Relationship Between Serum Amyloid A Level and Tanis/SelS mRNA Expression in Skeletal Muscle and Adipose Tissue From Healthy and Type 2 Diabetic Subjects

Håkan K.R. Karlsson, Hiroki Tsuchida, Staffan Lake, Heikki A. Koistinen, and Anna Krook

Tanis is a recently described protein reported to be a putative receptor for serum amyloid A and found to be dysregulated with diabetes in the Israeli sand rat Psammomys obesus. Tanis has also been identified as a selenoprotein, one of the first two identified membrane selenoproteins. We determined mRNA expression of the human homologue of Tanis, SelS/AD-015, in skeletal muscle and adipose tissue biopsies obtained from 10 type 2 diabetic patients and 11 age- and weight-matched healthy subjects. Expression of Tanis/SelS mRNA in skeletal muscle and adipose tissue biopsies was similar between diabetic and control subjects. A subset of subjects underwent a euglycemic-hyperinsulinemic clamp, and adipose tissue expression of Tanis/SelS was determined after in vivo insulin stimulation. Adipose tissue Tanis/SelS mRNA expression was unchanged after insulin infusion in control subjects, whereas Tanis/SelS mRNA increased in seven of eight subjects following insulin stimulation in diabetic subjects. Skeletal muscle and adipose tissue Tanis/SelS mRNA expression were positively correlated with plasma serum amyloid A. In conclusion, there is a strong trend toward upregulation of Tanis/SelS following insulin infusion in adipose tissue from type 2 diabetic subjects. Moreover, the positive relationship between Tanis mRNA and the acute-phase protein serum amyloid A suggests an interaction between innate immune system responses and Tanis expression in muscle and adipose tissue.

Diabetes 53:1424–1428, 2004

The development of type 2 diabetes is dependent on both environmental and genetic factors (1). Multiple approaches have been used to identify genetic mechanisms underlying type 2 diabetes, ranging from genome-wide scans, comparative genomics, and candidate gene approaches (2-4). Recently, Tanis, a putative receptor for serum amyloid A, has been identified (5) as a diabetes-associated gene in Psammomys obesus, a polygenic animal model of type 2 diabetes. Tanis is expressed in a range of tissues, including the insulin target tissues skeletal muscle, adipose, and liver (5). Tanis mRNA expression was reduced in liver of fasting diabetic animals (5). In vitro experiments reveal that expression of Tanis in 3T3 L1 adipocytes is decreased following exposure to high concentrations of insulin and glucose. Furthermore, overexpression of Tanis in hepatoma H4IE cells is associated with reduced glucose uptake, glycogen synthesis, and glycogen content, as well as impairment of the ability of insulin to suppress expression of the phosphoenolpyruvate carboxykinase (PEPCK) gene (6).

Tanis has recently been identified as a novel selenoprotein, SelS (7). Selenoproteins contain the amino acid selenocysteine (sec), which is encoded by UGA, which when present in a specific stem-loop structure is interpreted as sec instead of as a stop codon. Twenty-five selenoproteins have been identified in the human genome (7).

The human homologue for Tanis is AD-015/SelS. We hypothesized that Tanis/SelS expression may be altered in the context of human type 2 diabetes. We determined mRNA expression of Tanis/SelS in skeletal muscle and adipose tissue biopsies from patients with type 2 diabetes and age- and weight-matched healthy subjects. Furthermore, we examined whether acute insulin stimulation regulates Tanis/SelS mRNA in adipose tissue from type 2 diabetic and healthy subjects.

RESEARCH DESIGN AND METHODS

The institutional ethical committee of the Karolinska Institute reviewed and approved the study protocol. Informed consent was received from all subjects before participation. The clinical characteristics of the subjects are presented in Table 1. Ten male type 2 diabetic patients, with mean duration of disease of 4.8 years (range 2-11), were studied. Patients were treated with diet (n = 1); sulfonylureas (n = 5); metformin (n = 1); a combination of sulfonylurea, metformin, acarbose, and insulin (n = 1); a combination of sulfonylurea and metformin (n = 1); or insulin only (n = 1). The control group consisted of 11 healthy male subjects. All healthy subjects underwent an oral glucose tolerance test (8) to exclude diabetes or impaired glucose tolerance. None of the study participants were smokers or were taking any other medication. The subjects were instructed to avoid strenuous physical activity for a period of 72 h before the experiment. On the test day, subjects reported to the laboratory following an overnight fast and, in the case of the type 2 diabetic patients, before administration of any antidiabetic medication.

Muscle biopsy procedure. Skeletal muscle biopsies were obtained under local anesthesia from the vastus lateralis portion of the quadriceps femoris muscle (9,10). Samples were immediately stored in liquid nitrogen.
TABLE 1
Subject characteristics

<table>
<thead>
<tr>
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<th>Type 2 diabetic subjects</th>
<th>Nondiabetic subjects</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>59 ± 1</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>88.3 ± 4.2</td>
<td>86.3 ± 2.0</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>28.0 ± 1.0</td>
<td>26.8 ± 0.5</td>
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<tr>
<td>Duration of diabetes (years)</td>
<td>4.8 ± 1.0</td>
<td>—</td>
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<tr>
<td>V0₂max (ml · kg⁻¹ · min⁻¹)</td>
<td>26.9 ± 2.4</td>
<td>30.7 ± 1.3</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>13.2 ± 1.7*</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>9.4 ± 0.6*</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>6.0 ± 0.3*</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>Glucose uptake (μmol · kg⁻¹ · min⁻¹)</td>
<td>16.7 ± 2.6*</td>
<td>33.2 ± 1.8</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.48 ± 0.21</td>
<td>1.57 ± 0.22</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.60 ± 0.24*</td>
<td>5.64 ± 0.25</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.12 ± 0.07</td>
<td>1.31 ± 0.08</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.81 ± 0.15*</td>
<td>3.62 ± 0.22</td>
</tr>
<tr>
<td>C-peptide (nmol/l)</td>
<td>0.99 ± 0.11*</td>
<td>0.64 ± 0.05</td>
</tr>
<tr>
<td>Serum amyloid A (mg/l)</td>
<td>3.16 ± 0.54</td>
<td>2.22 ± 0.31</td>
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Data are means ± SE. *P < 0.05 vs. control subjects.

Euglycemic-hyperinsulinemic clamp and adipose tissue biopsy procedure. Insulin-mediated glucose utilization was determined on a separate occasion using the euglycemic-hyperinsulinemic clamp procedure (11,12). (After baseline blood samples were taken, insulin was infused at a rate of 40 mU · m⁻² · min⁻¹ for 180 min.) Adipose tissue biopsies were taken from subcutaneous abdominal adipose tissue at the level of the umbilicus under local anesthesia (mepivacain chloride 5 mg/ml) in the basal state and after 180 min of insulin infusion by needle aspiration (13).

Blood chemistry. Plasma glucose concentration was determined using a glucose oxidase method (Beckman Instruments, Fullerton, CA). Serum immunoreactive insulin and C-peptide concentrations were determined using a commercially available radioimmunoassay (Pharmacia, Uppsala, Sweden). HbA₁c was determined by specific ion-exchange chromatography, using a kit (Mono S HR 5/5; Pharmacia, Uppsala, Sweden). The normal range for HbA₁c in our laboratory is <5.2%. Plasma free fatty acid level was determined using a microfluorometric method (14).

Maximal oxygen uptake determination. On a separate occasion, maximal oxygen uptake (V₀₂max) was determined on a bicycle ergometer as described (15). V₀₂max was measured continuously with a breath-by-breath data collection technique (Erich Jaeger, Hoechberg, Germany) and calculated at each 20-s interval.

RNA extraction and analysis. Skeletal muscle biopsies (25–35 mg) and adipose tissue were removed from liquid nitrogen and homogenized using a homogeniser. Furthermore, primer sets generate an amplicon of 64 bp.

RESULTS
Subject characteristics are reported in Table 1. Age and BMI were similar between the type 2 diabetic and healthy subjects. Physical fitness as assessed by V₀₂max was not different between the two groups. Insulin-mediated peripheral glucose utilization, achieved during steady-state hyperinsulinemia, was reduced by 50% in type 2 diabetic subjects (P < 0.05 vs. healthy subjects). Serum and LDL cholesterol were lower in the type 2 diabetic subjects (P < 0.05 vs. healthy subjects). The metabolic control of the type 2 diabetic subjects was good (HbA₁c 6.0 ± 0.3%).

A search of the National Center for Biotechnology Information database revealed AD-015 to be the human homologue of Tanis. PCR primers were designed to determine mRNA expression of human Tanis/AD-015. Tanis/SelS mRNA expression in skeletal muscle was similar between healthy and type 2 diabetic subjects (Fig. 1). Furthermore, Tanis/SelS mRNA expression in adipose tissue biopsies was similar between healthy and type 2 diabetic subjects.

Insulin action on Tanis/SelS mRNA expression was determined. To correct the hyperglycemia in type 2 diabetic patients, insulin was infused for ~60 min before initiating the glucose infusion. Plasma free insulin concentrations during the insulin infusion were comparable between type 2 diabetic and healthy subjects (~60 mU/l, respectively). Steady-state plasma glucose concentration was maintained at 5.5 mmol/l in type 2 diabetic and healthy subjects. Following insulin infusion, Tanis/SelS mRNA increased in adipose tissue from type 2 diabetic subjects (P = 0.052) (Fig. 2), whereas mRNA levels in healthy subjects were unaltered.

Tanis has been proposed (5) to be a receptor for serum amyloid A. We assessed serum amyloid A concentration in healthy and type 2 diabetic subjects. When all subjects were analyzed collectively, plasma serum amyloid A levels

FIG. 1. Tanis/SelS mRNA levels in skeletal muscle from type 2 diabetic and healthy control subjects. Results are reported as arbitrary units after expressing and standardizing Tanis/SelS expression to β−globulin expression. The superimposed bars show mean ± SE for healthy (1.76 ± 0.49, n = 9; ○) and type 2 diabetic (1.94 ± 0.49, n = 10; ●) subjects, respectively.

to selectively detect cDNA from coding sequence mRNA and not DNA from potential contamination of genomic sequences. Probe: 5'-TAC ACC AGG GTG GGC TCC CTG CT-3'; 7-FAM; reporter: FAM; and quencher: 6-carboxytetramethylrhodamine (TAMRA). Forward primer: 5'-GGG GCG CCT GCT 5'-CTT C-3' TETRAMER. Reverse primer: 5'-GAT GTA CCA GCC ATA GTG GCC GTT C-3'. These primers generate an amplicon of 64 bp.

Statistical analysis. Data are presented as mean ± SE. Statistical differences were determined using Student’s unpaired t test or the Wilcoxon’s matched-pairs signed-rank test, as appropriate. The significance of correlations was determined using Pearson’s correlation analysis. P < 0.05 was considered statistically significant.
cells is associated with decreased insulin-stimulated glucose uptake and glycogen synthesis. The mechanism by which Tanis interferes with insulin action remains to be determined, as no effect on insulin receptor phosphorylation has been reported (6). Given that Tanis is associated with insulin resistance, we have determined Tanis/SelS mRNA expression in skeletal muscle and adipose tissue from type 2 diabetic and age- and weight-matched healthy subjects. Tanis/SelS mRNA expression in skeletal muscle and adipose tissue was similar between type 2 diabetic and healthy subjects. Moreover, plasma serum amyloid A was positively correlated with Tanis/SelS mRNA expression in both muscle and adipose tissue.

Tanis/SelS is one of 25 selenoproteins identified in the human genome and one of only two membrane selenoproteins that has been described (7). Selenoproteins are characterized by the presence of the amino acid selenocysteine, which in several cases has been found in the active sites of the enzyme. Selenoproteins include several glutathione peroxidases and thyroid hormone deiodinases (16). Selenoproteins are thought to be responsible for the majority of the biomedical effects of dietary selenium.

In contrast to the reduction in hepatic Tanis mRNA expression noted in fed diabetic Psammomys obesus rodents, Tanis/SelS mRNA expression was unaltered in skeletal muscle and adipose tissue from overnight-fasted type 2 diabetic patients as compared with control subjects. This could reflect tissue, nutritional, and/or species-specific differences. However, in Psammomys obesus rodents, fasting increased liver expression of Tanis in diabetic, but not in control, animals, such that the net effect was reflected as a similar level of Tanis expression between diabetic and healthy rodents under fasting conditions (5). Because skeletal muscle and adipose tissue biopsies in the present study were obtained from fasted subjects, a putative difference under fed conditions may have been masked.

We determined effect of short-term hyperinsulinemia on Tanis/SelS mRNA expression in adipose tissue obtained during a euglycemic-hyperinsulinemic clamp. During the euglycemic-hyperinsulinemic clamp procedure, fasting hyperglycemia in the type 2 diabetic patients was corrected by an insulin infusion for 60 min before commencing the glucose infusion (11). Thus, all subjects were studied...
under normoglycemic conditions (5.5 mmol/l). Tanis/SelS mRNA expression was increased under insulin-stimulated conditions in type 2 diabetic subjects, whereas levels were unchanged in control subjects. In vitro studies (5) in cultured cells have provided evidence that insulin and glucose suppress Tanis mRNA. However, these results could be misleading because results from these cell-based experiments were performed in cells incubated in insulin- and glucose-free media, a situation that is impossible to mimic in humans in vivo. In fact, apart from a baseline permissive effect, glucose and insulin do not suppress Tanis/SelS mRNA in adipose cells (5). Rather, our data provide evidence to suggest that in the context of type 2 diabetes, hyperinsulinemia may increase Tanis/SelS mRNA expression. Overexpression of Tanis in hepatoma cells leads to reduced insulin action on glucose uptake and glycogen expression. Whether increased Tanis/SelS provides evidence to suggest that in the context of type 2 diabetes, hyperinsulinemia may increase Tanis/SelS mRNA expression. Overexpression of Tanis in hepatoma cells leads to reduced insulin action on glucose uptake and glycogen synthesis (6). Whether increased Tanis/SelS mRNA in adipose cells in human type 2 diabetic subjects contributes to reduced insulin action remains to be determined.

Emerging evidence (17–19) suggests that type 2 diabetes is an inflammatory disorder with elevated circulating concentrations of several acute-phase reactants. Similarly, adiponectin, an adipocyte-derived hormone that has been shown to play important roles in the regulation of glucose and lipid metabolism, is structurally related to the complement 1q family (20), well-known players in the inflammatory processes. The relationship between Tanis mRNA and the acute-phase protein serum amyloid A suggests an interaction between innate immune system responses and Tanis expression in muscle and adipose tissue. Moreover, yeast 2 hybrid studies (5) reveal that Tanis binds serum amyloid A, suggesting that Tanis is the receptor for serum amyloid A. The positive correlation between plasma serum amyloid A and Tanis/SelS mRNA expression identified in the present study further supports this hypothesis. Thus, the serum amyloid A level is a surrogate marker for Tanis/SelS gene expression.

Selenium as a nutrient is related to immune system function (21). Selenium deficiency in AIDS patients is associated with increased mortality (16). Whether Tanis/SelS plays a role in selenium-mediated effects on the immune systems and inflammation in type 2 diabetes is an exciting hypothesis that requires further study. In summary, Tanis/SelS mRNA is expressed to a similar level in both skeletal muscle and adipose tissue from type 2 diabetic patients and age- and weight-matched healthy subjects. Expression of Tanis/SelS mRNA is positively correlated with serum amyloid A in type 2 diabetic and control subjects, supporting the hypothesis that Tanis/SelS may be the cell-surface receptor for serum amyloid A. Thus, serum amyloid A levels are also a good candidate surrogate marker for Tanis/SelS gene expression. Future studies are warranted to ascertain the functional role of Tanis/SelS in the regulation of glucose homeostasis in humans.

ACKNOWLEDGMENTS

This study was supported by grants from the Swedish Research Council, the Thürings Foundation, the Swedish Medical Association, Tore Nilsons Stiftelse, the Novo-Nordisk Foundation, Harald and Greta Jeansson Stiftelse, the Swedish Diabetes Association, the Markus and Amalia Wallenberg foundation, and the Swedish Fund for Research without Animal Experiments. H.A.K. was supported by fellowships from the Emil Aaltonen Foundation, the Finnish Academy of Science (Grant 52841), the Finnish Diabetes Research Foundation, the Finnish Medical Foundation, and the governmental subsidy for research of Helsinki University Central Hospital (EVO).

The authors would like to thank Professor Juleen R. Zierath for critical reading of the manuscript.

REFERENCES


