Factors in Serum from Type 2 Diabetes Patients Can Cause Cellular Insulin Resistance

Authors
F. Renström[1], J. Burén[1], M. K. Svensson[1, 2], J. W. Eriksson[1, 2, 3]

Affiliations
[1] Department of Public Health and Clinical Medicine, Medicine, Umeå University Hospital, Umeå, Sweden
[2] Department of Molecular and Clinical Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden
[3] AstraZeneca R & D, Mölndal, Sweden

Key words
- glucose transport
- insulin signaling
- human fat cells

Abstract

This pilot study was aimed to investigate whether there are humoral factors in serum from type 2 diabetic subjects that, in addition to glucose, insulin and free fatty acids are able to induce or contribute to peripheral insulin resistance with respect to glucose transport. Isolated subcutaneous adipocytes from 11 type 2 diabetic subjects and 10 nondiabetic controls were incubated for 24-h in medium supplemented with 25% serum from a control or a type 2 diabetic donor, in the presence of a low (5 mM) or a high (15 mM) glucose concentration, respectively. After the incubation period glucose uptake capacity was assessed. Serum from type 2 diabetic donors, compared to serum from controls, significantly reduced the maximal insulin effect to stimulate glucose uptake (~40%, $p < 0.05$) in adipocytes from control subjects, independent of surrounding glucose concentrations. Glucose uptake capacity in adipocytes isolated from type 2 diabetic subjects was similar regardless of culture condition. No significant alterations were found in cellular content of key proteins in the insulin signaling cascade (insulin receptor substrate-1 and -2, and glucose transporter 4) that could explain the impaired insulin-stimulated glucose transport in control adipocytes incubated with serum from type 2 diabetic donors. The present findings indicate the presence of biomolecules in the circulation of type 2 diabetic subjects, apart from glucose, insulin, and free fatty acids with the ability to induce peripheral insulin resistance. This further implies that even though normoglycemia is achieved other circulating factors can still negatively affect insulin sensitivity in type 2 diabetic patients.

received 17.10.2008
accepted 09.04.2009

Bibliography
DOI 10.1055/s-0029-1220751
Published online:
June 3, 2009
Horm Metab Res 2009;
41: 767–772

ISSN 0018-5043

Correspondence
Dr. F. Renström
Department of Public Health
and Clinical Medicine
Umeå University Hospital
6M, 4th floor
901 85 Umeå
Sweden
Tel.: +46/90/785 14 89
Fax: +46/90/785 44 00
frida.renstrom@
medicin.umu.se

Introduction

Insulin resistance is commonly the first measurable defect in the development of type 2 diabetes, where target tissues (i.e., muscle, liver, and fat) are unable to respond adequately to circulating levels of insulin. Our previous studies indicate that insulin resistance in subcutaneous adipocytes from type 2 diabetic subjects is reversible following 24-h incubation at a physiological glucose concentration [1]. Similar observations have been made regarding muscle cells [2]. Taken together such results indicate the possibility that in vivo circulating factors such as glucose could be the main explanation for the cellular insulin resistance observed. High blood glucose, insulin, or free fatty acids (FFAs) levels are all known to induce peripheral insulin resistance in vivo [3–5]. However, in our previous studies high glucose or FFA levels alone possessed only minor or no ability, respectively, to induce insulin resistance in primary adipocytes in vitro [6, 7]. It has recently been understood that the adipose tissue is an active endocrine organ producing and releasing adipokines that regulate whole-body energy homeostasis. The present study was conducted in order to investigate whether there might be additional biomolecules beside glucose, insulin, and FFAs in serum from type 2 diabetic subjects that are of importance for the development and maintenance of peripheral insulin resistance.

Research Design and Methods

Serum donors

Three nondiabetic control subjects and three subjects with type 2 diabetes came to the Metabolic Unit for serum donation after an overnight fast (≥10 h), without taking any medication in the morning. Each group comprised of one female and two males. Body composition was deter-

mined by the bioelectrical impedance analysis technique (BIA-101 RJL-systems, Detroit, MI, USA). An equation provided by the manufacturer was used for calculation of lean (fat free) and fat mass. The nondiabetic donors were 41.3 ± 11.3 years old with the following clinical characteristics; BMI 23.1 ± 1.3 kg/m^2, WHR 0.90 ± 0.00 (n = 2), fat mass 24.4 ± 9.0 %, and HbA_{1C} 4.3 ± 0.4 % (Swedish standard, normal range: 3.9–5.3 %). The type 2 diabetic donors age was 62.0 ± 6.4 years with a mean duration of diabetes of 8 years (range: 6–10). Their clinical characteristics were; BMI 26.4 ± 1.4 kg/m^2, WHR 1.05 ± 0.05 (n = 2), fat mass 31.8 ± 1.5 %, and HbA_{1C} 10.7 ± 1.8 %. Serum and plasma chemistry is shown in **Table 1**. There was one regular smoker and one tobacco snuff user in the type 2 diabetes group, but no tobacco use was allowed 24-h prior to serum donation. One serum donor in each group had a first-degree relative with type 2 diabetes. Among the three type 2 diabetic serum donors one subject had no antidiabetic medication, one was treated with insulin alone and one had insulin in combination with an oral antidiabetic drug (OAD) (sulfonylurea). Two of the type 2 diabetic subjects were treated with an ACE-inhibitor and one also with a statin. There was a 24-h period between the last intake of an ACE-inhibitor and serum donation. From each donor 100 ml venous blood was obtained, left for 30 min in room temperature to coagulate, centrifuged at 3000 rpm for 10 min at 4 °C, frozen at −80 °C in 10 ml aliquots and used for 3–4 experiments on isolated control and type 2 diabetic adipocytes, respectively.

Adipocyte donors

Ten nondiabetic control subjects and 11 type 2 diabetic subjects, respectively, came to the Metabolic Unit at 08.00. after an overnight fast (≥ 10 h) without taking any medication in the morning. Two abdominal subcutaneous needle biopsies were taken as previously described [1]. Clinical and biochemical characteristics of the subjects are shown in **Table 2**. Two type 2 diabetic subjects donated both adipocytes and serum, however, their adipocytes were not subjected to their own serum in any of the experimental settings. One of the control subjects used tobacco snuff and two of the type 2 diabetic subjects were regular smokers, but they refrained from tobacco use 24-h prior to biopsy. One of the control subjects was treated with a beta-blocker and a thiazide diuretic due to hypertension. Among the 11 type 2 diabetic subjects one subject had no antidiabetic medication and seven were treated with OADs, five of them had a single OAD (four metformin and one repaglinide) and two had two OADs combined (sulfonylurea and metformin). One subject was treated with insulin alone and two subjects had insulin in combination with an OAD (metformin or sulfonylurea). Six of the type 2 diabetic subjects were treated with antihypertensive agents; three subjects had ACE-inhibitors: one ACE-I in combination with diuretics, one calcium channel blocker and one angiotensin II receptor blocker in combination with a beta-blocker. Five of the type 2 diabetic subjects were also statin users. There was a 24-h period between the last intake of an ACE-inhibitor and biopsy. Individuals with stroke or myocardial infarction within the last 12 months, cancer or angina pectoris were excluded from the study. All participants gave their informed consent and the study was approved by the Umeå University Ethics Committee.

Blood chemistry

A blood sample from both adipocyte and serum donors was taken by standard venipuncture for analysis of blood chemistry.

Table 1 Fasting blood chemistry in control and type 2 diabetic serum donors

	Control (n = 3)	T2D (n = 3)
S-Insulin (mU/l)	5.3 ± 0.5	20.2 ± 10.9
S-Glucose (mmol/l)	5.8 ± 0.2	16.6 ± 2.3
S-Triglycerides (mmol/l)	0.9 ± 0.1	2.2 ± 0.8
S-Cholesterol (mmol/l)	4.1 ± 1.0	5.0 ± 0.7
S-LDL cholesterol (mmol/l)	2.7 ± 0.9	2.3 ± 0.8
S-HDL cholesterol (mmol/l)	1.1 ± 0.1	1.0 ± 0.1
S-hs-CRP (mg/l)	0.6 ± 0.2	4.1 ± 2.9
S-Cortisol (nmol/l)	400 ± 80	640 ± 60
P-FFA (mmol/l)	0.32 ± 0.06	0.38 ± 0.09
P-TNF-α (pg/ml)	0.62 ± 0.14	0.75 ± 0.09
P-IL-6 (pg/ml)	0.9 ± 0.5	2.0 ± 0.5
P-Adiponectin (µg/ml)	8.3 ± 0.9	5.3 ± 0.3
P-Leptin (ng/ml)	9.5 ± 6.1	10.5 ± 3.3

Data are number of subjects or means ± S.E.M. S: serum; P: plasma; hs-CRP: high sensitive-C-reactive protein

Table 2 Clinical characteristics and fasting blood chemistry of control and type 2 diabetic adipocyte donors

	Control (n = 10)	T2D (n = 11)
Sex (male/female)	6/4	6/5
Age (years)	60.9 ± 2.0	63.3 ± 1.9
Duration of diabetes (years)	n.a.	7.2 ± 1.2
BMI (kg/m^2)	28.9 ± 0.7	29.4 ± 0.9
WHR	0.95 ± 0.03	1.03 ± 0.02*
Fat mass (%)	36.0 ± 3.1	36.5 ± 2.2
Fat-free mass (%)	64.0 ± 3.1	63.5 ± 2.2
B-HbA_{1C} (%)†	4.4 ± 0.1	8.6 ± 0.7**
S-Insulin (mU/l)	9.3 ± 0.8	15.3 ± 3.4
S-Glucose (mmol/l)	5.4 ± 0.1‡	10.9 ± 1.1**
S-Triglycerides (mmol/l)	1.4 ± 0.1	2.8 ± 0.7
S-Cholesterol (mmol/l)	5.4 ± 0.3	5.3 ± 0.3
S-LDL cholesterol (mmol/l)	3.5 ± 0.3	3.2 ± 0.3§
S-HDL cholesterol (mmol/l)	1.3 ± 0.1	0.9 ± 0.1*
S-hs-CRP (mg/l)	1.2 ± 0.3‡	3.5 ± 2.0
S-Cortisol (nmol/l)	418 ± 38	490 ± 60
P-FFA (mmol/l)	0.41 ± 0.05	0.48 ± 0.05
P-TNF-α (pg/ml)	0.88 ± 0.11	1.10 ± 0.15
P-IL-6 (pg/ml)	1.9 ± 0.2	2.0 ± 0.3
P-Adiponectin (µg/ml)	9.0 ± 0.7	7.5 ± 1.3
P-Leptin (ng/ml)	18.9 ± 4.7	11.6 ± 2.2

Data are number of subjects or means ± S.E.M. n.a.: not applicable; B: blood; S: serum; P: plasma; hs-CRP: high sensitive-C-reactive protein. † Swedish standard, normal range 3.9–5.3 %. ‡ n = 9, § n = 10, * $p < 0.05$, ** $p < 0.001$

HbA_{1C} was measured by high-pressure liquid chromatography (HPLC) (Integral 4000, BioRad, Anaheim, California, USA) and calibrated according to the Swedish standard (reference 3.9–5.3 %). Commercial kits were used according to the manufacturer's instructions as follows: Serum insulin concentrations were determined by microparticle enzyme immunoassay (Abbott Imx, Abbott Laboratories, Abbott Park, IL, USA) and high sensitive C-reactive protein (hs-CRP) by CRP (Latex) HS Tinaquant kit (Roche Diagnostics Co., Indianapolis, IN, USA). FFA was determined using a commercial enzymatic assay, NEFA-C (Wako Chemical USA Inc., Richmond, VA, USA). A RIA and ELISA kit were used to determine leptin and adiponectin, respectively (LINCO Research, St. Charles, MO, USA) and plasma tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) levels using high-sensitive immunoassays (Qantikine HS Human TNF-α Immunoassay

and Qantikine HS Human IL-6 Immunoassay, R & D Systems Inc., Minneapolis, MN, USA). Serum cortisol was measured by chemiluminescent immunometric assay (IMMULITE, BioRad, Anaheim, CA, USA). All other measurements were done according to the routine methods at the Department of Clinical Chemistry, Umeå University Hospital.

Chemicals

Collagenase A and Adenosine deaminase (ADA) were purchased from Boehringer Mannheim (Mannheim, Germany). BSA (fraction V) and N^6-(*R*-phenylisopropyl)adenosine (PIA) were from Sigma Chemical Co. (St. Louise, MO, USA). Human insulin (Actrapid®) was from Novo Nordisk A/S (Copenhagen, Denmark) [^{14}C];-U-D-Glucose (specific activity 200–300 mCi/mmol) was from Amersham Pharmacia Biotech (Freiburg, Germany). DMEM, penicillin/streptomycin (PEST) and medium 199 were from Gibco BRL, Life Technologies (Paisley, UK). Anti-IRS-1 and anti-IRS-2 polyclonal antibodies were from Upstate Biotechnology Inc. (Lake Placid, NY, USA), anti-actin monoclonal antibody and GLUT4 polyclonal antibody from Chemicon International Inc. (Temecula, CA, USA). Secondary antibodies were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA).

Adipocyte isolation and incubation

Adipocytes were isolated as previously described [8] and equally divided between four polystyrene flasks containing penicillin (100 units/ml) and streptomycin (100 µg/ml). Five ml serum (25% of final volume) from a control or a type 2 diabetic donor with known glucose concentration was added to two flasks, respectively. To each of the two flasks, 15 ml (75% of final volume) of a mixture of DMEM containing 0 or 25 mM glucose, respectively, was added to reach a final glucose level of 5 and 15 mM, respectively, thus adjusting for the ambient serum glucose concentration. Adipocytes were incubated for 24-h at 37 °C with gentle agitation (~30 rpm) in a humidified atmosphere composed of 95% air and 5% CO_2. After the incubation period, aliquots of media were frozen at −80 °C. The FFA level was subsequently determined in media from control adipocytes cultured in 5 mM glucose with control or type 2 diabetic serum, respectively. Cell size was determined on isolated adipocytes as previously described [9] and did not differ between control and type 2 diabetic subjects or between the incubation conditions used (data not shown). Cell viability following culture was confirmed by trypan blue exclusion test.

Glucose uptake assay

After a 24-h incubation period, cells were washed and after an initial 15-minute pretreatment period glucose uptake capacity was assessed during the following 45-minutes by stimulation with 0, 5, 25, and 1000 µU/ml insulin, respectively, as previously described [10]. Measurements were performed in duplicate for all treatments.

Western blot analysis

After the incubation period, subsequent cell lysates and immunoblotting with appropriate antibodies were conducted essentially as previously described [6].

Statistical analyses

Statistical analyses were performed using the SPSS package (SPSS Inc., Chicago, IL, USA). Results are given as means ± S.E.M. Statistical significance of differences in clinical characteristics and blood chemistry was determined using one-way ANOVA. HbA_{1C}, insulin, glucose, hs-CRP and leptin were logarithmically transformed due to skewed distributions. Statistical significance of differences in glucose transport in freshly isolated adipocytes was determined using two-way ANOVA. Kruskal–Wallis nonparametric test with Wilcoxon signed ranks as post hoc test was used when analyzing within subject differences in glucose transport, FFA level, and cellular IRS-1, IRS-2 and GLUT4 content. Mann–Whitney was used as post hoc test when comparing differences in glucose transport between control and type 2 diabetic subjects after 24-h incubation. A p value of <0.05 was considered statistically significant.

Results

Glucose transport

Freshly isolated adipocytes from type 2 diabetic subjects displayed a significant impairment in glucose transport compared to controls ($p<0.001$, **Fig. 1a**), and this was true for both basal glucose transport (by ~50%, $p<0.01$) and insulin responsiveness, calculated as insulin-stimulated glucose transport as per cent of basal transport ($p=0.001$). The observed differences in glucose transport capacity between freshly isolated adipocytes from control and T2DM subjects were still present after 24-h incubation in the presence of control serum and low glucose levels (**Fig. 1b**).

Compared to control serum, type 2 diabetic serum significantly reduced (by ~40%, $p<0.05$) the maximal insulin effect to stimulate glucose uptake capacity in control adipocytes (**Fig. 2**). Similarly, high glucose per se also reduced the maximal effect of insulin to stimulate glucose uptake capacity in control adipocytes (by ~40%, $p<0.05$). There was a tendency (NS) of an additive effect of the combination of high glucose with diabetic serum to impair the insulin effect on glucose uptake (in total ~60% relative reduction, $p<0.05$). There were no significant alterations in basal glucose transport between the different culture conditions (data not shown).

Adipocytes isolated from type 2 diabetic subjects displayed no significant alterations in basal or insulin-stimulated glucose uptake capacity between the different culture conditions (**Fig. 2**). No significant difference in EC_{50} values for insulin's effect on glucose uptake was observed in adipocytes from control or type 2 diabetic subjects, respectively following the different culture conditions (data not shown). Thus, control adipocytes exposed to either a high glucose level or to type 2 diabetic serum displayed unresponsiveness to insulin, that is, an impaired efficacy. In contrast, there was no consistent lateral shift in the dose-response curve for insulin suggesting that the potency was left intact [11].

Cellular content of insulin signaling proteins

To investigate the mechanism behind the insulin resistance induced by type 2 diabetic serum, cellular levels of key proteins in the insulin signaling cascade were assessed in control adipocytes. When control adipocytes were cultured in type 2 diabetic serum at 5 or 15 mM glucose, the IRS-1 protein level was 120 ± 22% and 109 ± 22%, respectively ($p=$ ns, $n=8$), as compared to the protein level in control cells cultured in control serum at low glucose. Similarly, IRS-2 protein levels was 93 ± 12 and 90 ± 13% ($p=$ ns, $n=5$) and GLUT4 levels was 89 ± 23 and 90 ± 26% ($p=$ ns, $n=5$) of control adipocyte levels.

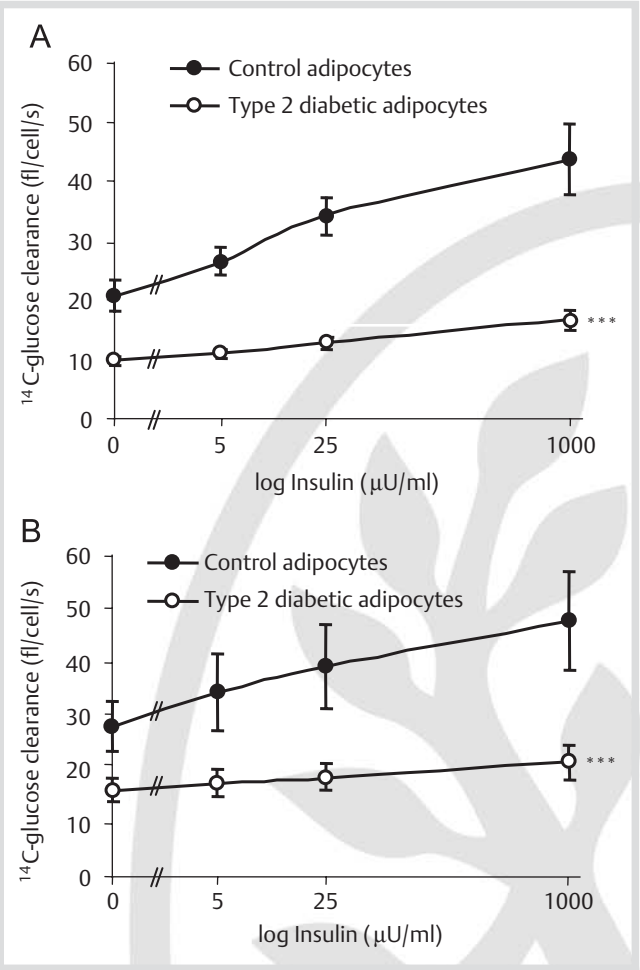

Fig. 1 Glucose uptake capacity in freshly isolated adipocytes (**A**) and after 24-h incubation with control serum at a low (5 mM) glucose level (**B**). Results are expressed as means ± S.E.M of 7–10 separate experiments. Black circles: control adipocytes, white circles: type 2 diabetic adipocytes. *** $p<0.001$ vs. control adipocytes.

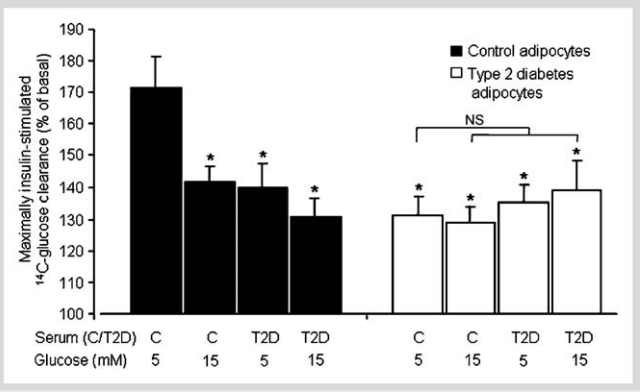

Fig. 2 Insulin-stimulated glucose uptake capacity in adipocytes cultured with control or type 2 diabetic serum at a low or high glucose concentration. Subcutaneous adipocytes isolated from 10 control (black bars) and 11 type 2 diabetic (white bars) subjects, respectively, were isolated and cultured for 24-h in medium supplemented with 25% serum from a control (C) or a type 2 diabetic (T2D) subject, in the presence of 5 or 15 mM glucose, respectively. Maximally insulin-stimulated (1000 µU/ml) glucose uptake rates are presented as per cent of basal glucose uptake (no insulin) for control or T2D adipocytes, respectively. Data are expressed as means ± S.E.M. * $p<0.05$ vs. control adipocytes cultured with control serum at 5 mM glucose.

FFA level in media

After culturing control adipocytes for 24-h in 5 mM glucose with 25% serum from a control or a type 2 diabetic donor the level of FFAs in the medium rose from 0.08.±0.02 to 0.56±0.06 and 0.10±0.02 to 0.69±0.07 mmol/l (n=9), respectively. Thus the relative rise in media FFA during the incubation period was similar irrespective of type 2 diabetic or control serum supplementation, and this was compatible with similar rates of adipocyte lipolysis.

Discussion

The present study indicates that serum from type 2 diabetic subjects induce insulin resistance in primary human adipocytes irrespective of the ambient glucose concentration. This finding has clinical relevance, since it suggests that factors other than glucose in the circulation may negatively affect insulin sensitivity in type 2 diabetes patients.

Although chronic hyperinsulinemia is considered to impair cellular glucose transport capacity, the final insulin concentrations (range: 0.7–10.0 µU/ml) in culture media were too low to have any major impact [12]. Glucose concentration in the serum was adjusted for when preparing the culture media so that fixed glucose levels were set, and hence glucose could be discarded as a sole critical factor. It can, however, not be excluded that long-term hyperglycemia preceding serum donation might indirectly be responsible for the observed effect of type 2 diabetic serum on glucose uptake capacity. In fact, hyperglycemia has previously been associated with increased circulating levels of inflammatory mediators and cortisol that can promote insulin resistance [13].

Type 2 diabetes is often associated with low grade inflammation and macrophage infiltration in the adipose tissue [14–16]. The proinflammatory cytokines TNF-α and IL-6 produced by macrophages and adipocytes have been shown to affect insulin sensitivity [17,18] and the expression of these proteins are positively correlated with degree of adiposity and insulin resistance [18,19]. However, due to the 1:4 dilution of the serum the final concentration of TNF-α and IL-6 in culture media supplemented with type 2 diabetic serum was below the plasma levels observed in control subjects, that is, concentrations that do not produce detectable biological responses in vitro [20–23]. It therefore seems unlikely that either of these proteins contributed to the impaired insulin responsiveness induced by type 2 diabetic serum.

Adiponectin and leptin have both established insulin-sensitizing effects: adiponectin increase FFA oxidation in muscle and inhibit hepatic glucose production [24] while leptin is involved in the regulation of feeding and peripheral insulin sensitivity through neural actions [25]. Insulin resistant states are associated with decreased adiponectin levels [26], as observed in serum from type 2 diabetic donors in the present study (**Table 1**). However, due to the four fold dilution the final concentration of adiponectin in the culture media was very low (between 1–2 µg/ml) regardless of whether the serum donor were type 2 diabetic or non-diabetic. Adiponectin is thus unlikely to be responsible for the impaired insulin-stimulated glucose uptake capacity observed when control adipocytes were exposed to serum from type 2 diabetic donors compared to nondiabetic donors, although it can not be completely excluded. No significant differences in

leptin levels were observed between type 2 diabetic and nondiabetic serum donors (**Table 1**).

Considering that the plasma FFA levels were within normal range and did not differ between controls and type 2 diabetic donors, plus the 4-fold dilution of supplemented serum, it seems unlikely that the final concentration of FFAs in culture media contributed to the impaired insulin responsiveness caused by type 2 diabetic serum. This is further supported by in vitro studies investigating the effect of FFAs on glucose uptake capacity in adipocytes [7,27]. Normal human serum has been shown to increase lipolysis in primary rat adipocytes [28] and this is compatible with the observed rise of FFAs in media during culture with both control and type 2 diabetic serum. Transferrin and iron present in normal serum have been shown to impair insulin-stimulated glucose transport [29], but no difference in the iron level or total iron-binding capacity (TIBC) was evident between control and type 2 diabetic serum in our study (data not shown).

There was a tendency of increased morning cortisol levels in serum from type 2 diabetic compared to control donors. Elevated glucocorticoid levels, even within the normal fluctuation observed in its daily rhythm, has been shown to induce peripheral insulin resistance in vivo [30,31], but the underlying cellular mechanism is not fully understood. Treatment with the glucocorticoid analogue dexamethasone in vitro has induced defects in insulin-stimulated glucose transport in 3T3-F442A, 3T3-L1, primary rat and also human omental adipocytes [8,32,33]. In contrast, human subcutaneous adipocytes are reported to be unaffected, arguing against a direct effect of cortisol per se in the present study [34].

When comparing blood chemistry between type 2 diabetic donors and control donors serum we were unable to identify any evident factor that could be responsible for the observed impairment in insulin-stimulated glucose transport. It can, however, not be excluded that the rather large difference in age between the control and type 2 diabetic serum donor group might be of importance. Interestingly, despite individual differences in anthropometric and biochemical variables, the respective sera from the three different type 2 diabetic donors displayed similar impairment on adipocyte glucose uptake.

The impairment in insulin-stimulated glucose transport seen in control adipocytes cultured with serum from type 2 diabetic donors was not associated with any alterations in the measured cellular levels of key proteins in the insulin signaling cascade. Altered phosphorylation and/or activity status of the IRSs that affect downstream insulin signaling [35–37] could be of importance but was not assessed in the present study. 15 versus 5 mM glucose decreased insulin-stimulated glucose transport but did not alter the protein level of IRS-1 and IRS-2 in control adipocytes cultured with control serum, and this is in agreement with our previous work [6]. These data further support our previous findings that decreased IRS-1 protein is a secondary phenomenon in insulin resistance [6,10]. Interestingly, serum from type 2 diabetic donors or high glucose levels per se had similar negative effects on insulin-stimulated glucose transport in control adipocytes. When they were combined the effects appeared to be slightly additive, although nonsignificantly. If so, this could indicate that different pathways leading to impairment of the glucose transport machinery might be utilized.

The insulin-stimulated glucose uptake in adipocytes from type 2 diabetic subjects remained significantly impaired compared to control adipocytes following culture in control serum with low glucose. Although this finding is supported by previous studies where 6–24-h incubations at a physiological glucose level were unable to improve insulin action with respect to glucose uptake in adipocytes from type 2 diabetic subjects [38,39] this seemingly is at variance with previous data obtained by our group [1]. Since 10% fetal calf serum was used in the previous study, but 25% human in the present, one possible explanation is that species of origin or the concentrations of serum supplemented to culture medium, could have significant impact on cellular insulin action. Clinical and biochemical characteristics of the subjects were fairly similar between the two studies. However, in the previous study basal glucose transport in freshly isolated adipocytes from type 2 diabetic subjects was similar to control adipocytes, but was significantly impaired (by ~50%, $p < 0.01$) in the present study. Thus, differences in adipocyte phenotypes between the two groups of type 2 diabetic subjects might contribute to differences in results between the two studies with respect to normalization of insulin responsiveness.

Future studies, for example, utilizing fractionation by molecular weight of serum prior to cell incubations, might further characterize the nature of the biomolecule(s) with the ability to induce cellular insulin resistance. These molecules are expected to be present in high concentration(s) or, alternatively, should be very potent since the effect on insulin sensitivity was significant although the serum was diluted four times. Candidate factors such as retinol binding protein-4 (RBP4), serum amyloid A (SAA), monocyte chemoattractant protein 1 (MCP-1), soluble adhesion molecules, resistin, endothelin-1, and IGF-binding protein-related protein 1 (IGFBP-rP1) should be investigated [40–46].

In conclusion, the present pilot study indicates the presence of biomolecules in the blood of type 2 diabetic subjects, apart from glucose, insulin, FFA, IL-6, and TNF-α with the ability to induce peripheral insulin resistance. The impairment of insulin action was not associated with alterations in the cellular levels of the insulin signaling proteins IRS-1 and -2.

Acknowledgments

We gratefully acknowledge the assistance of Ewa Strömqvist-Engbo and the staff at the Metabolic Unit at the Umeå University Hospital. We thank Dr. Mikael Dohlsten for initial ideas on this study. This work was supported by grants from the Swedish Research Council (Medicine, project 14287), the Swedish Diabetes Association, AstraZeneca R & D, the Faculty of Medicine at Umeå University, the Sigurd and Elsa Golje, and the Torsten and Ragnar Söderberg Foundations.

References

1 *Buren J, Lindmark S, Renström F, Eriksson JW*. 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? Metabolism 2003; 52: 239–245
2 *Zierath JR, Galuska D, Nolte LA, Thorne A, Kristensen JS, Wallberg-Henriksson H*. Effects of glycaemia on glucose transport in isolated skeletal muscle from patients with NIDDM: in vitro reversal of muscular insulin resistance. Diabetologia 1994; 37: 270–277
3 *Rossetti L, Giaccari A, DeFronzo RA*. Glucose toxicity. Diabetes Care 1990; 13: 610–630
4 *Rizza RA, Mandarino LJ, Genest J, Baker BA, Gerich JE*. Production of insulin resistance by hyperinsulinaemia in man. Diabetologia 1985; 28: 70–75
5 *Boden G, Chen X*. Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. J Clin Invest 1995; 96: 1261–1268

6 *Renström F*, *Buren J*, *Svensson M*, *Eriksson JW*. Insulin resistance induced by high glucose and high insulin precedes insulin receptor substrate 1 protein depletion in human adipocytes. Metabolism 2007; 56: 190–198
7 *Lundgren M*, *Eriksson JW*. No in vitro effects of fatty acids on glucose uptake, lipolysis or insulin signaling in rat adipocytes. Horm Metab Res 2004; 36: 203–209
8 *Buren J*, *Liu HX*, *Jensen J*, *Eriksson JW*. Dexamethasone impairs insulin signalling and glucose transport by depletion of insulin receptor substrate-1, phosphatidylinositol 3-kinase and protein kinase B in primary cultured rat adipocytes. Eur J Endocrinol 2002; 146: 419–429
9 *Smith U*, *Sjöström L*, *Björnstorp P*. Comparison of two methods for determining human adipose cell size. J Lipid Res 1972; 13: 822–824
10 *Renström F*, *Buren J*, *Eriksson JW*. Insulin receptor substrates-1 and -2 are both depleted but via different mechanisms after down-regulation of glucose transport in rat adipocytes. Endocrinology 2005; 146: 3044–3051
11 *Kahn CR*. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 1978; 27: 1893–1902
12 *Garvey WT*, *Olefsky JM*, *Marshall S*. Insulin induces progressive insulin resistance in cultured rat adipocytes. Sequential effects at receptor and multiple postreceptor sites. Diabetes 1986; 35: 258–267
13 *Lindmark S*, *Buren J*, *Eriksson JW*. Insulin resistance, endocrine function and adipokines in type 2 diabetes patients at different glycaemic levels: potential impact for glucotoxicity in vivo. Clin Endocrinol (Oxf) 2006; 65: 301–309
14 *Bouloumie A*, *Curat CA*, *Sengenes C*, *Lolmede K*, *Miranville A*, *Busse R*. Role of macrophage tissue infiltration in metabolic diseases. Curr Opin Clin Nutr Metab Care 2005; 8: 347–354
15 *Forouhi NG*, *Sattar N*, *MacKeigue PM*. Relation of C-reactive protein to body fat distribution and features of the metabolic syndrome in Europeans and South Asians. Int J Obes Relat Metab Disord 2001; 25: 1327–1331
16 *Pannacciulli N*, *Cantatore FP*, *Minenna A*, *Bellacicco M*, *Giorgino R*, *De Pergola G*. C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. Int J Obes Relat Metab Disord 2001; 25: 1416–1420
17 *Hotamisligil GS*, *Murray DL*, *Choy LN*, *Spiegelman BM*. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci USA 1994; 91: 4854–4858
18 *Rotter V*, *Nagaev I*, *Smith U*. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem 2003; 278: 45777–45784
19 *Hotamisligil GS*, *Arner P*, *Caro JF*, *Atkinson RL*, *Spiegelman BM*. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995; 95: 2409–2415
20 *Green A*, *Dobias SB*, *Walters DJ*, *Brasier AR*. Tumor necrosis factor increases the rate of lipolysis in primary cultures of adipocytes without altering levels of hormone-sensitive lipase. Endocrinology 1994; 134: 2581–2588
21 *Ryden M*, *Dicker A*, *van Harmelen V*, *Hauner H*, *Brunnberg M*, *Perbeck L*, *Lönnqvist F*, *Arner P*. Mapping of early signaling events in tumor necrosis factor-alpha -mediated lipolysis in human fat cells. J Biol Chem 2002; 277: 1085–1091
22 *Paysant J*, *Blanque R*, *Vasse M*, *Soria C*, *Soria J*, *Gardner CR*. Factors influencing the effect of the soluble IL-6 receptor on IL-6 responses in HepG2 hepatocytes. Cytokine 2000; 12: 774–779
23 *Senn JJ*, *Klover PJ*, *Nowak IA*, *Mooney RA*. Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes 2002; 51: 3391–3399
24 *Yamauchi T*, *Kamon J*, *Minokoshi Y*, *Ito Y*, *Waki H*, *Uchida S*, *Yamashita S*, *Noda M*, *Kita S*, *Ueki K*, *Eto K*, *Akanuma Y*, *Froguel P*, *Foufelle F*, *Ferre P*, *Carling D*, *Kimura S*, *Nagai R*, *Kahn BB*, *Kadowaki T*. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002; 8: 1288–1295
25 *Faraj M*, *Lu HL*, *Cianflone K*. Diabetes, lipids, and adipocyte secretagogues. Biochem Cell Biol 2004; 82: 170–190
26 *Hotta K*, *Funahashi T*, *Arita Y*, *Takahashi M*, *Matsuda M*, *Okamoto Y*, *Iwahashi H*, *Kuriyama H*, *Ouchi N*, *Maeda K*, *Nishida M*, *Kihara S*, *Sakai N*, *Nakajima T*, *Hasegawa K*, *Muraguchi M*, *Ohmoto Y*, *Nakamura T*, *Yamashita S*, *Hanafusa T*, *Matsuzawa Y*. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000; 20: 1595–1599
27 *Van Epps-Fung M*, *Williford J*, *Wells A*, *Hardy RW*. Fatty acid-induced insulin resistance in adipocytes. Endocrinology 1997; 138: 4338–4345
28 *Rumberger JM*, *Peters T*, *Jr*, *Burrington C*, *Green A*. Transferrin and iron contribute to the lipolytic effect of serum in isolated adipocytes. Diabetes 2004; 53: 2535–2541
29 *Green A*, *Basile R*, *Rumberger JM*. Transferrin and iron induce insulin resistance of glucose transport in adipocytes. Metabolism 2006; 55: 1042–1045
30 *Amatruda JM*, *Livingston JN*, *Lockwood DH*. Cellular mechanisms in selected states of insulin resistance: human obesity, glucocorticoid excess, and chronic renal failure. Diabetes Metab Rev 1985; 1: 293–317
31 *Dinneen S*, *Alzaid A*, *Miles J*, *Rizza R*. Metabolic effects of the nocturnal rise in cortisol on carbohydrate metabolism in normal humans. J Clin Invest 1993; 92: 2283–2290
32 *Saad MJ*, *Folli F*, *Araki E*, *Hashimoto N*, *Csermely P*, *Kahn CR*. Regulation of insulin receptor, insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-F442A adipocytes. Effects of differentiation, insulin, and dexamethasone. Mol Endocrinol 1994; 8: 545–557
33 *Sakoda H*, *Ogihara T*, *Anai M*, *Funaki M*, *Inukai K*, *katagiri H*, *Fukushima Y*, *Onishi Y*, *Ono H*, *Fujishiro M*, *Kikuchi M*, *Oka Y*, *Asano T*. Dexamethasone-induced insulin resistance in 3T3-L1 adipocytes is due to inhibition of glucose transport rather than insulin signal transduction. Diabetes 2000; 49: 1700–1708
34 *Lundgren M*, *Buren J*, *Ruge T*, *Myrnas T*, *Eriksson JW*. Glucocorticoids down-regulate glucose uptake capacity and insulin-signaling proteins in omental but not subcutaneous human adipocytes. J Clin Endocrinol Metab 2004; 89: 2989–2997
35 *Paz K*, *Hemi R*, *LeRoith D*, *Karasik A*, *Elhanany E*, *Kanety H*, *Zick Y*. A molecular basis for insulin resistance. Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. J Biol Chem 1997; 272: 29911–29918
36 *Zick Y*. Ser/Thr phosphorylation of IRS proteins: a molecular basis for insulin resistance. Sci STKE 2005; 2005: pe4
37 *Danielsson A*, *Ost A*, *Nyström FH*, *Strålfors P*. Attenuation of insulin-stimulated insulin receptor substrate-1 serine 307 phosphorylation in insulin resistance of type 2 diabetes. J Biol Chem 2005; 280: 34389–34392
38 *Danielsson A*, *Ost A*, *Lystedt E*, *Kjolhede P*, *Gustavsson J*, *Nyström FH*, *Strålfors P*. Insulin resistance in human adipocytes occurs downstream of IRS1 after surgical cell isolation but at the level of phosphorylation of IRS1 in type 2 diabetes. FEBS J 2005; 272: 141–151
39 *Smith U*. Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance – is insulin resistance initiated in the adipose tissue? Int J Obes Relat Metab Disord 2002; 26: 897–904
40 *Yang Q*, *Graham TE*, *Mody N*, *Preitner F*, *Peroni OD*, *Zabolotny JM*, *Kotani K*, *Quadro L*, *Kahn BB*. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005; 436: 356–362
41 *Yang RZ*, *Lee MJ*, *Hu H*, *Pollin TI*, *Ryan AS*, *Nicklas BJ*, *Snitker S*, *Horenstein RB*, *Hull K*, *Goldberg NH*, *Goldberg AP*, *Shuldiner AR*, *Fried SK*, *Gong DW*. Acute-phase serum amyloid A: an inflammatory adipokine and potential link between obesity and its metabolic complications. PLoS Med 2006; 3: e287
42 *Sartipy P*, *Loskutoff DJ*. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci USA 2003; 100: 7265–7270
43 *Bluher M*, *Unger R*, *Rassoul F*, *Richter V*, *Paschke R*. Relation between glycaemic control, hyperinsulinaemia and plasma concentrations of soluble adhesion molecules in patients with impaired glucose tolerance or Type II diabetes. Diabetologia 2002; 45: 210–216
44 *Steppan CM*, *Wang J*, *Whiteman EL*, *Birnbaum MJ*, *Lazar MA*. Activation of SOCS-3 by resistin. Mol Cell Biol 2005; 25: 1569–1575
45 *Ishibashi KI*, *Imamura T*, *Sharma PM*, *Huang J*, *Ugi S*, *Olefsky JM*. Chronic endothelin-1 treatment leads to heterologous desensitization of insulin signaling in 3T3-L1 adipocytes. J Clin Invest 2001; 107: 1193–1202
46 *Lopez-Bermejo A*, *Khosravi J*, *Fernandez-Real JM*, *Hwa V*, *Pratt KL*, *Casamitjana R*, *Garcia-Gil MM*, *Rosenfeld RG*, *Ricart W*. Insulin resistance is associated with increased serum concentration of IGF-binding protein-related protein 1 (IGFBP-rP1/MAC25). Diabetes 2006; 55: 2333–2339