

Elevation in Tanis Expression Alters Glucose Metabolism and Insulin Sensitivity in H4IIE Cells

Yuan Gao,¹ Ken Walder,¹ Terry Sunderland,¹ Lakshmi Kantham,¹ Helen C. Feng,¹ Melissa Quick,¹ Natalie Bishara,¹ Andrea de Silva,¹ Guy Augert,² Janette Tenne-Brown,¹ and Gregory R. Collier¹

Increased hepatic glucose output and decreased glucose utilization are implicated in the development of type 2 diabetes. We previously reported that the expression of a novel gene, Tanis, was upregulated in the liver during fasting in the obese/diabetic animal model *Psammomys obesus*. Here, we have further studied the protein and its function. Cell fractionation indicated that Tanis was localized in the plasma membrane and microsomes but not in the nucleus, mitochondria, or soluble protein fraction. Consistent with previous gene expression data, hepatic Tanis protein levels increased more significantly in diabetic *P. obesus* than in nondiabetic controls after fasting. We used a recombinant adenovirus to increase Tanis expression in hepatoma H4IIE cells and investigated its role in metabolism. Tanis overexpression reduced glucose uptake, basal and insulin-stimulated glycogen synthesis, and glycogen content and attenuated the suppression of PEPCK gene expression by insulin, but it did not affect insulin-stimulated insulin receptor phosphorylation or triglyceride synthesis. These results suggest that Tanis may be involved in the regulation of glucose metabolism, and increased expression of Tanis could contribute to insulin resistance in the liver. *Diabetes* 52:929–934, 2003

A major factor that contributes to hyperglycemia in type 2 diabetes is excessive hepatic glucose production. Depending on the concentrations of insulin and glucagon in the portal blood and the level of glucose itself, the liver either stores glucose in the form of glycogen or, upon the depletion of glycogen, synthesizes glucose via the gluconeogenic pathway for export. In many subjects with type 2 diabetes, hepatic glucose utilization, associated with reduced glucokinase activity, is diminished (1). Furthermore, insulin is less effective in suppressing gluconeogenesis in these subjects, leading to elevated hepatic glucose output (2,3). The implication of these defects in the development of hyperglycemia has been supported by studies of transgenic mice

that overexpress hepatic PEPCK or glucose-6-phosphatase. A modest increase in their activities (two- to threefold) caused hyperglycemia, hyperinsulinemia, impaired glucose tolerance, and decreased glycogen storage (4–6). Conversely, increased hepatic glucose utilization through glucokinase overexpression lowered plasma glucose concentration, improved insulin sensitivity, and increased glycogen storage in rats (7,8). These findings strongly suggest a critical role of the liver in maintaining glucose homeostasis.

As both genetic and environmental factors play a role in the development of type 2 diabetes (9), we have used a polygenic animal model, *Psammomys obesus* (Israeli sand rat), to identify genes that may contribute to its pathogenesis. When housed in the laboratory and maintained on an ad libitum diet of standard rodent food, a proportion of the animals spontaneously develop metabolic abnormalities, including obesity and type 2 diabetes (10–12). The distributions of body weight, blood glucose, and insulin levels are continuous across the *P. obesus* population in a manner analogous to those observed in human populations. This makes *P. obesus* a unique animal model for studies of type 2 diabetes. We previously identified a number of genes that were dysregulated in diabetic *P. obesus*. For instance, the expression of the beacon gene in the hypothalamus was positively correlated with percentage of body fat, and intracerebroventricular infusion of the protein resulted in a dose-dependent increase in food intake and body weight and an increase in the hypothalamic expression of neuropeptide Y (13). More recently, we reported that a gene, Tanis, which encoded a protein of 188 amino acids with a putative transmembrane domain, was upregulated after fasting in the liver of diabetic *P. obesus* but not in nondiabetic controls, suggesting that Tanis may play an important role in liver metabolism (14). Here we studied the cellular localization and changes in Tanis protein levels in the liver of *P. obesus* and investigated the effects of Tanis overexpression on glucose metabolism in H4IIE cells using a recombinant adenovirus (Ad).

RESEARCH DESIGN AND METHODS

Experimental animals. The use of all animals in this study was approved by Deakin University Animal Ethics Committee. A colony of *P. obesus* was maintained as previously described (14). Animals were given a standard laboratory diet, from which 12% of energy was derived from fat, 63% was derived from carbohydrate, and 25% was derived from protein (Barastoc, Pakenham, Australia). Under such conditions, a proportion of the animals developed obesity and diabetes. At 16 weeks of age, whole blood glucose and plasma insulin concentrations were determined using an automated glucose analyzer and radioimmunoassay, respectively (14). The animals were classi-

From the ¹Metabolic Research Unit, School of Health Sciences, Deakin University, Victoria, Australia; and ²Merck Santé, Lyon, France.

Address correspondence and reprint requests to Yuan Gao, Metabolic Research Unit, School of Health Sciences, Deakin University, Pigdons Road, Victoria 3217, Australia. E-mail: gao@deakin.edu.au.

Received for publication 22 August 2002 and accepted in revised form 7 January 2003.

Ad, adenovirus; DMEM, Dulbecco's modified Eagle's medium; GFP, green fluorescence protein; HDM, high-density microsomes; IR, insulin receptor; IRS, IR substrate; LDM, low-density microsome; M/N, mitochondria/nucleus; MOI, multiplicity of infection; PM, plasma membrane; SAA-1 β , serum amyloid A-1 β .

fied as nondiabetic (euglycemia [≤ 8 mmol/l], euinsulinemia [≤ 150 mU/l]) or type 2 diabetic (hyperglycemia [≥ 8 mmol/l], hyperinsulinemia [≥ 150 mU/l]) as previously described (11).

Reagents. All tissue culture media, supplements, and FBS were obtained from Life Technologies (Melbourne, Australia). Antibodies for phosphotyrosine (PY20), insulin receptor substrate (IRS)-1, and insulin receptor (IR)- β were from Santa Cruz (Santa Cruz, CA) and were used according to the manufacturer's recommendations. ^{14}C -glucose and ^{14}C -glycerol were from Amersham Pharmacia Biotech (NSW, Australia). Hexokinase, amyloglucosidase, and glucose-6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*) were from Sigma (NSW, Australia). Reagents for protein concentration determination were from Bio-Rad (NSW, Australia).

Cell fractionation. For determining Tanis protein content and its cellular localization, nondiabetic and diabetic animals (18 weeks old) were either fed ad libitum or fasted for 24 h before anesthesia. Fresh liver tissue was homogenized in a glass douncer and fractionated by differential centrifugation into plasma membrane, high-density microsomes, low-density microsomes, nuclei/mitochondria, and soluble proteins as described (15). In some cases, the high-density and low-density microsomes were pooled in one fraction designated as microsomal proteins.

Generation of Tanis polyclonal antibody and immunoblots. The cDNA encoding the COOH-terminus of Tanis from *P. obesus* (amino acids 54–188) was subcloned into the pGEX-5X-1 expression vector (Amersham Pharmacia Biotech). The COOH-terminus of Tanis was expressed as a glutathione S-transferase fusion protein in *Escherichia coli* strain BL21, purified over glutathione-Sepharose 4B and then separated on preparative 12.5% SDS-PAGE. The protein bands in the gels (containing 5 mg of recombinant protein) were excised, homogenized, and used to immunize two New Zealand rabbits. Before use, Tanis-specific antibody was purified from the antiserum against its antigen covalently coupled to N-hydroxysuccinimide-activated Sepharose (Amersham Pharmacia Biotech). This antibody was found to cross-react with Tanis from human, mouse, and rat. For Western blot, protein samples from animal tissues or cultured cells were separated on 12.5% SDS-PAGE and transferred to polyvinylidene difluoride membrane. The membrane was incubated with the primary antibody and then an appropriate secondary antibody. Immunoreactive proteins were visualized using an enhanced chemiluminescent detection system (Amersham Pharmacia Biotech).

Construction of recombinant Ad. Recombinant Ad for the overexpression of Tanis (Ad-Tanis) was constructed using the AdEasy kit supplied by Q-biogene (Carlsbad, CA). Briefly, the entire coding sequence of *P. obesus* Tanis cDNA (0.56 kb) was subcloned into the transfer vector pShuttle-CMV. This recombinant plasmid and the adenoviral DNA were introduced together into *E. coli* strain BJ5181 for homologous recombination to take place. The recombinant viral DNA was linearized with *PacI*. The large fragment was gel purified and transfected into 293A cells for plaque formation. Plaques were screened for Tanis expression in 293A cells by Western blots with the Tanis antibody described above. Recombinant Ad-Tanis was amplified in 293A cells, purified over a CsCl gradient, and dialyzed in a buffer of 10 mmol/l Tris (pH 8.0), 2 mmol/l MgCl₂, and 5% (wt/vol) sucrose. The titer of viral solution was estimated by measuring absorbance at 280 nm. One optical density unit corresponded to 1.1×10^{12} viral particles/ml. A control Ad overexpressing green fluorescence protein (Ad-GFP) was purchased from Q-biogene, amplified, and purified as above. All viral solutions were aliquotted and stored at -80°C until use.

Cell culture. Hepatoma H4IIE cells were cultured in monolayer in Dulbecco's modified Eagle's medium (DMEM; 4.5 g/l glucose) containing 10% heat-inactivated FBS and antibiotics (penicillin, 75 $\mu\text{g/ml}$; streptomycin, 50 $\mu\text{g/ml}$) in 5% CO₂/95% air at 37°C. For Ad-mediated overexpression studies, $\sim 90\%$ confluent H4IIE cells in six-well plates were infected with no virus (Control), Ad-GFP, or Ad-Tanis in 400 μl of growth medium for 5 h at 37°C, and then 2–3 ml of growth medium was added to each well. Thirty hours after the start of infection, the cells were incubated in serum-free DMEM (1 g/l glucose) overnight before assays were performed. Thus, all assays were done 2 days after viral infection, by which time maximal overexpression of Tanis was observed (see RESULTS). Daily microscopic examination indicated that cell survival was not affected by Ad infection for at least 10 days after infection.

Glycogen synthesis and content. Glycogen synthesis was measured by the incorporation of [^{14}C]glucose into glycogen as described (16). Briefly, Ad-infected cells were serum-starved overnight and then treated with serum-free DMEM containing 5.5 mmol/l glucose and 0.5 $\mu\text{Ci/ml}$ [U- ^{14}C]-D-glucose in the absence or presence of insulin (10, 100, or 1,000 nmol/l) for 2 h. The cells were then washed three times in ice-cold PBS, solubilized in 1 mol/l potassium hydroxide (KOH), and heated at 95°C for 10 min. Glycogen was precipitated twice with acetone, resuspended in water, and counted for ^{14}C using a liquid scintillation counter. For determining glycogen content, cells were incubated for 5 h with insulin. Glycogen was extracted with 0.1 mol/l KOH. After the

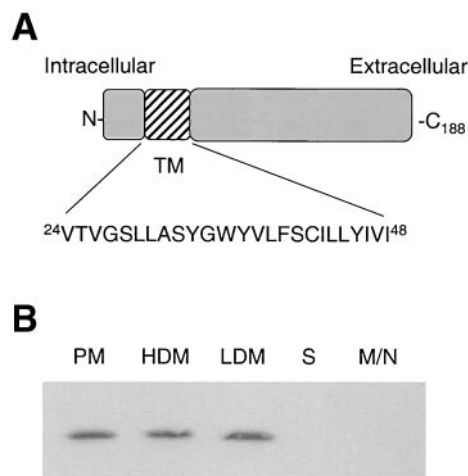


FIG. 1. Tanis protein (A) and its cellular localization as determined by cell fractionation and Western blot (B). A: Sequence of the hydrophobic transmembrane (TM) domain and orientation of the protein in the membrane are depicted. B: Liver cells from nondiabetic *P. obesus* were fractionated into PM, HDM, LDM, M/N, and soluble (S) proteins. Forty micrograms of protein from each fraction was separated on 12.5% SDS-PAGE, transferred to polyvinylidene difluoride (PVDF), and probed in a Western blot with a Tanis-specific polyclonal antibody.

extract was adjusted to pH 4.5 with 3 mol/l acetic acid, glycogen was digested with amyloglucosidase (17). The released glucose was measured enzymatically with hexokinase and glucose-6-phosphate dehydrogenase (18).

Glucose uptake. Two days after Ad infection, H4IIE cells were incubated with 0 or 100 nmol/l insulin in Krebs Ringer phosphate buffer for 30 min at 37°C. [^{14}C] 2-deoxyglucose (1 $\mu\text{Ci/ml}$) was then added to the cells to a final concentration of 2 mmol/l. The cells were incubated for an additional 15 min at 37°C, washed three times in ice-cold PBS, and lysed in 1% SDS. An aliquot was counted for ^{14}C .

Quantification of PEPCK mRNA. H4IIE cells were infected with Ad and treated with 0 or 100 nmol/l insulin for 2 h. Total RNA was isolated using Trizol (Life Technologies), and concentration was determined using an Agilent 2100 Bioanalyser. cDNA was synthesized using Superscript II (Life Technologies). PEPCK transcript was quantified using real-time PCR technology on an ABI Prism 7700 sequence detector using the forward primer 5'-AGCCATGTG CAACTCATGCA-3' and the reverse primer 5'-CTCTGGTGCACCTGAAACA-3'. PCR conditions were 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. PEPCK mRNA content was normalized against cyclophilin transcript in the same samples quantified under the same conditions.

Triglyceride synthesis. Triglyceride synthesis was assayed using a modified method (19). Briefly, after infection with appropriate Ad and overnight serum starvation, H4IIE cells were incubated with DMEM (5.5 mmol/l glucose) containing 0.8 $\mu\text{Ci/ml}$ [^{14}C]glycerol for 6 h at 37°C. The cells were then rapidly washed twice in ice-cold PBS, scraped, and pelleted by centrifugation. Cells were lysed in 1 ml of hexane:isopropanol (3:2, vol/vol), and then 0.5 ml of H₂O was added. The mixture was vortexed vigorously and centrifuged at 4,000g for 10 min. The upper organic phase containing triglycerides was counted for ^{14}C incorporation.

Statistical analysis. All experiments were done at least twice each with three replicates. Data were expressed as mean \pm SE and were analyzed by ANOVA. Differences were considered significant at $P < 0.05$.

RESULTS

Cellular distribution of Tanis. The Tanis protein contains a highly hydrophobic region near the NH₂-terminus ($^{24}\text{VTVGSLASYGWYVLFSCILLYIVI}^{48}$; Fig. 1A). Bioinformatic analysis using a number of computer programs indicates that this region is a putative transmembrane domain, suggesting that Tanis may be a membrane protein. For confirming its cellular localization, liver cells from nondiabetic *P. obesus* were separated into five fractions by differential centrifugation: plasma membrane (PM), high-density microsomes (HDM), low-density microsome

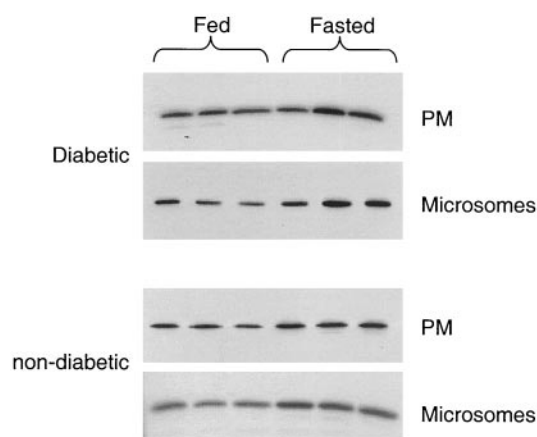


FIG. 2. Tanis protein levels in the liver of nondiabetic and diabetic *P. obesus* that had been fed ad libitum or fasted for 24 h before anesthesia. PM and microsomal proteins (containing both high-density and low-density) were isolated. Forty micrograms of protein was separated on 12.5% SDS-PAGE, transferred to PVDF, and probed in a Western blot with a Tanis-specific polyclonal antibody. Three animals in each treatment for both phenotypes were analyzed.

(LDM), mitochondria/nucleus (M/N), and soluble proteins. Western blot of these fractions found Tanis in the PM, HDM, and LDM fractions but not in the M/N or soluble proteins (Fig. 1B). As another approach to establish its localization, we immunofluorescently stained H4IIE cells with the Tanis antibody, using IR antibody (anti-IR β) as control in separate stainings. The staining patterns were found to be similar for both Tanis and IR (data not shown). These data indicate that Tanis is a PM protein; however, a proportion of the protein is associated with intracellular microsomes.

Increase in Tanis protein levels in fasted *P. obesus*.

Previous studies indicated that hepatic Tanis gene expression in diabetic *P. obesus* and nondiabetic controls was similar in the fed state, but fasting increased the expression levels, particularly in the diabetic animals (14). Consequently, we compared Tanis protein levels in the fed and fasted states in diabetic and nondiabetic *P. obesus*. PM and microsomes (containing both HDM and LDM) were prepared from liver cells of *P. obesus* that had been fed ad libitum or fasted for 24 h. Figure 2 shows that Tanis protein levels increased in both the PM and the microsomal fractions in fasted animals in comparison with fed controls. However, the increase was stronger in diabetic than in nondiabetic animals, particularly in the microsomal fraction. Consistent with Tanis gene expression studies (14), the protein levels seemed not to be significantly different in the fed state between diabetic and nondiabetic *P. obesus*.

Ad-mediated Tanis overexpression in H4IIE cells.

The regulation of Tanis gene expression and protein content in the liver during fasting suggests that this gene may be involved in metabolism. To further study such a possibility, we overexpressed Tanis in H4IIE cells using a recombinant Ad. The recombinant Ad for the expression of Tanis (Ad-Tanis) and the control Ad expressing the protein GFP (Ad-GFP) are replication-deficient in target hosts other than 293A cells. High multiplicity of infection (MOI), typically >100, is often required to achieve desirable levels of overexpression (20,21). We first titrated the amount of virus on cells grown in six-well plates using

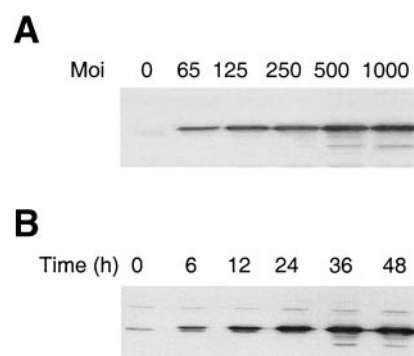


FIG. 3. Ad-mediated Tanis overexpression in H4IIE cells. **A:** Ninety percent confluent cells were infected with varying MOI of the Ad-Tanis and harvested 48 h after infection. **B:** Cells were infected with an MOI of 250 and harvested at different time points after infection. In both cases, Tanis protein was detected by Western blots.

varying amounts of virus. Tanis expression was detectable in cells infected with an MOI of 65, and the efficiency plateaued at 250–500 MOI (Fig. 3A). Using an MOI of 250, Tanis expression was evident 6 h after infection and peaked at ~36 h (Fig. 3B). Therefore, all subsequent overexpression studies were done using an MOI of 250 and cells were analyzed 48 h after infection.

Tanis overexpression reduced glycogen synthesis and glycogen content.

Glycogen synthesis was assayed in cells 2 days after infection with the recombinant virus in the presence or absence of insulin. Consistent with previous reports (22), glycogen synthesis in control cells was responsive to insulin stimulation, increasing by 51, 62, and 74% at 10, 100, and 1,000 nmol/l insulin, respectively, when compared with 0 nmol/l insulin (Fig. 4A). Infecting the cells with Ad-GFP had no effect on basal or insulin-stimulated glycogen synthesis. In contrast, basal glycogen synthesis in Ad-Tanis-infected cells was slightly but significantly (12%) lower than in control cells, and this

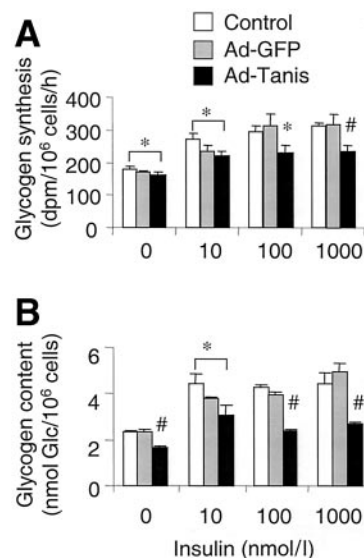


FIG. 4. Effect of Tanis overexpression on glycogen synthesis (**A**) and glycogen content (**B**) in H4IIE cells infected with no virus (control), Ad-GFP, or Ad-Tanis. Glycogen synthesis was measured by the incorporation of [¹⁴C]glucose, and glycogen content was determined enzymatically. Both experiments were repeated three times each with three replicates. Similar results were obtained from each experiment. * $P < 0.05$, # $P < 0.01$.

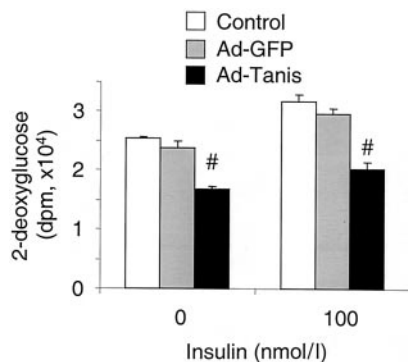


FIG. 5. Effect of Tanis overexpression on glucose uptake in H4IIE cells. Glucose uptake assays were performed using [¹⁴C]2-deoxyglucose (2 mmol/l, 1 μ Ci/ml) for 15 min at 37°C after insulin treatment for 30 min at 37°C. The experiment was repeated three times with similar results. #*P* < 0.01.

difference became more profound in the presence of insulin. For example, at 100 nmol/l insulin, glycogen synthesis was decreased by 21% as compared with control cells.

Consistent with the glycogen synthesis data, infection with Ad-GFP had no effect on glycogen content, whereas infection with Ad-Tanis significantly decreased glycogen content. Glycogen content in Tanis overexpressing cells was decreased relative to control or Ad-GFP-infected cells. For example, glycogen in Ad-Tanis-infected cells was 71, 70, 57, and 61% of the control cells treated with 0, 10, 100, and 1,000 nmol/l insulin, respectively (Fig. 4B). The effect of insulin on glycogen content in cells overexpressing Tanis seemed to be maximal at 10 nmol/l. There was a trend for a slight decrease in the effect of insulin from 10 to 100 or 1,000 nmol/l in Ad-Tanis-infected cells; however, this decrease did not reach statistical significance.

Tanis overexpression reduced glucose uptake. We next examined the effect of Tanis overexpression on glucose uptake in the cells. Infecting cells with the control virus Ad-GFP had no effect on glucose uptake (Fig. 5). However, Ad-Tanis infection consistently decreased glucose uptake by ~35%, both with or without insulin. As expected, insulin had little effect on glucose uptake because the liver-specific Glut2, unlike Glut4 in fat and muscle, does not translocate to the cell surface in response to insulin.

Effect of Tanis overexpression on PEPCK gene expression. PEPCK, which catalyzes the rate-limiting step in

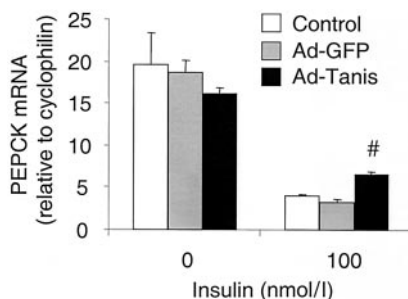


FIG. 6. Effect of Tanis overexpression on PEPCK gene expression in H4IIE cells. Ad-infected cells were treated with insulin for 2 h before RNA isolation. PEPCK transcript was quantified by real-time RT-PCR and normalized against cyclophilin transcript. The experiment was repeated twice with similar results. #*P* < 0.01.

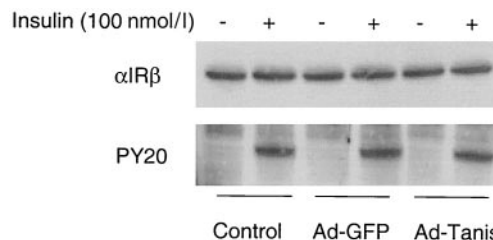


FIG. 7. Tanis overexpression did not affect IR phosphorylation. H4IIE cells were infected with Ad for 48 h and then treated with 0 or 100 nmol/l insulin for 15 min at 37°C. IR protein and its tyrosine phosphorylation were detected in Western blots with an IR antibody (α IR β) and PY20, respectively.

hepatic gluconeogenesis, is regulated on the transcriptional level; its expression is suppressed by insulin or stimulated by glucagon and cAMP. PEPCK transcript levels were similar in control, Ad-GFP-infected, and Ad-Tanis-infected cells in the absence of insulin, and the levels were suppressed by 79 and 82% by 100 nmol/l insulin in control and Ad-GFP infected cells, respectively (Fig. 6). However, insulin decreased the transcript by only 60% in Ad-Tanis-infected cells. As a result, these cells had twice as much PEPCK mRNA as compared with control or Ad-GFP-infected cells in the presence of insulin.

Insulin receptor phosphorylation was not affected by Tanis overexpression. Tanis-induced decrease in insulin-stimulated glycogen synthesis in Fig. 4A may be a result of impaired insulin signaling. However, the overexpression did not alter IR content or insulin-induced tyrosine phosphorylation in the cells (Fig. 7). Furthermore, the phosphorylation of IRS-1 was unaltered (data not shown). These data indicate that Tanis overexpression did not compromise the initial events of insulin signaling.

Triglyceride synthesis was not affected by Tanis overexpression. We examined whether Tanis overexpression altered triglyceride synthesis in the cells by the incorporation of [¹⁴C]glycerol. Figure 8 shows that Tanis expression had little effect on triglyceride synthesis. These data also indicate that the effects of Tanis on glycogen synthesis and glucose uptake were specific and not due to any impaired cell viability.

DISCUSSION

In this report, we extend our earlier findings on the novel gene, Tanis, which was discovered in the diabetic animal

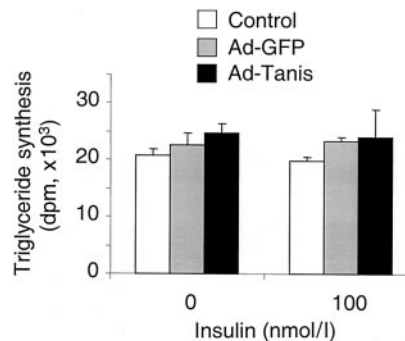


FIG. 8. Triglyceride synthesis was not affected by Tanis overexpression in H4IIE cells. H4IIE cells were infected with Ad for 48 h. Triglyceride synthesis was measured by the incorporation of [¹⁴C]glycerol at 0 or 100 nmol/l insulin for 6 h at 37°C. The experiment was repeated three times with similar results.

model *P. obesus* (14). We established Tanis cellular localization, confirmed the differential regulation of Tanis protein levels with fasting in diabetic *P. obesus*, and demonstrated that Tanis overexpression in H4IIE cells led to insulin resistance with respect to glucose uptake, glycogen synthesis, and PEPCK regulation.

As the Tanis protein has a putative hydrophobic transmembrane domain, we first determined its cellular localization. Cell fractionation of *P. obesus* liver cells and immunofluorescence of H4IIE cells consistently showed Tanis on the plasma membrane, although some of the protein was associated with intracellular microsomes. In agreement with our previous data that hepatic Tanis gene expression increased twofold in the diabetic animals but only 50% in nondiabetic *P. obesus* during fasting (14), we also confirmed that Tanis protein in the liver changed in a similar manner, increasing more significantly in diabetic animals but to a lesser extent in nondiabetic controls after fasting.

That Tanis gene expression was dysregulated in the liver of diabetic *P. obesus* and that the expression levels were associated with blood glucose concentration in vivo and inversely related to glucose concentrations in the media of hepatoma cells suggest that Tanis expression was regulated by glucose and may play a role in hepatic glucose metabolism (14). H4IIE hepatoma cells, which have many properties similar to those of hepatocytes in vivo, including the regulation of PEPCK and insulin-stimulated glycogen synthesis (23,24), were used as a model system to further study the function of Tanis. Ad-mediated Tanis overexpression decreased glucose uptake, glycogen synthesis, and glycogen storage. Such changes were not due to impairment in cell viability, as triglyceride synthesis was unaffected by the overexpression. It is notable that Tanis overexpression decreased insulin-stimulated glycogen synthesis more than basal glycogen synthesis, suggesting the presence of insulin resistance. As Tanis overexpression did not affect IR and IRS-1 protein content or their phosphorylation, the decrease in insulin sensitivity seemed to have occurred farther downstream in the signaling pathway.

Elevation in Tanis expression also attenuated the suppression of PEPCK gene expression by insulin. PEPCK catalyzes the rate-limiting step in hepatic gluconeogenesis. Without any known allosteric effectors, its activity is regulated at the transcriptional level and is suppressed by insulin or stimulated by glucagon and cAMP (25). Studies with transgenic animals have established that even modest elevations in PEPCK gene expression can cause abnormalities, including glucose intolerance and hyperglycemia (4,5). Although Tanis overexpression had no effect on PEPCK expression in the absence of insulin, it attenuated the suppression of the expression by insulin. As such, PEPCK mRNA in Ad-Tanis-infected cells was twofold higher when compared with control or Ad-GFP-infected cells in the presence of 100 nmol/l insulin (Fig. 6).

These in vitro results are consistent with studies in *P. obesus*, which have shown that diabetic animals had decreased glycogen synthase activity and glycogen storage but increased PEPCK activity (11,26–28). Together these results provide additional evidence that Tanis may play a role in hepatic glucose metabolism, and its overexpression

in the postprandial state could contribute to hepatic insulin resistance characterized by impaired glucose utilization and elevated glucose output. However, it should be noted that hepatic Tanis gene expression and protein levels were similar in the fed state between diabetic and nondiabetic *P. obesus*. The differential regulation was observed only in the fasted state, with stronger upregulation in the diabetic animals. Additional studies are required to clarify further the role of Tanis in the development of insulin resistance in vivo.

How Tanis induces such changes in glucose metabolism is unknown. However, a number of novel membrane proteins have recently been implicated in lipid metabolism, adipogenesis, and/or obesity (29,30). One of these proteins is the membrane glycoprotein PC-1, which, when highly expressed, causes insulin resistance in muscle and skin fibroblasts (31–34). It is interesting that Tanis and PC-1 have some features in common. Both proteins have a wide range of tissue distribution, are localized in plasma and intracellular membranes, and have similar topology in the plasma membrane (Fig. 1) (14,35). Their role in metabolism also seems to be tissue-specific. PC-1 is not differentially expressed in adipose tissue of high-fat-fed or streptozotocin-induced diabetic rats and does not induce insulin resistance in 3T3-L1 adipocytes (36). Similarly, the expression of Tanis is not affected in adipocytes of fasted *P. obesus*, and its overexpression has no effect on glucose uptake, lipogenesis, or fatty acid uptake in differentiated 3T3-L1 cells (data not shown). Despite such similarities, the mechanisms by which they cause insulin resistance are different. PC-1 acts by impairing insulin-stimulated IR tyrosine phosphorylation, whereas Tanis seems to affect downstream insulin signaling events (Fig. 7) (33).

One possibility for Tanis to exert its function in vivo is by interacting with other proteins. In this regard, Tanis was found to bind the acute-phase protein serum amyloid A-1 β (SAA-1 β) by yeast two hybrid screening, and this interaction was confirmed in vitro using purified Tanis and SAA (14). It is possible that Tanis acts as a receptor for SAA1 β . The circulating levels of acute phase proteins, including SAA and C-reactive protein, are known to be increased in many subjects with diabetes and may be implicated in the development of this disease (37–39). The significance of the Tanis-SAA1 β interaction in metabolism is a focus of future studies.

In conclusion, we have shown that Tanis protein levels are altered in the liver of diabetic *P. obesus* after fasting, and elevation in Tanis expression impairs glucose metabolism in H4IIE cells. The in vitro results suggest that elevated Tanis levels may contribute to hyperglycemia in patients with type 2 diabetes by decreasing hepatic glucose utilization and increasing hepatic glucose output.

ACKNOWLEDGMENTS

This research was funded by Autogen, Melbourne, Australia.

REFERENCES

1. Seoane J, Barbera A, Telemaque-Potts S, Newgard CB, Guinovart JJ: Glucokinase overexpression restores glucose utilization and storage in cultured hepatocytes from male Zucker diabetic fatty rats. *J Biol Chem* 274:31833–31838, 1999
2. DeFronzo RA, Ferrannini E, Simonson DC: Fasting hyperglycemia in

- non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism* 38:387–395, 1989
3. Friedman JE, Yun JS, Patel YM, McGrane MM, Hanson RW: Glucocorticoids regulate the induction of phosphoenolpyruvate carboxykinase (GTP) gene transcription during diabetes. *J Biol Chem* 268:12952–12957, 1993
 4. Valera A, Pujol A, Pelegrin M, Bosch F: Transgenic mice overexpressing phosphoenolpyruvate carboxykinase develop non-insulin-dependent diabetes mellitus. *Proc Natl Acad Sci U S A* 91:9151–9154, 1994
 5. Sun Y, Liu S, Ferguson S, Wang L, Klepcyk P, Yun JS, Friedman JE: Phosphoenolpyruvate carboxykinase overexpression selectively attenuates insulin signaling and hepatic insulin sensitivity in transgenic mice. *J Biol Chem* 277:23301–23307, 2002
 6. Trinh KY, O'Doherty RM, Anderson P, Lange AJ, Newgard CB: Perturbation of fuel homeostasis caused by overexpression of the glucose-6-phosphatase catalytic subunit in liver of normal rats. *J Biol Chem* 273:31615–31620, 1998
 7. Niswender KD, Shiota M, Postic C, Cherrington AD, Magnuson MA: Effects of increased glucokinase gene copy number on glucose homeostasis and hepatic glucose metabolism. *J Biol Chem* 272:22570–22575, 1997
 8. O'Doherty RM, Lehman DL, Telemaque-Potts S, Newgard CB: Metabolic impact of glucokinase overexpression in liver: lowering of blood glucose in fed rats is accompanied by hyperlipidemia. *Diabetes* 48:2022–2027, 1999
 9. Kahn CR, Vicent D, Doria A: Genetics of non-insulin-dependent (type-II) diabetes mellitus. *Annu Rev Med* 47:509–531, 1996
 10. Shafir E, Gutman A: *Psammomys obesus* of the Jerusalem colony: a model for nutritionally induced, non-insulin-dependent diabetes. *J Basic Clin Physiol Pharmacol* 4:83–99, 1993
 11. Barnett M, Collier GR, Collier FM, Zimmet P, O'Dea K: A cross-sectional and short-term longitudinal characterisation of NIDDM in *Psammomys obesus*. *Diabetologia* 37:671–676, 1994
 12. Walder KR, Fahey RP, Morton GJ, Zimmet PZ, Collier GR: Characterization of obesity phenotypes in *Psammomys obesus* (Israeli sand rats). *Int J Exp Diabetes Res* 1:177–184, 2000
 13. Collier GR, McMillan JS, Windmill K, Walder K, Tenne-Brown J, de Silva A, Trevaskis J, Jones S, Morton GJ, Lee S, Augert G, Civitarese A, Zimmet PZ: Beacon: a novel gene involved in the regulation of energy balance. *Diabetes* 49:1766–1771, 2000
 14. Walder K, Kantham L, McMillan JS, Trevaskis J, Kerr L, De Silva A, Sunderland T, Godde N, Gao Y, Bishara N, Windmill K, Tenne-Brown J, Augert G, Zimmet PZ, Collier GR: Tanis: a link between type 2 diabetes and inflammation? *Diabetes* 51:1859–1866, 2002
 15. Simpson IA, Yver DR, Hissin PJ, Wardzala LJ, Karnieli E, Salans LB, Cushman SW: Insulin-stimulated translocation of glucose transporters in the isolated rat adipose cells: characterization of subcellular fractions. *Biochim Biophys Acta* 763:393–407, 1983
 16. Huang D, Cheung AT, Parsons JT, Bryer-Ash M: Focal adhesion kinase (FAK) regulates insulin-stimulated glycogen synthesis in hepatocytes. *J Biol Chem* 277:18151–18160, 2002
 17. van Dijk TH, van der Sluijs FH, Wiegman CH, Baller JF, Gustafson LA, Burger HJ, Herling AW, Kuipers F, Meijer AJ, Reijngoud DJ: Acute inhibition of hepatic glucose-6-phosphatase does not affect gluconeogenesis but directs gluconeogenic flux toward glycogen in fasted rats: a pharmacological study with the chlorogenic acid derivative S4048. *J Biol Chem* 276:25727–25735, 2001
 18. Bergmeyer HU: *Methods of Enzymatic Analysis*. New York, Academic Press, 1985
 19. Grunfeld C, Dinarello CA, Feingold KR: Tumor necrosis factor- α , interleukin-1, and interferon α stimulate triglyceride synthesis in HepG2 cells. *Metabolism* 40:894–898, 1991
 20. Slosberg ED, Desai UJ, Fanelli B, St Denny I, Connelly S, Kaleko M, Boettcher BR, Caplan SL: Treatment of type 2 diabetes by adenoviral-mediated overexpression of the glucokinase regulatory protein. *Diabetes* 50:1813–1820, 2001
 21. Bai J, Cederbaum AI: Adenovirus-mediated overexpression of catalase in the cytosolic or mitochondrial compartment protects against cytochrome P450 2E1-dependent toxicity in HepG2 cells. *J Biol Chem* 276:4315–4321, 2001
 22. Hofmann C, Marsh JW, Miller B, Steiner DF: Cultured hepatoma cells as a model system for studying insulin processing and biologic responsiveness. *Diabetes* 29:865–874, 1980
 23. Valera A, Bosch F: Glucokinase expression in rat hepatoma cells induces glucose uptake and is rate limiting in glucose utilization. *Eur J Biochem* 222:533–539, 1994
 24. Duong DT, Waltner-Law ME, Sears R, Sealy L, Granner DK: Insulin inhibits hepatocellular glucose production by utilizing liver-enriched transcriptional inhibitory protein to disrupt the association of CREB-binding protein and RNA polymerase II with the phosphoenolpyruvate carboxykinase gene promoter. *J Biol Chem* 277:32234–32242, 2002
 25. Magnuson MA, Quinn PG, Granner DK: Multihormonal regulation of phosphoenolpyruvate carboxykinase-chloramphenicol acetyltransferase fusion genes: insulin's effects oppose those of cAMP and dexamethasone. *J Biol Chem* 262:14917–14920, 1987
 26. Ziv E, Kalman R, Hershkop K, Barash V, Shafir E, Bar-On H: Insulin resistance in the NIDDM model *Psammomys obesus* in the normoglycaemic, normoinsulinaemic state. *Diabetologia* 39:1269–1275, 1996
 27. Kalderon B, Gutman A, Levy E, Shafir E, Adler JH: Characterization of stages in development of obesity-diabetes syndrome in sand rat (*Psammomys obesus*). *Diabetes* 35:717–724, 1986
 28. Marquie G, Duhault J, Jacotot B: Diabetes mellitus in sand rats (*Psammomys obesus*): metabolic pattern during development of the diabetic syndrome. *Diabetes* 33:438–443, 1984
 29. Baulande S, Lasnier F, Lucas M, Pairault J: Adiponutrin, a transmembrane protein corresponding to a novel dietary- and obesity-linked mRNA specifically expressed in the adipose lineage. *J Biol Chem* 276:33336–33344, 2001
 30. Karpichev IV, Cornivelli L, Small GM: Multiple regulatory roles of a novel *Saccharomyces cerevisiae* protein, encoded by YOL002c, in lipid and phosphate metabolism. *J Biol Chem* 277:19609–19617, 2002
 31. Youngren JF, Maddux BA, Sasson S, Sbraccia P, Tapscott EB, Swanson MS, Dohm GL, Goldfine ID: Skeletal muscle content of membrane glycoprotein PC-1 in obesity: relationship to muscle glucose transport. *Diabetes* 45:1324–1328, 1996
 32. Frittitta L, Spampinato D, Solini A, Nosadini R, Goldfine ID, Vigneri R, Trischitta V: Elevated PC-1 content in cultured skin fibroblasts correlates with decreased in vivo and in vitro insulin action in nondiabetic subjects: evidence that PC-1 may be an intrinsic factor in impaired insulin receptor signaling. *Diabetes* 47:1095–1100, 1998
 33. Maddux BA, Goldfine ID: Membrane glycoprotein PC-1 inhibition of insulin receptor function occurs via direct interaction with the receptor α -subunit. *Diabetes* 49:13–19, 2000
 34. Pender C, Ortmeier HK, Hansen BC, Goldfine ID, Youngren JF: Elevated plasma cell membrane glycoprotein levels and diminished insulin receptor autophosphorylation in obese, insulin-resistant rhesus monkeys. *Metabolism* 51:465–470, 2002
 35. Goldfine ID, Maddux BA, Youngren JF, Frittitta L, Trischitta V, Dohm GL: Membrane glycoprotein PC-1 and insulin resistance. *Mol Cell Biochem* 182:177–184, 1998
 36. Sakoda H, Ogihara T, Anai M, Funaki M, Inukai K, Katagiri H, Fukushima Y, Onishi Y, Ono H, Yazaki Y, Kikuchi M, Oka Y, Asano T: No correlation of plasma cell 1 overexpression with insulin resistance in diabetic rats and 3T3-L1 adipocytes. *Diabetes* 48:1365–1371, 1999
 37. Kumon Y, Suehiro T, Itahara T, Ikeda Y, Hashimoto K: Serum amyloid A protein in patients with non-insulin-dependent diabetes mellitus. *Clin Biochem* 27:469–473, 1994
 38. Lin Y, Rajala MW, Berger JP, Moller DE, Barzilay N, Scherer PE: Hyperglycemia-induced production of acute phase reactants in adipose tissue. *J Biol Chem* 276:42077–42083, 2001
 39. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD, O'Reilly DS, Packard CJ, Sattar N: C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes* 51:1596–1600, 2002