stimulate glucose uptake in vitro ($r = -0.36$, $P = .064$ and $r = -0.41$, $P < .05$, respectively) in all groups taken together. In the in vivo situation, fasting blood glucose, HbA_{1c}, and serum insulin were all negatively correlated to insulin sensitivity (M-value; $r = -0.62$, $P < .001$, $r = -0.61$, $P < .001$, and $r = -0.56$, $p < .01$, respectively). Cell size, waist-to-hip ration (WHR), and BMI correlated negatively with insulin's effect to stimulate glucose uptake both in vitro ($r = -0.55$, $P < .01$, $r = -0.54$, $P < .01$, and $r = -0.43$, $P < .05$, respectively) and in vivo ($r = -0.43$, $P < .05$, $r = -0.50$, $P < .01$, and $r = -0.36$, $P < .05$, respectively). Multiple regression analyses revealed that adipocyte cell size and WHR independently predicted insulin resistance in vitro. Furthermore, insulin sensitivity in vivo could be predicted by fasting blood glucose and serum insulin levels. We conclude that insulin resistance in fat cells from type 2 diabetes patients is fully reversible following incubation at physiological glucose concentrations. Thus, cellular insulin resistance may be mainly secondary to the hyperglycemic state in vivo.

DIABETES is a metabolic disorder defined as an altered glucose metabolism leading to chronic hyperglycemia. Type 2 diabetes is characterized by insulin resistance in muscle, fat, and liver, along with a decreased ability of the β cells of the pancreas to respond properly to elevated glucose levels. In vivo studies performed in type 1 diabetes patients[1] and rats[2] demonstrated that chronic hyperglycemia is an independent factor in the development of insulin resistance. This phenomenon is commonly referred to as glucose toxicity.[3] In a study by Rossetti et al, correction of hyperglycemia by phlorizin normalized insulin sensitivity in diabetic rats without altering insulin secretion.[2] The concept of glucose toxicity is well documented, but the underlying mechanisms are not well understood. Marshall et al[4-6] performed rat studies that led them to suggest that an increase in glucose flux through the hexosamine biosynthesis pathway contributed to glucose-induced insulin desensitization. Glutamine fructose-6-phosphate amidotransferase (GFAT) is the key enzyme that regulates the flux through the hexosamine pathway, where the first step is the conversion of glutamine and fructose-6-phosphate to glucoseamine-6-phosphate. Products of the hexosamine pathway are believed to accumulate and impair insulin-stimulated glucose transporter 4 (GLUT4) translocation[7,8] and glycogen synthase activation,[9-11] perhaps secondary to impaired insulin signaling[12-14] and protein kinase C (PKC) activation.[15] In this way, the cells could potentially be protected from too high and possibly harmful intracellular levels of glucose and its metabolites.

The "glucotoxic effect" seems to be reversible at least in muscle since insulin-resistant muscle strips from type 2 diabetes patients incubated in a "euglycemic" milieu recover their insulin sensitivity.[16] Consequently, an important way to reduce insulin resistance in type 2 diabetes is to treat hyperglycemia by any means that can normalize glucose levels. However, the blood glucose level is probably not the only factor of importance to cause insulin resistance since other humoral as well as neural factors may be of importance, and primary cellular mechanisms could also be involved.[17,18]

The aim of this study was to investigate whether cellular insulin resistance is related to the glycemic level in vivo and whether it is reversible. Therefore, fat cells from type 2 diabetes patients with varying degree of metabolic control (good and poor metabolic control) and healthy control subjects were investigated. Glucose uptake capacity was determined both in freshly obtained fat cells and also following in vitro incubation

From the Department of Medicine, Umeå University Hospital, Umeå, Sweden.

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Address reprint requests to Jonas Burén, MSc, Department of Medicine, UKBF 3B, Umeå University Hospital, S-901 85 Umeå, Sweden.

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Table 1. Clinical Characteristics of the Study Participants

	Poor (P)	Good (G)	Control (C)
Sex (male/female)	6/4	6/4	6/4
Age (yr)	58.6 ± 2.4	58.4 ± 2.2	58.5 ± 2.5
BMI (kg/m^2)	27.3 ± 0.9	27.5 ± 1.0	27.3 ± 1.1
Body fat (%)	23.8 ± 1.7	25.5 ± 2.2	23.1 ± 2.1
LBM (%)	76.2 ± 1.7	74.5 ± 2.2	76.9 ± 2.1
Duration of diabetes (yr)	5.4 ± 1.4	5.0 ± 1.0	
Fasting blood glucose (mmol/L)	13.1 ± 0.8*	7.8 ± 0.7†	5.1 ± 0.1
HbA_{1c}(%)	8.9 ± 0.5*	5.5 ± 0.2*	4.5 ± 0.1*
Fasting serum insulin (mU/L)	8.5 ± 0.8	8.5 ± 1.2	12.5 ± 2.5
Fasting plasma free fatty acid (μmol/L)	723 ± 76	527 ± 88	556 ± 65
Plasma free fatty acid during clamp (μmol/L)	62 ± 7	41 ± 9	46 ± 7
M-value (mg/kg LBM/min)	5.4 ± 0.9‡	7.5 ± 1.0	9.0 ± 0.9

NOTE. Data are number of subjects or means ± SEM.
*$P < .001$ *v* all other groups.
†$P < .01$ *v* C.
‡$P < .05$ *v* C.

at a physiological glucose level for 24 hours. We also assessed if there were any differences between the groups in basal and stimulated lipolysis, as well as in the antilipolytic effect of insulin. Associations between insulin action in vivo and in adipocytes in vitro were also determined.

MATERIALS AND METHODS

Subjects

The 3 study groups consisted of 10 individuals matched for age, body mass index (BMI), and sex; their clinical and biochemical characteristics are listed in Table 1. Two groups consisted of type 2 diabetes patients, according to the 1998 World Health Organization (WHO) criteria,[19] with either good (hemoglobin A_{1c} [HbA_{1c}] < 7%, reference value < 5.3%, G) or poor (HbA_{1c} > 7.5%, P) metabolic control, and 1 group consisted of healthy control subjects (C). Eight subjects in P and 5 subjects in G were on oral antidiabetic medication and the other diabetes patients had no medication. The Umeå University Ethics Committee approved the study protocol. All subjects gave their informed consent.

Protocol

Following an overnight fast, a subcutaneous needle biopsy from the lower part of the abdomen was taken at the clinic at 8 AM after dermal local anesthesia with lidocain (Xylocain, Astrazeneca, Södertälje, Sweden). Venous blood samples were taken in the fasting state for analysis of blood chemistry by routine methods at the Department of Clinical Chemistry, Umeå University Hospital as previously described.[20] Plasma free fatty acids (FFA) were determined using an enzymatic assay, NEFA C (Wako Chemicals USA, Richmond, VA). Body composition was determined by the bioelectrical impedance analysis technique (BIA-101 RJL-systems, Detroit, MI).[21] Lean body mass (LBM) and body fat were calculated from these measurements.

In Vivo Insulin Sensitivity

On a separate day, the hyperinsulinemic, euglycemic clamp technique was used to assess insulin sensitivity[22] and it was performed essentially as previously described.[20] Subjects arrived at the laboratory after an overnight fast since 10 PM. A 2-hour hyperinsulinemic, euglycemic clamp was started at 8 AM, and after initial priming, a constant infusion of short-acting insulin (Actrapid) was administered at 56 mU/m^2 body surface/min. The glucose infusion was adjusted to maintain blood glucose at 6.0 mmol/L. Insulin sensitivity was assessed as glucose uptake at steady state during the time period 60 to 120 minutes, ie, the M-value, and it was expressed as mg glucose infused/kg LBM/min.

Reagents

Medium 199, Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), and penicillin/streptomycin (PEST) were from Gibco BRL, Life Technologies (Paisley, UK). Bovine serum albumin (fraction V, BSA), N^6-(R-phenylisopropyl) adenosine (PIA) and 8-Br-cAMP were purchased from Sigma Chemical Co (St Louis, MO). Collagenase A, adenosine deaminase (ADA), and glycerokinase were purchased from Boehringer Mannheim (Mannheim, Germany). Human insulin (Actrapid) was purchased from Novo Nordisk A/S (Copenhagen, Denmark). [^{14}C]-U-D-glucose (specific activity, ~200 to 300 mCi/mmol) was from Amersham Pharmacia Biotech, Freiburg, Germany. [γ-^{32}P]adenosine triphosphate (ATP) (specific activity, 5 Ci/μmol) was from Amersham Pharmacia Biotech (Bucks, UK).

Adipocyte Preparation and Incubation

Needle biopsies of abdominal subcutaneous fat tissue were obtained at 8 AM after an overnight fast. The fat tissue was washed with prewarmed medium and blood clots were taken away. Isolated fat cells were prepared by shaking the tissue in polypropylene containers at 37°C for 40 to 50 minutes in medium 199 containing 5.6 mmol/L glucose with 40 mg/mL BSA and 0.6 mg/mL collagenase. Cells were filtered through nylon mesh and washed 4 times with fresh medium. A fraction of the isolated adipocytes was used for immediate experiments and the remaining cells were cultured in Teflon flasks containing DMEM with 6 mmol/L (initial concentration) D-glucose, 10% FCS, penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were incubated at 37°C for 24 hours under a gas phase of 95% O_2 and 5% CO_2. Cell size was determined in isolated adipocytes as previously described[23] and did not differ between freshly isolated cells and cells that had been incubated for 24 hours (data not shown); cell size was similar in the 3 groups of subjects (cell diameter, 105.2 ± 3.4 μm in P, 103.6 ± 2.3 μm in G, and 101.8 ± 4.0 μm in C). Cell viability was verified by trypan-blue exclusion tests.

Glucose Uptake Assay

Fresh adipocytes and adipocytes that had been incubated for 24 hours, respectively, were washed 4 times and glucose uptake was assessed as previously described.[17,24] In brief, adipocytes (lipocrit 3%

to 5%) were incubated in medium 199 without glucose with 4% BSA, ADA (1 U/mL), and PIA (1 μmol/L) for 15 minutes at 37°C in the presence or absence of insulin at the indicated concentrations. After that, ^{14}C-U-D-glucose (0.86 μmol/L) was added. The cells were separated from the incubation medium after 1 hour by centrifugation through silicone oil and the radioactivity associated with the cells was measured by scintillation counting. Under these experimental conditions, glucose uptake is mainly determined by the rate of transmembrane glucose transport.[25] The cellular clearance of glucose from the medium was calculated according to the following formula and taken as an index of the rate of glucose uptake: Cellular clearance of medium glucose = (cpm cells × volume)/(cpm medium × cell number × time).

Lipolysis

Freshly isolated adipocytes were washed 4 times and incubated at lipocrit 1% to 3% in medium 199 containing 5.6 mmol/L glucose, 4% BSA, ADA (1 U/mL), PIA (1 μmol/L), with and without 8-bromo-cAMP (5 mmol/L) and various concentrations of insulin (0 to 100 μU/mL) for 60 minutes at 37°C. After 1 hour, the reaction was stopped by transferring cells and medium to prechilled tubes on ice; adipocytes were rapidly separated from the medium by centrifugation through silicone oil. The rate of lipolysis was then assessed by measurement of the glycerol content in the medium according to Bradley and Kaslow.[26] In brief, glycerol was phosphorylated in the presence of glycerokinase and [γ-^{32}P]ATP for 30 minutes at 37°C. Residual [γ-^{32}P]ATP was then hydrolyzed in perchloric acid at 95°C for 60 minutes. Free ^{32}P-phosphate was precipitated on ice in the presence of ammonium molybdate and triethylamine. After centrifugation, radioactivity of the supernatant reflecting phosphorylated glycerol was measured.

Statistics

Statistical analyses were performed using the SPSS package (SPSS Inc, Chicago, IL). Results are given as means ± SEM; statistical significance was determined using 1-way analysis of variance (ANOVA) with the Bonferroni test as a post hoc test or Kruskal-Wallis nonparametric test for unpaired values with the Mann-Whitney test as a post hoc test. When appropriate, P values were adjusted for multiple comparisons and $P < .05$ was considered statistically significant. Associations between variables were analyzed with simple linear regression. We used stepwise multiple regression to assess independent associations between variables. Values for insulin response in vitro were logarithmically transformed in all regression analyses due to skewed distribution. For data not normally distributed, Spearman's rho correlation was used.

RESULTS

Glucose Uptake

Glucose uptake measurements were performed in all patients except 3 (1 P and 2 C, due to very small amounts of fat tissue in the biopsies). In fresh adipocytes, basal non-stimulated glucose uptake varied greatly within each group but did not differ among the 3 study groups (11.7 ± 2.8, 10.9 ± 2.4, and 7.0 ± 1.1 fL/cell/s for P, G, and C, respectively). Glucose uptake measurements in vitro suggested that adipocytes from diabetes patients with poor metabolic control did not respond properly to insulin and the relative increase induced by 1,000 μU/mL insulin was ~55% lower ($P < .05$) in P compared to C (Fig 1A). Adipocytes from diabetes patients with poor metabolic control were significantly more insulin-resistant than adipocytes from diabetes patients with good metabolic control at a low (5 μU/mL) but not at a high (1,000 μU/mL) insulin concentrations ($P < .05$ and P = not significant [NS], respectively). Taken together, P and G, ie, adipocytes from diabetes patients, had a lower maximal insulin response than C cells ($P < .05$).

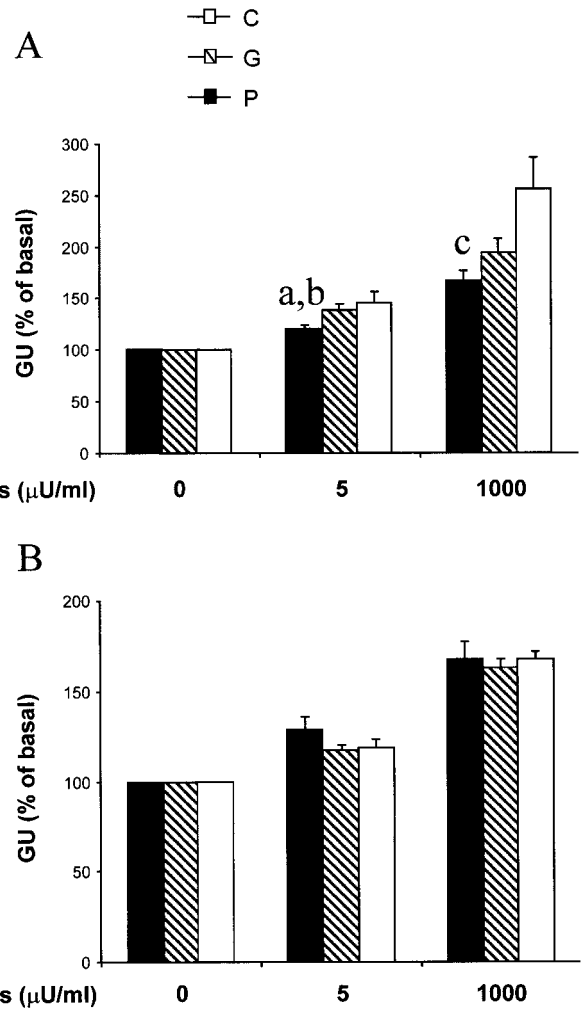

Fig 1. Glucose uptake in fresh fat cells (A) and in cells that had been incubated during 24 hours at a physiological glucose concentration (6 mmol/L) (B). Subcutaneous adipocytes from type 2 diabetes patients with good (G) and poor (P) metabolic control and from control (C) subjects were prepared as described in the Methods. Insulin at concentrations of 0, 5, and 1,000 μU/mL was added for 15 minutes and then ^{14}C-glucose was added and glucose uptake assessed over 60 minutes. Data are means ± SEM (n = 9, 10, and 8 for P, G, and C, respectively) and are expressed as % of glucose uptake (GU) in the basal nonstimulated condition. [a]$P < .05$ *v* G; [b]$P = .078$ *v* C; [c]$P < .05$ *v* C.

Following incubation of fat cells at a physiological glucose level (6 mmol/L) for 24 hours, basal glucose uptake was higher ($P < .01$) than in freshly isolated cells and it was similar in all 3 groups (15.8 ± 3.3, 19.8 ± 6.3, and 15.4 ± 2.8 fL/cell/s for P, G, and C, respectively; NS). The relative insulin response was overall slightly lower ($P < .01$) than in fresh cells, but there were no differences among the 3 groups at insulin concentrations of 5 or 1,000 μU/mL (Fig 1B).

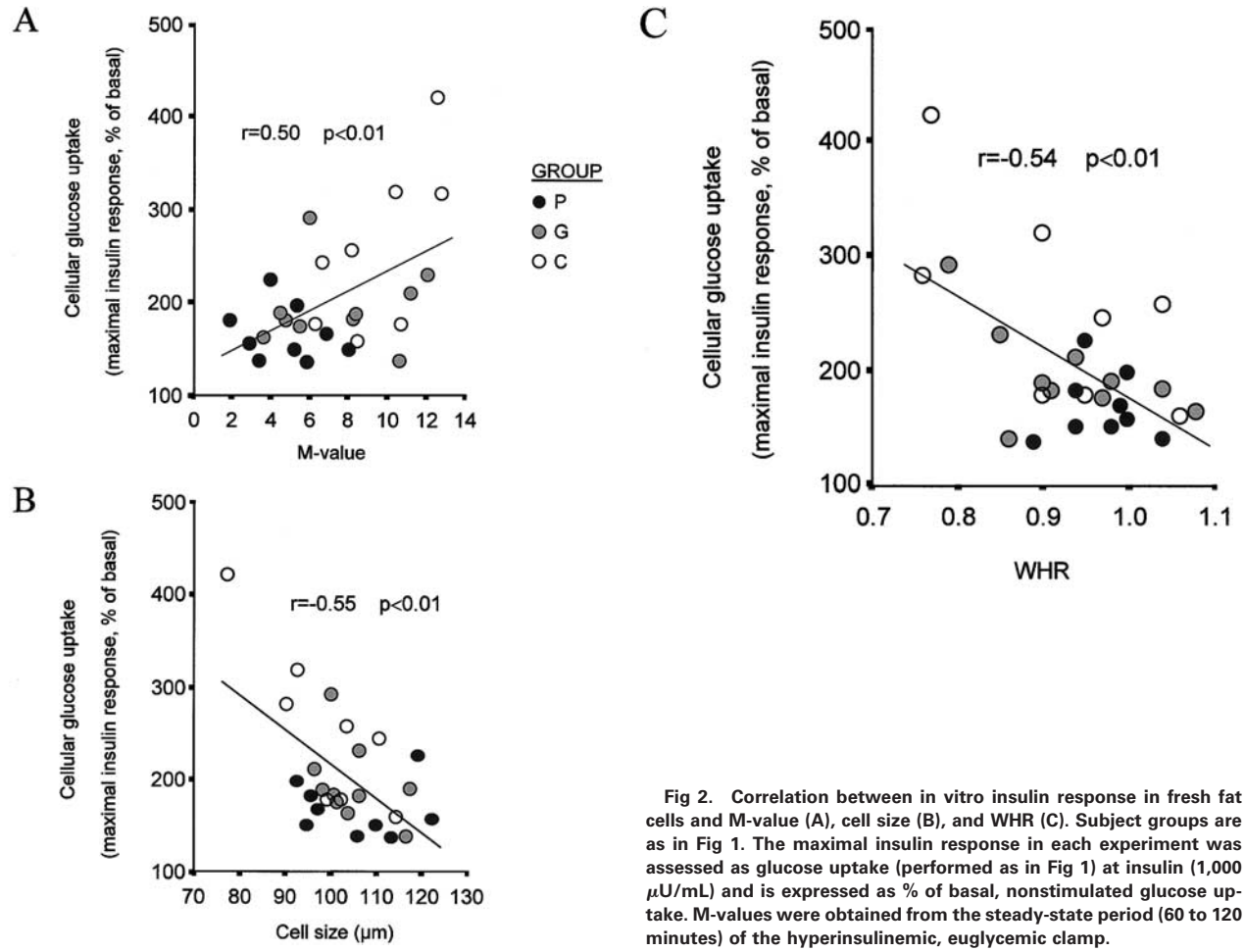

Fig 2. Correlation between in vitro insulin response in fresh fat cells and M-value (A), cell size (B), and WHR (C). Subject groups are as in Fig 1. The maximal insulin response in each experiment was assessed as glucose uptake (performed as in Fig 1) at insulin (1,000 μU/mL) and is expressed as % of basal, nonstimulated glucose uptake. M-values were obtained from the steady-state period (60 to 120 minutes) of the hyperinsulinemic, euglycemic clamp.

Lipolysis

This assay was performed in fresh adipocytes from a subset of the subjects, n = 5 in each group, due to limited amounts of fat tissue in the biopsies. Basal lipolysis did not differ among the 3 groups (glycerol release, 2.1 ± 0.9, 2.3 ± 0.6, and 1.7 ± 1.0 nmol/10^5 cells for C, G, and P, respectively; NS). Adipocytes from diabetes patients, especially those from the group with good metabolic control, tended to be more responsive to the stimulating effect of the cyclic adenosine monophosphate (cAMP) analog 8-bromo-cAMP; stimulated lipolysis was 236% ± 46%, 543% ± 200%, and 457% ± 191% of nonstimulated lipolysis for C, G, and P, respectively. However, these differences were not statistically significant. The ability of insulin to inhibit cAMP-stimulated lipolysis was not affected by diabetes or by the metabolic state; maximal inhibition was ~55% to 70% (P = NS) for all 3 groups at a maximal antilipolytic concentration of insulin (100 μU/mL).

Correlation Analyses

Insulin sensitivity in vivo, calculated as the M-value, was strongly and positively correlated with the effect of insulin to stimulate glucose uptake in fresh adipocytes in vitro ($r = 0.50$, $P < .01$, Fig 2A). Fasting blood glucose at the time of the biopsy, but not serum insulin levels, was negatively and near-significantly correlated with insulin response in vitro ($r = -0.36$, $P = .064$). In addition, long-term glycemia reflected by HbA_{1c} also correlated negatively to insulin's effect to stimulate glucose uptake in vitro ($r = -0.41$, $P < .05$).

Adipocyte cell size, WHR, and BMI predict cellular insulin resistance, since these parameters correlated negatively with the cellular insulin response on glucose uptake ($r = -0.55$, $P < .01$ for adipocyte cell size; $r = -0.54$, $P < .01$ for WHR; and $r = -0.43$, $P < .05$ for BMI; Fig 2B and C). Fat mass itself (as percent of total body mass) did not correlate with the effect of insulin to stimulate glucose uptake in vitro. The only parameter that correlated with fat mass was adipocyte cell size ($r = 0.44$, $P < .05$). We used stepwise multiple regression to find as few explanatory variables as possible for cellular insulin response in vitro. The variables insulin sensitivity in vivo, fasting blood glucose, HbA_{1c}, adipocyte cell size, WHR, and BMI were included in the equation. Adipocyte cell size together with WHR as independent variables created a regression equation in which cellular insulin response can be predicted with $R^2 = 0.44$ ($P < .01$).

Fasting blood glucose, HbA_{1c}, and serum insulin strongly correlated with insulin sensitivity in vivo calculated as the

M-value ($r = -0.62$, $P < .001$ for fasting blood glucose; $r = -0.61$, $P < .001$ for HbA_{1c}, and $r = -0.56$, $P < .01$ for serum insulin). Cell size, WHR, and BMI also correlated negatively with insulin sensitivity in vivo ($r = -0.43$, $P < .05$ for adipocyte cell size; $r = -0.50$, $P < .01$ for WHR; and $r = -0.36$, $P < .05$ for BMI). Fat mass was not associated with insulin sensitivity in vivo. The variables above that correlated significantly to insulin sensitivity in vivo were included in a multiple regression model. Fasting blood glucose and serum insulin created a regression equation in which the M-value can be predicted with $R^2 = 0.70$ ($P < .001$).

DISCUSSION

The present study demonstrates that cellular insulin resistance in subcutaneous fat cells from type 2 diabetes patients is related to the degree of metabolic control in vivo and that it is fully reversible after a 24-hour incubation in vitro at a physiological glucose level. After the incubation, adipocytes from type 2 diabetes patients displayed similar insulin sensitivity and responsiveness as control cells when assessed as the rate of glucose uptake. Our data thus suggest that fat cell insulin resistance is mainly secondary to the hyperglycemic or diabetic state in vivo.

Cellular insulin resistance is a consistent finding in type 2 diabetes.[27] Subcutaneous adipocytes from diabetes patients are insulin-resistant, and in the present study insulin sensitivity in vitro assessed as glucose uptake correlated to insulin sensitivity in vivo assessed by hyperinsulinemic, euglycemic clamp. A 24-hour incubation of adipocytes at a physiological glucose level abolished the differences in insulin-stimulated glucose uptake between diabetes and control cells, suggesting that high glucose or other circulating factors in vivo is the main explanation for cellular insulin resistance. The association between glycemia in vivo, ie, fasting glucose and HbA_{1c}, and insulin action in vitro found in linear regression analyses suggests that hyperglycemia could be a critical mechanism. This concept is also supported by studies on subjects with type 2 diabetes, indicating that normalization of blood glucose levels by insulin therapy or dietary control restores insulin action in adipocytes.[28-30] Our data can obviously not be directly transferred to other insulin-sensitive tissues, ie, liver and muscle. However, there is support in the literature that cellular insulin resistance in muscle may be secondary to the hyperglycemic state in vivo. In a previous study in muscle, where muscle biopsies were taken from insulin-resistant type 2 diabetes patients, incubation at 4 mmol/L glucose reversed muscular insulin resistance.[16] Further support in our study that hyperglycemia could be a critical mechanism in cellular insulin resistance is the strong association between glycemia and insulin sensitivity in vivo. Multiple regression analyses revealed that fasting glucose and insulin levels may predict insulin sensitivity in vivo with a high degree of accuracy.

Peripheral insulin resistance could potentially develop as a physiological response to protect insulin-sensitive tissues from a glucose overload by downregulation of cellular glucose transport.[1,3,31] Hyperglycemia increases the activity of GFAT, an enzyme in the hexosamine pathway. GFAT activity is increased in hyperglycemic obese mice and in hyperglycemic type 2 diabetes subjects.[32-34] Activation of this pathway has also been suggested to activate different PKC isoforms[15] that could play a role in the effect of the hexosamine pathway to induce insulin resistance.

In rat adipocytes, we recently found that high glucose per se can induce impaired glucose uptake capacity and lead to depletion of cellular insulin receptor substrate 1[35] supporting the role of glucotoxicity in fat cells. However, a major concern in our present study is the possibility that the absence of a hormone or some metabolite other than glucose in the culture medium might explain the recovery of insulin responsiveness in the fat cells from diabetes patients. This concept may be supported by our multiple regression analyses where glycemia in vivo did not display a significant association with the insulin response in vitro. However, the number of subjects in the present study is small, which may explain the failure to establish glucose level per se as an independent factor. To further explore this issue we are now undertaking another larger study in which the role of glucotoxicity as well as other humoral factors, eg, fatty acids, insulin-antagonistic hormones, and cytokines, are specifically addressed.

No significant alterations in basal and cAMP-stimulated lipolysis were found between control cells and cells from diabetes patients. The present study suggests that insulin's ability to inhibit cAMP-stimulated lipolysis in adipocytes in vitro is intact in type 2 diabetes and this is compatible with a previous study from our group.[24] However, due to a very limited number of experiments and a great variation between subjects in the present study, lipolysis regulation should be examined more thoroughly.

Cell size, WHR, and BMI, but not fat mass, correlated negatively with insulin's ability to stimulate glucose uptake both in vitro and in vivo. We used stepwise multiple regression, in which several possible independent variables were entered, to explain the degree of insulin responsiveness. Adipocyte cell size and abdominal obesity, reflected by WHR, were found to be independent factors determining insulin action in vitro. The literature reports that a central localization of fat distribution is associated with a higher degree of insulin resistance in vivo.[36] It is also reported that subjects with enlarged subcutaneous abdominal fat cell size on average are more prone to hyperinsulinemia and glucose intolerance than subjects with comparable adiposity,[37-39] and that enlarged subcutaneous abdominal fat size is an independent predictor of type 2 diabetes.[40] Although the diabetes patients in our study tended to have slightly larger adipocytes, the difference compared with cells from control subjects was not significant.

In conclusion, insulin resistance in adipocytes from type 2 diabetes patients can be reversed by incubation of the cells at a physiological glucose level. We suggest that cellular insulin resistance in type 2 diabetes is mainly secondary to the hyperglycemic state in the in vivo situation and this is further supported by the fact that fasting and long-term blood glucose was negatively correlated to insulin's ability to stimulate glucose uptake in vitro. Glycemia was also

associated with insulin sensitivity in vivo. However, it cannot be excluded that other factors apart from glucose that are present in the diabetic milieu in vivo could be important for whole body and cellular insulin resistance. Thus, cellular insulin resistance may not be a primary perturbation and the underlying mechanisms could involve early neuroendocrine dysregulation followed by metabolic perturbations, eg, hyperglycemia, which in turn may aggravate insulin resistance.

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