Tanis: A Link Between Type 2 Diabetes and Inflammation?

Ken Walder,[1] Lakshmi Kantham,[1] Janine S. McMillan,[1] James Trevaskis,[1] Lyndal Kerr,[1] Andrea de Silva,[1] Terry Sunderland,[1] Nathan Godde,[1] Yuan Gao,[1] Natalie Bishara,[1] Kelly Windmill,[1] Janette Tenne-Brown,[1] Guy Augert,[2] Paul Z. Zimmet,[3] and Greg R. Collier[1,4]

Here we describe a novel protein, which we have named Tanis, that is implicated in type 2 diabetes and inflammation. In *Psammomys obesus*, a unique polygenic animal model of type 2 diabetes and the metabolic syndrome, Tanis is expressed in the liver in inverse proportion to circulating glucose ($P = 0.010$) and insulin levels ($P = 0.004$) and in direct proportion with plasma triglyceride concentrations ($P = 0.007$). Hepatic Tanis gene expression was markedly increased (3.1-fold) after a 24-h fast in diabetic but not in nondiabetic P. obesus. In addition, glucose inhibited Tanis gene expression in cultured hepatocytes ($P = 0.006$) as well as in several other cell types ($P = 0.001–0.011$). Thus, Tanis seems to be regulated by glucose and is dysregulated in the diabetic state. Yeast-2 hybrid screening identified serum amyloid A (SAA), an acute-phase inflammatory response protein, as an interacting protein of Tanis, and this was confirmed by Biacore experiments. SAA and other acute-phase proteins have been the focus of recent attention as risk factors for cardiovascular disease, and we contend that Tanis and its interaction with SAA may provide a mechanistic link among type 2 diabetes, inflammation, and cardiovascular disease. *Diabetes* 51:1859–1866, 2002

Elevated hepatic glucose output is an early pathological event in the development of type 2 diabetes (1–3) and may result primarily from increased rates of gluconeogenesis (4,5). Together with reduced clearance of glucose from the blood, increased hepatic glucose output is a significant contributor to the rise in blood glucose concentration, which is characteristic of type 2 diabetes. We hypothesized that the identification of genes that are differentially expressed in the liver of type 2 diabetic animals may provide new insights into hepatic metabolic pathways that contribute to type 2 diabetes and its complications.

To test this, we conducted differential display PCR experiments using RNA obtained from the livers of fed and fasted *Psammomys obesus* (Israeli sand rats). *P. obesus* is a unique, polygenic animal model of type 2 diabetes and the metabolic syndrome (6–12). In their natural desert habitat, these animals remain lean and healthy on a low-energy diet of saltbush (*Atriplex halimus*) (6). However, when housed in a laboratory setting and given ad libitum access to standard rodent laboratory diet, a proportion of the animals develop a range of metabolic abnormalities, including insulin resistance, obesity, type 2 diabetes, and dyslipidemia (6–12). The distributions of body weight, blood glucose, and plasma insulin concentrations are continuous across the population of *P. obesus* (7,10) and closely approximate the distributions observed in human cross-sectional data (13,14).

Previous studies of hepatic metabolism in *P. obesus* identified a number of factors that could contribute to hepatic insulin resistance in these animals. Compared with albino rats, *P. obesus* have low numbers of hepatocyte insulin receptors (15,16), increased gluconeogenesis and PEPCK activity (17), and elevated hepatic glucose output (18). Compared with their lean, nondiabetic littermates, *P. obesus* with type 2 diabetes have increased hepatic glucose output (18) and hepatic lipogenesis (17). Thus, hepatic metabolism in type 2 diabetic *P. obesus* exhibits a number of similarities with that observed in humans with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Experimental animals. A colony of *P. obesus* is maintained at Deakin University (Geelong, Australia). Breeding pairs are fed a diet of lucerne and standard diet ad libitum. Experimental animals were weaned at 4 weeks of age and given a standard laboratory diet, from which 12% of energy was derived from fat, 63% from carbohydrate, and 25% from protein (Barastoc, Pakenham, Australia). Animals were housed in a temperature-controlled room (22 ± 1°C) with a 12-h light-dark cycle (light 0600–1800). Animals were classified as normal glucose tolerant (NGT), impaired glucose tolerant (IGT), or type 2 diabetic at 16 weeks of age according to their blood glucose and plasma insulin concentrations as previously described (7). Whole-blood glucose was measured using an enzymatic glucose analyzer (Model 27, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentrations were determined using a double-antibody solid-phase radioimmunoassay (Phadeseph, Kabi Pharmacia Diagnostics, Uppsala, Sweden).

Differential display PCR. At 18 weeks of age, animals ($n = 6$ from each group; NGT, IGT, and type 2 diabetic) were randomly assigned and either fasted for 24 h or fed ad libitum. After 24 h, the animals were killed, and the tissues were immediately removed and frozen in liquid nitrogen. RNA was extracted from tissues using RNAzol B (Tel-Test, Friendswood, TX) and

From the [1]Metabolic Research Unit, School of Health Sciences, Deakin University, Waurn Ponds, Victoria, Australia; [2]Lipha SA Research and Development, Lyon, France; the [3]International Diabetes Institute, Caulfield, Australia; and [4]Autogen, South Melbourne, Australia.

Address correspondence and reprint requests to Ken Walder, School of Health Sciences, Deakin University, Waurn Ponds, Victoria 3217, Australia. E-mail: walder@deakin.edu.au.

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3AT, 3-amino-1,2,4-triazole; IGT, impaired glucose tolerant; NGT, normal glucose tolerant; RU, resonance units; SAA, serum amyloid A; SPR, surface plasmon resonance.

reverse-transcribed using Superscript II (Invitrogen Life Technologies, Rockville, MD). Differential display PCR was performed on liver cDNA using an RNAimage mRNA Differential Display System (GenHunter, Nashville, TN). The Tanis gene was identified using a G-anchored primer (5′-aag ctt ttt ttt ttg-3′) and an arbitrary primer (5′-aag ctt ctc aac g-3′).

Sequencing. DNA sequencing was performed using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and a 373 automated fluorescent DNA sequencer (PE Applied Biosystems). The 5′ and 3′ ends of the transcript were determined by RACE of a Marathon cDNA Library (Clontech, Palo Alto, CA).

Measurement of Tanis gene expression. The level of Tanis gene expression in each cDNA sample was quantified using Taqman PCR technology on an ABI Prism 7700 sequence detector. β-Actin was used as an internal standard to normalize the amount of cDNA in a reaction. Primer sequences were as follows: Tanis gene forward, 5′-gat gcg ttc aat gat gtc ttc ct-3′; Tanis gene reverse, 5′-ga agc aaa ccc cat caa ctg t-3′; β-actin forward, 5′-gca aag acc tgt atg cca aca c-3′; β-actin reverse, 5′-gcc aga gca gtg atc tct ttc tg-3′. Fluorogenic probe sequences were 5′-cac atc agt aat cct cac tgg tgg gct ca-3′ for the Tanis gene and 5′-tgc tgg cac cag act tgc cct c-3′ for the β-actin gene. The Tanis and β-actin probes had the reporter dyes FAM and VIC, respectively, attached to the 5′ end, and both probes had the quencher dye TAMRA attached to the 3′ end. PCR conditions were 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Cell culture. Tanis gene expression was studied in three cell lines: HepG2 hepatocytes (European Collection of Cell Cultures), C2C12 myotubes (American *Type Culture* Collection), and 3T3-L1 adipocytes (a gift supplied by Dr. Lance Macaulay, CSIRO, Parkville, Australia). All cells were routinely cultured in Dulbecco's modified Eagle's medium (5–25 mmol/l glucose), 10% FCS serum, and antibiotics (Life Technologies, Melbourne, Australia).

Yeast-2 hybrid screen. Yeast-2 hybrid screening was performed using the ProQuest Two Hybrid System (Life Technologies). The coding sequence of Tanis was cloned into the yeast vector pDBLeu and transformed into DH5α cells by electroporation. Recombinant clones were detected by PCR using vector-specific primers (forward 5′-gaa taa gtg cga cat cat cat c-3′; reverse 5′-gta aat ttc tgg caa ggt aga c-3′). One positive clone, pDBB559, was selected for use in yeast-2 hybrid screening. The sequence of the insert was confirmed to be 100% homologous to the Tanis cDNA sequence and cloned in frame with the GAL4 DNA binding domain of pDBLeu. pDBB559 was transformed into the yeast strain MaV203, and the amount of 3-amino-1,2,4-Triazole (3AT) required for suppression of basal HIS3 expression of the transformants was determined by titration of cell growth on plates containing varying amounts of 3AT (0–100 mmol/l). MaV203 cell growth was inhibited at 3AT concentrations >10 mmol/l, and all plates used in the subsequent yeast-2 hybrid library screen contained 25 mmol/l 3AT to suppress basal HIS3 expression induced by GAL4DB-Tanis. MaV203 cells harboring the pDBB559 plasmid were specially prepared for large-scale transformation with a commercially available cDNA expression library. Specifically 18 μg of plasmid DNA, harvested from a ProQuest human liver cDNA library, was transformed into MaV203 cells containing the pDBB559 plasmid. Approximately 6.0×10^5 transformants were plated onto selective media containing 25 mmol/l 3AT but lacking leucine, tryptophan, and histidine. Transformants that induced the HIS3 reporter gene were predicted to contain potential interacting proteins and were selected for additional analysis. Putative HIS-positive transformants were tested for induction of two other associated reporters, URA3 and lacZ. Of the transformants initially identified as HIS+, four clones were also found to be positive in inducing expression of URA3 and lacZ. The plasmids isolated from these four clones were then sequenced using standard methods.

Expression and purification of recombinant Tanis and serum amyloid A proteins. The cDNA encoding the complete 189 amino acid Tanis protein, the cDNA corresponding to the region coding for a COOH-terminal (amino acids 54–189, termed Tanis-C) fragment of Tanis and cDNA encoding the mature sequence of serum amyloid A (SAA)1β (amino acids 19–122) were ligated into the pGEX-5X-1 expression vector (Amersham Pharmacia Biotech, Buckinghamshire, U.K.). The GST, GST–full-length Tanis, GST-Tanis-C, and GST-SAA proteins were expressed in the BL21 strain of *Escherichia coli* and affinity-purified using Glutathione Sepharose beads (Amersham Pharmacia Biotech). The quality and quantity of the expressed proteins were checked by SDS-PAGE and Coomassie blue staining. The GST, GST-Tanis-C, and GST-SAA expressed and purified well, whereas GST–full-length Tanis showed weak expression, and only a small amount of protein was recovered on purification. GST protein served as a control in all experiments involving recombinant GST fusion proteins.

Surface plasmon resonance analysis. The real-time protein–protein interactions were examined by surface plasmon resonance (SPR) analysis using a Biacore J instrument purchased from Biacore AB (Uppsala, Sweden). The system detects binding between specific pairs of molecules where one (ligand) is attached to the surface of a sensor chip and the other (analyte) present in a sample solution is passed over the surface. HEPES-buffered saline buffer (10 mmol/l HEPES [pH 7.4], 150 mmol/l NaCl, 3 mmol/l EDTA, 0.005% polysorbate 20) was used as running buffer in all SPR experiments. SAA, purified from human plasma (95% pure), was purchased from Trace Scientific (Melbourne, Australia). The SAA or GST-Tanis-C or GST-SAA was immobilized covalently to a sensor chip (CM5) via amine coupling by carbodiimide chemistry using the reagents supplied (Amine coupling kit, Biacore AB). Preconcentration tests were performed and found pH 4.0 to be most suitable for coupling ligands to CM5 chips. To test for interaction, we diluted the analyte samples (GST, GST-Tanis, GST-Tanis-C, or GST-SAA) into running buffer and injected them into the system when the sensorgram exhibited a stable baseline with noise levels <2 resonance units (RU). The chips were regenerated between uses by injection of 10 mmol/l Glycine-HCl (pH 2.0) for 4 min.

Statistical analysis. All data are expressed as means ± SE. Comparisons between groups were made by ANOVA with post hoc least-significant difference tests. Differences were considered significant at $P < 0.05$.

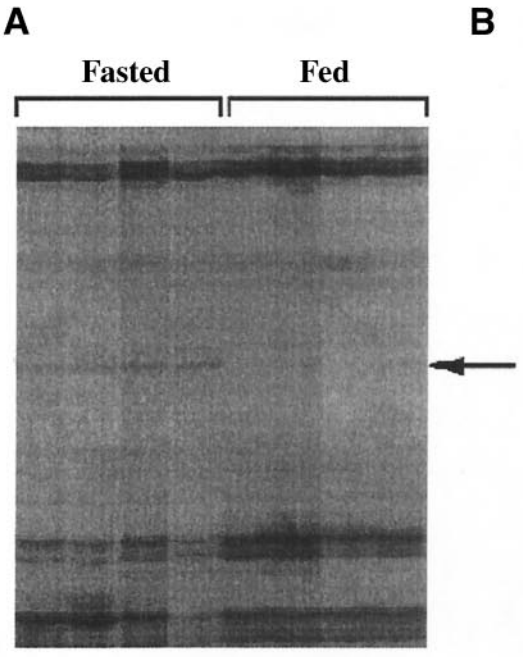
RESULTS

Differential display PCR was used to identify a gene whose expression was elevated in the liver of fasted *P. obesus* relative to fed controls (Fig. 1*A*). This band was excised and sequenced, revealing it to be a novel *P. obesus* gene with no apparent homologues in the public databases. We named this gene "Tanis," a Hebrew word for fasting. Subsequently, several apparently homologous sequences have been submitted to Genbank (e.g., accession nos. AF157317 and AF335543). The entire *P. obesus* Tanis mRNA sequence, obtained using RACE, is shown in Fig. 1*B*. The predicted Tanis amino acid sequence is given in Fig. 1*C*, along with alignments to apparently homologous genes from other species. A high level of identity was evident between species (Table 1), indicative of a conserved gene with important physiological function.

P. obesus mRNA for Tanis consists of 1,155 nucleotides and encodes a protein of 189 amino acids. Sequence analysis of the predicted Tanis protein suggested a single transmembrane region (amino acids 26–48), a dileucine motif, and a coiled-coil region.

Analysis using Expasy software tools (http://www.expasy.ch) predicted eight possible serine phosphorylation sites, one threonine phosphorylation site, three possible O-glycosylation sites, and four possible protein kinase C phosphorylation sites. Tanis could not be assigned to any known gene families and was predicted to have an overall composition of 44% α-helix, 17% extended sheet, and 39% random coil.

The genomic structure of the *P. obesus* Tanis gene was determined by direct sequencing of gDNA and cDNA samples and is shown in Fig. 1*D*. The gene consists of six exons ranging in size from 76 to 660 nucleotides. Exon 6 includes coding sequence for the COOH-terminal 25 amino acids and 585 nucleotides of 3′ untranslated region. The corresponding human gene, known as AD-015, was derived by automated computational analysis of genomic sequence at the National Center for Biotechnology Information (National Institutes of Health, Bethesda, MD) using gene prediction. The contig containing this sequence was localized to human chromosome 15q26.3 in the interval D15S157-qTEL, with the nearest marker identified as stSG26005. Of interest is that the syntenic chromosomal region in mice and pigs contains four obesity-related quantitative trait loci: Qw7 (19), Bw61 (20), Pfat1 (21), and SSC7 (22), suggesting that a gene in this region affects body fat accumulation.



B

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L
gagaccgagggcctgaggttcctgcacgtcacagtgggctccctgctggccagctatggc
 E  T  E  G  L  R  F  L  H  V  T  V  G  S  L  L  A  S  Y  G
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C

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Tanis     MESAEEPLPA RPALETEGLR FLHVTVGSLL ASYGWYVLFS CILLYIVIQK
 H47      .DRD... S. .......S.. .......... .......... .........R
AD-015    ..RQ..SLS. .......... ...T...... .T....IV.. .....V.F..
          51                                                  100
 Tanis    LSVRLRVLRQ RQLDQADAVL EPDAVVKRQE ALAAARLRMQ EDLNAQVEKH
 H47      ..L...A... ......ET.. ...V...... .......... ..........
AD-015    ..A...A... ....R.A.AV ...V...... .......K.. .E........
          101                                                 150
 Tanis    KEKLRQLEEE KRRQKIEMWD SMQEGRSYRR NPGRPQEEDG PGPSTSSSVT
 H47      .......... .......... .........K. .S........ .....-...I
AD-015    ....K..... .......... .....K..KG .AKK.....S .........LK
          151                                        188
 Tanis    RKGKSDKKPL RGNGYNPLTG EGGGTCAWRPG RRGPSSGG
 H47      P......... ..G....... .......S.... ........
AD-015    -R....R... ..G.....S. ....A.S.... ........
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D

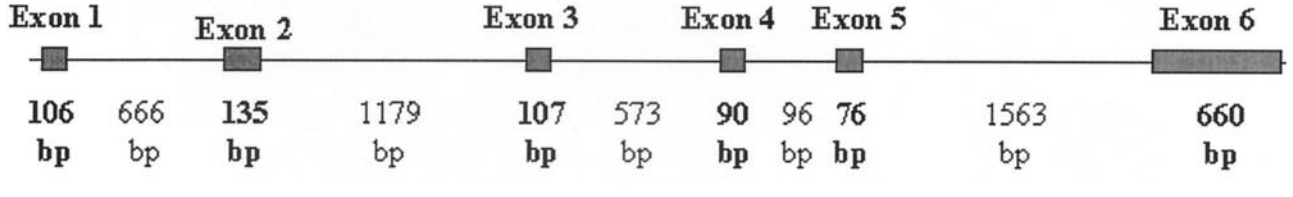

FIG. 1. ***A*****: Autoradiograph of ddPCR gel showing upregulated Tanis gene expression in the liver of** ***P. obesus*** **in the fasted state (arrow shows location of band corresponding to the Tanis gene).** ***B*****: Nucleotide and amino acid sequences of the** ***P. obesus*** **Tanis gene. Putative transmembrane sequence is underlined.** ***C*****: Amino acid sequence of the** ***P. obesus*** **Tanis gene aligned with corresponding genes from human (AD-015) and mouse (H47).** ***D*****: Genomic structure of the** ***P. obesus*** **Tanis gene.**

TABLE 1
Conservation (% identity) of Tanis at the nucleotide and amino acid levels in various species

	Nucleotide				Amino acid			
	Po	Hs	Rr	Mm	Po	Hs	Rr	Mm
P. obesus (Tanis)	—	*84*	*93*	*90*	—	*80*	*91*	*90*
Homo sapiens (AD-015)	*84*	—	*85*	*84*	*80*	—	*86*	*81*
Rattus rattus (EST)	*93*	*85*	—	*96*	*91*	*86*	—	*96*
Mus musculus (H47)	*90*	*84*	*96*	—	*90*	*81*	*96*	—

Accession numbers: *P. obesus* (Po; Tanis); *Homo sapiens* (Hs, AD-015), XM007631; *Rattus rattus* (Rr), AA893841; *Mus musculus* (Mm; H47), AF335543.

Hepatic Tanis gene expression was increased 2.2-fold after a 24-h fast in *P. obesus* ($P < 0.001$). Within the subgroups of animals, the increase in hepatic gene expression of Tanis after fasting was significant only in the diabetic animals (3.1-fold increase; $P = 0.010$) (Fig. 2). In ad libitum–fed animals, expression of the Tanis gene in the liver was reduced in both IGT ($P = 0.039$) and type 2 diabetic *P. obesus* ($P = 0.015$) relative to their NGT littermates (Fig. 2). In addition, linear correlations were observed between Tanis expression and circulating triglyceride concentrations (Pearson $r = 0.593$, $P = 0.007$); (Fig. 3), as well as blood glucose (Spearman $r = -0.378$, $P = 0.010$) and insulin concentrations (Spearman $r = -0.416$, $P = 0.004$) in ad libitum–fed *P. obesus*. There was also evidence of a correlation between hepatic Tanis gene expression and the change in blood glucose (Spearman $r = 0.395$, $P = 0.010$) and insulin concentrations (Pearson $r = 0.374$, $P = 0.015$) after 24 h of fasting. Multiple linear regression analysis indicated that only the change in blood glucose concentration was independently associated with Tanis gene expression ($P = 0.004$), suggesting a relationship between these two variables. In addition, when dietary energy restriction was continued for a period of 2 weeks (at 67% of normal dietary intake), Tanis gene expression in the liver was increased 2.2-fold relative to ad libitum–fed control animals (1.91 ± 0.29 vs. 0.87 ± 0.08 arbitrary units, $P = 0.006$).

In accordance with the results obtained in vivo, cell culture experiments showed that Tanis gene expression in HepG2 hepatocytes was profoundly regulated by media glucose concentration ($P = 0.006$) (Fig. 4). Increasing the concentration of glucose in the media caused a dose-dependent reduction in the levels of Tanis gene expression, with a maximal effect observed at 12.5 mmol/l glucose of ~90% suppression.

We tested for Tanis gene expression in tissues other than liver using both Taqman PCR and Northern blots. Tanis gene expression was detected by Taqman PCR in all tissues examined, including hypothalamus, liver, skeletal muscle, adipose tissue, testes, heart, and kidney. Northern blotting revealed a single band of the expected size (1,155 nucleotides) in a range of tissues, including liver, adipose tissue, hypothalamus, and skeletal muscle (Fig. 5).

Expression profiling of the Tanis gene in nonhepatic tissues revealed no effect of fasting on Tanis gene expression in adipose tissue, muscle, or hypothalamus (data not shown). However, in vitro Tanis gene expression was suppressed in a dose-dependent manner by glucose in 3T3-L1 adipocytes (Fig. 6) and C2C12 muscle cells (maximum effect of 50% suppression at 25 mmol/l glucose; $P = 0.002$). Tanis gene expression was also decreased by

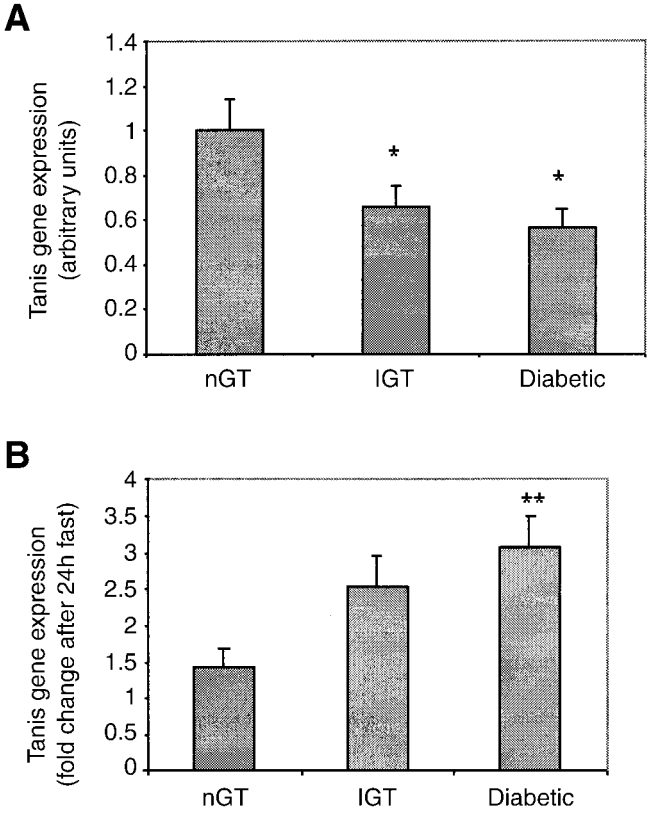

FIG. 2. Hepatic Tanis gene expression in *P. obesus*. *A*: Reduced Tanis gene expression in the liver of IGT and type 2 diabetic *P. obesus* in the fed state. *B*: Fold increase in Tanis gene expression in the liver of *P. obesus* after 24-h fast compared with the fed state. *Significantly different from the NGT group ($P < 0.05$); **significant increase compared with the fed diabetic group ($P = 0.010$).

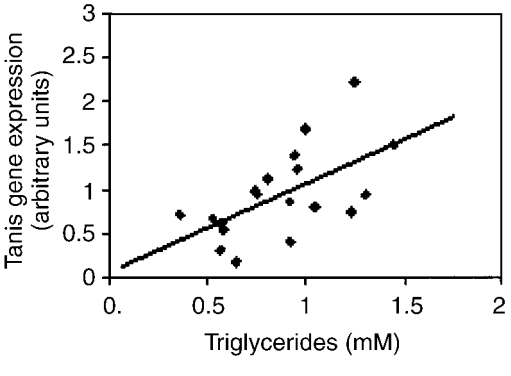

FIG. 3. Linear correlation between hepatic Tanis gene expression and circulating triglyceride concentrations in *P. obesus* ($r = 0.593$, $P = 0.007$).

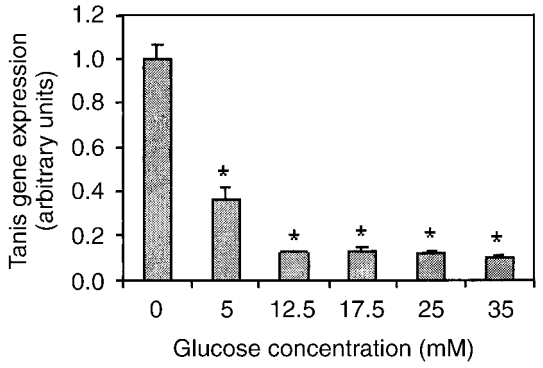

FIG. 4. Effects of increasing glucose concentration on Tanis gene expression in HepG2 hepatocytes. *Significantly different from glucose concentration of 0 mmol/l ($P < 0.001$).

insulin in 3T3-L1 cells (Fig. 6) and in C2C12 cells (data not shown).

To examine further the physiological role of the Tanis protein, we conducted a yeast-2 hybrid screen to identify interacting proteins. Using Tanis as bait, ~600,000 transformants from a human liver cDNA library were screened. Expression analysis of three different reporter genes independently confirmed four clones to be positive, with each showing evidence of interaction of intermediate strength. Sequencing of these clones revealed that three of the four encoded SAA, an acute-phase inflammatory response protein. The entire nucleotide sequence of all three positive clones identified in the yeast-2 hybrid screen matched with the known sequence of human SAA1β, an allele of the SAA1 gene (Genbank accession no. CAA39974).

The putative interaction between Tanis and SAA was confirmed by SPR analysis. A CM5 sensor chip with 4,737 RU of human plasma SAA coupled to its surface was initially used for testing interaction with GST–full-length Tanis. The sensorgram revealed a binding phenomenon with GST-Tanis (data not shown). GST–full-length Tanis, which contains the predicted transmembrane domain, was

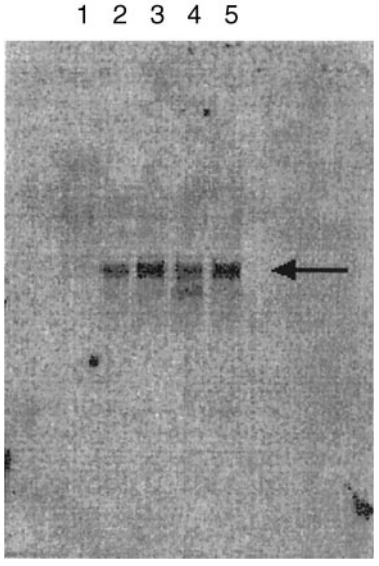

FIG. 5. Northern blot for Tanis in *P. obesus*. *Lane 1*, size marker; *lane 2*, adipose tissue; *lane 3*, hypothalamus; *lane 4*, liver; *lane 5*, skeletal muscle. Arrow indicates position of the Tanis gene.

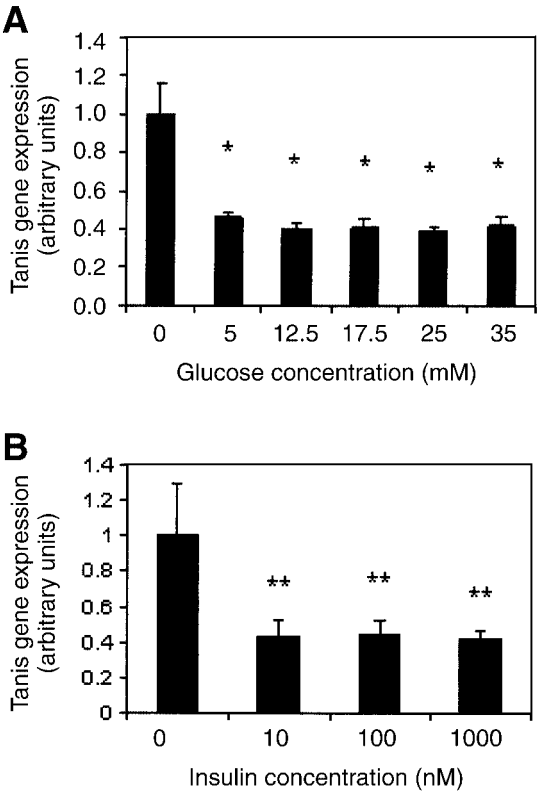

FIG. 6. Effects of glucose (*A*) and insulin (*B*) concentrations on the expression of the Tanis gene in 3T3-L1 adipocytes. *Significantly different from glucose concentration of 0 mmol/l ($P < 0.001$); **significantly different from insulin concentration of 0 nmol/l ($P = 0.020$).

difficult to express and purify to a satisfactory degree. Therefore, we expressed and purified the COOH-terminal 136–amino acid fragment of Tanis, which does not include the transmembrane region. The GST-Tanis-C protein was expressed and purified at satisfactory levels. In the SPR binding analysis, GST-Tanis-C demonstrated binding with human plasma SAA, which was concentration-dependent (Fig. 7*A* and *B*). Interaction was shown to be positive even in the reverse situation, where GST-Tanis was bound to the sensor chip as a ligand and SAA was passed through as an analyte (Fig. 7*C*).

SAA purified from human plasma is a heterogeneous sample likely to contain all of the forms of SAA present in circulating blood. To examine interactions with a homogeneous sample, we expressed and purified SAA1β as a GST fusion protein and demonstrated its ability to interact with Tanis by SPR analysis (Fig 7*D*).

DISCUSSION

We have identified Tanis, a novel hepatic receptor for SAA that is regulated by glucose and differentially expressed in type 2 diabetes. Tanis therefore provides a potential link among diabetes, the acute inflammatory response, and cardiovascular disease.

We searched specifically, using differential display PCR, for genes in the liver that exhibit an inappropriate response to fasting in diabetic *P. obesus* and identified the Tanis gene. Tanis is composed of six exons and five introns and encodes a cDNA product of 1,155 nucleotides. The human Tanis gene (AD-015) is located at 15q26.3.

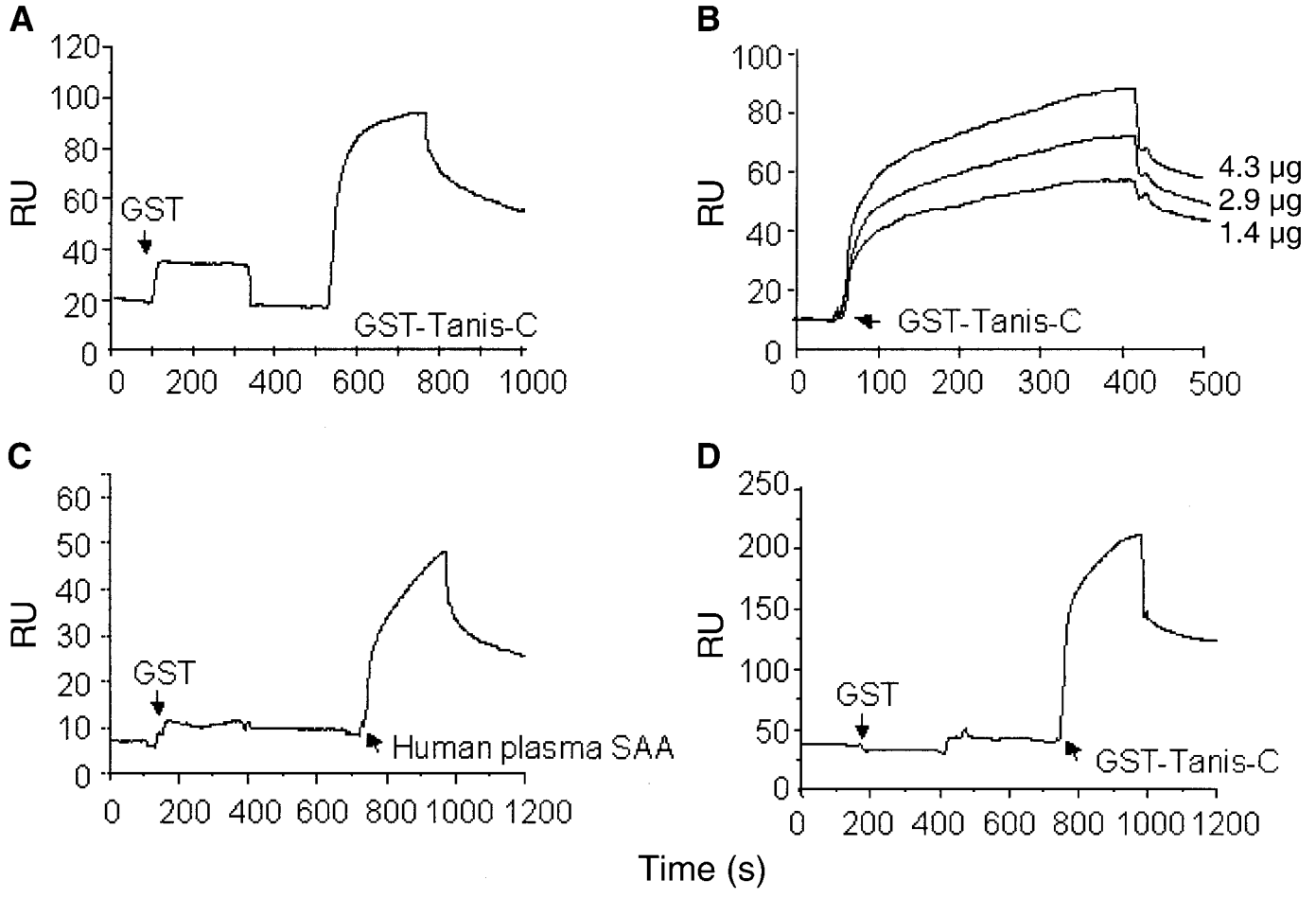

FIG. 7. Real-time interaction between Tanis and SAA by SPR analysis. The ligand (*A* and *B*: human plasma SAA; *C*: GST-Tanis-C; *D*: GST-SAA) was immobilized onto the CM5 sensor chip, and the analyte (*A*, *B*, and *D*: GST-Tanis-C, 5 µg; *C*: human plasma SAA 5 µg) diluted in binding buffer was passed over the chip. The change in SPR was indicated in RU. The samples were injected over 4 (*A*, *C*, and *D*) or 6 (*B*) min. The injection points are indicated by arrows. Injection of GST control protein alone (*A*, *B*, and *D*) did not produce a binding phenomenon.

Although this region has not been previously identified in genome-wide linkage scans for diabetes-related phenotypes in human populations, the syntenic regions in mice and pigs contain several quantitative trait loci for phenotypes related to obesity (19–22). The protein product of the Tanis gene is 189 amino acids in length and is predicted to have a single transmembrane region close to the NH_2-terminus (amino acids 26–48) (Fig. 1*C*), as well as numerous possible phosphorylation and glycosylation sites. The Tanis protein could not be assigned to any known families. The high degree of identity at both the nucleotide and amino acid levels for the Tanis gene (Table 1) indicate a high degree of conservation that suggests that Tanis is a protein of considerable physiological importance.

Results from the differential display PCR suggested that hepatic expression of the Tanis gene in response to 24 h of fasting was dysregulated in *P. obesus* with type 2 diabetes (Fig. 1*A*). Profound changes in carbohydrate and lipid metabolism occur in the liver during fasting, such as an increase in gluconeogenesis and fatty acid oxidation, and reduced glycogenolysis (23–25). Fasting also reduces glycemia and insulinemia and results in increased circulating free fatty acid concentrations. Tanis gene expression in the liver was increased 3.1-fold after 24 h of fasting in diabetic *P. obesus* but was not significantly increased in nondiabetic control animals. There was also a tendency for increased Tanis gene expression after fasting in the IGT group (2.5-fold increase; $P = 0.072$). Thus, we hypothesized that Tanis may be regulated by circulating metabolites such as glucose, insulin, or free fatty acid and may be involved in metabolic processes important in the development of type 2 diabetes.

Subsequent expression profiling identified negative correlations between hepatic Tanis gene expression and circulating glucose and insulin concentrations and a positive association with plasma triglyceride concentration. Multiple linear regression analysis showed that only blood glucose concentration was independently associated with Tanis gene expression. Furthermore, hepatic expression of the Tanis gene was significantly reduced in type 2 diabetic *P. obesus* relative to their lean, healthy littermates. Cell culture experiments showed that glucose strongly inhibited Tanis gene expression in HepG2 hepatocytes, as well as in 3T3-L1 adipocytes and C2C12 muscle cells. Taken together, these results provide strong evidence that glucose is a key regulator of Tanis gene expression in the liver. In general, genes that are transcriptionally or translationally regulated by glucose tend to be involved in glucose/energy homeostasis, such as glucokinase (26) and fatty-acid synthase (27), or diseases associ-

ated with diabetes, such as atherosclerosis (CD36 gene (28). Regulation of Tanis gene expression by glucose could partially explain the differential response observed in fasted *P. obesus*, as the 24-h fast reduced blood glucose by only 0.85 mmol/l in NGT animals (4.23 ± 0.19 to 3.38 ± 0.25 mmol/l) compared with 8.64 mmol/l in diabetic *P. obesus* (13.48 ± 0.89 to 4.85 ± 0.32 mmol/l). Linear regression analysis confirmed that Tanis gene expression was associated with the change in glycemia after fasting and that this relationship was independent of body weight, glucose, and insulin concentrations at baseline and the change in insulinemia after fasting.

The observations that Tanis gene expression in the liver is regulated by glucose, that the expression of Tanis is decreased in *P. obesus* with type 2 diabetes, and that there is exaggerated Tanis gene expression in response to fasting in these animals suggest that this gene may be important in key hepatic metabolic pathways. Tanis has characteristics consistent with a cell-surface receptor, and its mouse homologue (H47) was recently shown to reside at the cell surface membrane (29). We hypothesize that the increase in Tanis gene expression may correspond with increased appearance of this receptor at the cell surface of hepatocytes after fasting, which could in turn be important in the metabolic response to reduced energy intake. As most proteins exert their biological functions by interacting with other proteins, we reasoned that by knowing with which proteins Tanis interacts, we might be able to predict its function or assign a position in a known metabolic or signal transduction pathway.

Yeast-2 hybrid screening of a human liver cDNA library resulted in identification of four independent positive clones. Additional sequencing revealed SAA as the main protein interacting with Tanis. SAA was considered to be a true positive as the interaction-mediated expression was positive for three built-in reporter genes for each of the clones. One could argue that the nuclear localization of two proteins expressed in yeast might give rise to false-positive interactions between proteins. To verify whether the observed interaction is true and occurs in a cell-free environment, we applied SPR analysis and examined interaction using purified Tanis and SAA proteins. A native form of SAA purified from human plasma as well as recombinant SAA, purified from bacteria as a GST-fusion protein, showed clear binding with recombinant Tanis, as observed by SPR in real time. Increasing amounts of Tanis passed over SAA immobilized on a sensor chip exhibited a concentration-dependent increase in SPR signal. The recombinant Tanis devoid of putative NH_2-terminal and transmembrane regions also interacted with SAA, raising the possibility that the binding site is located within 136 amino acid residues in the COOH-terminal region. SAA comprises a family of acute-phase inflammatory response proteins that are found in all vertebrates and are expressed primarily in the liver. They are classified as acute-phase A-SAAs or constitutive C-SAAs on the basis of their response to inflammatory stimuli. When challenged with inflammation under a variety of conditions, the concentration of A-SAAs in the circulation increases up to 1,000-fold, at times reaching 1 mg/ml.

The relationship between inflammation, in particular the acute-phase response, and chronic diseases such as type 2 diabetes and atherosclerosis has been the subject of recent investigation. Blood concentrations of markers of the acute-phase response, including SAA, C-reactive protein, and interleukin-6, are elevated in type 2 diabetes (30–34). In addition, a recent prospective study showed that elevated circulating acute-phase response proteins predicted the development of type 2 diabetes in a cohort of >27,000 U.S. women (34), supporting a role for inflammation in diabetogenesis. Increased circulating levels of these proteins have also been associated with increased overall mortality and cardiovascular mortality (35–38). Indeed, it has been suggested that type 2 diabetes and atherosclerosis both may represent chronic inflammatory processes that are exacerbated by increased circulating levels of these acute-phase proteins (35,39). SAA is synthesized in the liver and incorporated in HDL cholesterol when released into the circulation. Alteration of HDL metabolism and cholesterol transport that could promote a proatherogenic phenotype has been proposed as a possible mechanism to explain the link between type 2 diabetes and atherosclerosis (40,41). Therefore, we hypothesize that Tanis is a novel hepatic receptor for SAA and that its expression is regulated by glucose. Both the Tanis receptor itself and its interaction with SAA represent new targets with great potential for the development of treatment and/or preventive strategies in type 2 diabetes, dyslipidemia, and cardiovascular disease, as well as other diseases with chronic inflammatory components, such as rheumatoid arthritis. Additional investigation into the relationship between Tanis and SAA may also lead to the identification of a biochemical mechanism that explains the link between inflammation and type 2 diabetes.

In summary, we identified a novel gene (Tanis) whose expression is markedly influenced by glucose and is dysregulated after fasting in the diabetic state. The Tanis protein seems to be a receptor that binds to SAA, an acute-phase inflammatory response protein previously implicated in the development of type 2 diabetes and atherosclerosis. We suggest that Tanis represents a new target for the development of therapeutic agents for type 2 diabetes and cardiovascular disease.

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