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ORIGINAL ARTICLE

Decorin is a secreted protein associated with obesity and type 2 diabetes

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Objective: To characterize the expression of the small leucine-rich glycoprotein decorin in adipose tissue.
Design: Real-time PCR was used to measure decorin gene expression in adipose tissue from normal glucose tolerant (NGT), impaired glucose tolerant and type 2 diabetic (T2D) *Psammomys obesus*. Adipose tissue was fractionated to determine which cells were responsible for decorin expression. The location of decorin protein expression in adipose tissue was determined using immunohistochemistry. Real-time PCR was used to measure decorin mRNA levels in human adipose tissue from 16 insulin-sensitive, 16 insulin-resistant and 6 T2D human subjects. Circulating plasma decorin concentrations were measured by enzyme-linked immunosorbent assay in 145 NGT and 141 T2D human individuals from a large-scale epidemiological study in Mauritius.
Results: Decorin mRNA was found to be highly expressed in adipose tissue, and decorin gene expression was significantly higher in visceral than that in subcutaneous adipose tissue depots in both *P. obesus* and human subjects ($P=0.002$ and $P=0.001$, respectively). Decorin mRNA was predominantly expressed by stromal/vascular cells of adipose tissue, and decorin protein in adipose tissue was primarily detected adjacent to blood vessels. Circulating plasma decorin levels in humans were elevated by 12% in T2D ($P=0.049$) compared to NGT subjects. There was a significant independent correlation between plasma decorin levels and waist-to-hip ratio (WHR, $P=0.024$). In male subjects, plasma decorin levels were significantly correlated with WHR ($P=0.006$), and fasting and 2-h glucose levels in an oral glucose tolerance test ($P=0.027$ and $P=0.001$, respectively).
Conclusions: Decorin expression in adipose tissue was markedly upregulated in the obese state and may therefore play a role in adipose tissue homeostasis or in pathophysiology associated with obesity.
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Keywords: decorin; *Psammomys obesus*; adipose tissue

Introduction

Secreted proteins, hormones and cytokines produced by pancreatic β-cells and insulin-responsive tissues such as muscle, liver and adipose tissue are key regulators of energy metabolism and glucose homeostasis.[1] Insulin is the principal hormone that regulates glucose metabolism. It is secreted by pancreatic β-cells after food ingestion, enabling the body to store fuel by stimulating glucose uptake in skeletal muscle and adipose tissue as well as glycogen synthesis in muscle and liver and by suppressing gluconeogenesis in the liver.[2] In recent years, the identification of novel secreted proteins such as leptin, ghrelin, adiponectin, visfatin and retinol binding protein 4 has revolutionized our understanding of the role of circulating proteins in energy balance and glucose homeostasis.[3–7] Moreover, these secreted proteins have been shown to be integral to the pathophysiology of metabolic abnormalities such as glucose intolerance (type 2 diabetes, impaired glucose tolerance or impaired fasting glycaemia), insulin resistance, central obesity, dyslipidaemia and hypertension, which are associated with the metabolic syndrome.[8,9]

We have sought to identify secreted proteins that have not previously been associated with the development of obesity, type 2 diabetes and the metabolic syndrome. We have developed a signal sequence trap (SST) using RNA from *Psammomys obesus* (a unique polygenic animal model of obesity and type 2 diabetes[10]) to generate libraries of genes

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encoding proteins secreted from a range of tissues, including skeletal muscle, adipose tissue and liver. These SST clones were screened using cDNA microarray technology to identify genes that were differentially expressed between lean normal glucose tolerant (NGT), overweight and impaired glucose tolerant (IGT) and obese type 2 diabetic (T2D) *P. obesus*. Using this strategy, we have found that the small leucine-rich proteoglycan decorin was highly expressed in adipose tissue and that decorin expression was markedly upregulated in skeletal muscle and adipose tissue from obese *P. obesus*. The aim of this study was to characterize decorin expression in *P. obesus* and in human subjects.

Materials and methods

Experimental animals

A colony of *P. obesus* was maintained at Deakin University (Geelong, VIC, Australia). Animals were fed *ad libitum* a standard rodent laboratory diet from which 63% of energy was derived from carbohydrate, 25% from protein and 12% from fat (Barastoc, Pakenham, VIC, Australia). Animals were maintained in a temperature-controlled room ($22 \pm 1\,^{\circ}C$) with a 12–12 h light–dark cycle (0600–1800 light). At 16 weeks of age, animals were classified into three groups according to their blood glucose and plasma insulin concentrations as previously described.[10] The three groups were classified as follows: lean and NGT, overweight and impaired glucose tolerant (IGT) and obese, T2D. Whole blood glucose was measured using an enzymatic glucose analyser (model 27, Yellow Springs Instruments, Yellow Springs, OH, USA), and plasma insulin concentrations were determined using a double antibody solid-phase radioimmunoassay kit (Linco, Billerica, MA, USA).

At 18 weeks of age, male *P. obesus* were separated into two treatment groups, either 'fed' where animals have access to chow *ad libitum* or 'fasted' whereby animals were fasted for 24 h. The characteristics of each group are represented in Supplementary Table 1. Animals were killed by anaesthetic overdose (pentobarbitone, $120\,mg\,kg^{-1}$; Sigma, St Louis, MO, USA) and tissues were rapidly excised, snap frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ for subsequent RNA extraction. All experiments were conducted according to strict National Health and Medical Research Council guidelines and approved by the Deakin University Animal Welfare Committee.

RNA extraction

Total RNA was extracted from *P. obesus* tissues using TRIzol (Invitrogen, Carlsbad, CA, USA) in conjunction with RNeasy columns (Qiagen, Hilden, Germany). The quality and concentration of RNA was determined using the RNA 6000 Nano Assay and an Agilent 2100 Bioanalyser (Agilent Technologies, Santa Clara, CA, USA).

SST and cDNA microarray technology to identify differential expression

The SST was used in conjunction with cDNA microarray technology to identify decorin as previously described.[11] Standard *t*-tests comparing NGT vs IGT and NGT vs T2D were used to identify differential expression in skeletal muscle between the *P. obesus* groups. Gene expression was considered significantly different at $P<0.05$ level.

Real-time PCR

Total RNA was extracted from *P. obesus* and quantified as described above. First-strand cDNA was generated from RNA using SuperScript First-Strand Synthesis System for real-time PCR (RT-PCR) (Invitrogen). Decorin gene expression levels were quantitated using SYBR Green PCR Master Mix (Applied Biosystems, Worthington, UK) with an ABI PRISM 7700 Sequencing Detector (Perkin Elmer, Waltham, MA, USA). RT-PCR primers were as follows: *P. obesus* decorin forward 5′-TTCACCACTCGAAGATGACAC-3′, reverse 5′-TTCTGGGA TGAGCGAGGAAG-3′.

Stromal/vascular fractionation

P. obesus were killed by anaesthetic overdose and the epididymal adipose tissues rapidly excised. Approximately 1 g tissue was placed in 10 ml of Krebs Ringer phosphate buffer and minced using scissors. The minced tissue was washed several times with Krebs Ringer phosphate buffer and digested by the addition of type I collagenase ($0.75\,mg\,ml^{-1}$; Worthington, Lakewood, NJ, USA) for 20 min in a rocking water bath at $37\,^{\circ}C$. The digested tissue was filtered through nylon mesh into Krebs Ringer phosphate buffer and centrifuged at $300\,g$ for 10 min at room temperature. The floating adipocyte layer was collected and the supernatant aspirated to reveal the pellet fraction. Both fractions were snap frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ for subsequent RNA extraction and RT-PCR. RT-PCR primers to confirm correct fractionation were as follows: leptin forward 5′-TCGCGGTCTACCAACACATC-3′, leptin reverse 5′-TCTC TAGGTCGTTGGATATTTGCA-3′, CD68 forward 5′-GGACAG CTTACCTTTGGATTCAA-3′, CD68 reverse 5′-CTGTGGGAA GGACACATTGTATTC-3′.

Immunohistochemical analysis of decorin

Lean NGT and overweight IGT *P. obesus* were killed by anaesthetic overdose and epididymal, visceral (mesenteric) and subcutaneous (subscapular) adipose tissues rapidly excised and placed in 4% paraformaldehyde overnight at $4\,^{\circ}C$. Tissues were processed into paraffin using a Thermo-shandon Hypercentre XP (Shandon Scientific Ltd, Cheshire, England) and Tissuetek Embedding Centre (Miles Scientific, Naperville, IL, USA). Sections (5 μm thick) were cut and mounted onto polysine-coated slides (Menzel-Glaser, Braunschweig, Germany). After dewaxing and hydrating

through a graded alcohol series, the sections were quenched in a 3% H_2O_2, 50% methanol, 50% dH_2O solution for 5 min. Sections were then blocked with 10% normal rabbit serum/0.05% Tween-20/phosphate-buffered saline (PBS) for 30 min followed by 5 μg ml^{-1} anti-mouse decorin in 5% rabbit serum/0.05% Tween-20/PBS for 1 h. Next, the sections were washed in 0.05% Tween-20/PBS and then incubated with a rabbit anti-goat biotin antibody (DakoCytomation, Glostrup, Denmark) at a 1:200 dilution for 1 h. After washing, sections were incubated with a horseradish peroxidase–streptavidin (DakoCytomation) antibody at a 1:200 dilution for 1 h and washed, and DAB (DakoCytomation) was added and incubated for 5 min. Sections were washed in dH_2O and counterstained with haematoxylin for 1 min, dehydrated, mounted using DePeX (BDH, Poole, UK) and dried overnight. Negative control sections were incubated with 5% rabbit serum/0.05% Tween-20/PBS, substituting the decorin antibody for 5 μg ml^{-1} goat IgG (Zymed, South San Francisco, CA, USA).

Enzyme-linked immunosorbent assay

A sandwich enzyme-linked immunosorbent assay (ELISA) was developed using commercially available unlabelled and biotinylated human decorin affinity-purified polyclonal antibody (R&D Systems, Minneapolis, MN, USA). The unlabelled decorin antibody was diluted to 1 μg ml^{-1} in sodium carbonate/sodium bicarbonate coating buffer and added to Maxisorp ELISA plates (NUNC, Roskilde, Denmark). Decorin levels in plasma were assayed in duplicate. Serially diluted recombinant human decorin (R&D Systems) was used as a standard. The biotinylated decorin antibody was diluted to 1 μg ml^{-1} in PBS containing 0.05% Tween-20, and the detection reagent, extrAvidin-peroxidase (Sigma), was diluted to 0.4 μg ml^{-1} in PBS containing 0.05% Tween-20. The assay was developed with the addition of 3,3′,5,5′-tetramethylbenzidine (Sigma) dissolved in horseradish peroxidase substrate buffer (0.2 M Na_2PO_4, 0.1 M citric acid, pH 5.0). The reaction was stopped with the addition of 1 M H_3PO_4 after 6 min incubation. A BioRad microtiter plate reader (model 550) measured the optical density of developed assays at 450 nm with a subtracted background reference of 630 nm.

Human adipose tissue samples

Paired abdominal subcutaneous and visceral adipose samples were obtained from 38 patients undergoing elective abdominal surgery and immediately frozen in liquid nitrogen. The study protocol was approved by the Human Research Ethics Committee at St Vincent's Hospital, Sydney, Australia, and subjects provided written informed consent. Before surgery, blood samples, weight and waist circumference were obtained for baseline characterization. Subjects were divided into groups of people with type 2 diabetes ($n=6$) and normoglycaemic individuals ($n=32$). The normoglycaemic group was further classified into insulin-sensitive (IS) or insulin-resistant (IR) groups based on median HOMA-IR[12] (Table 1). Total RNA was extracted from adipose tissue samples using Tri-reagent (Sigma) in conjunction with RNeasy Mini kit columns (Qiagen). cDNA was synthesized (Omniscript RT; Qiagen) and RT-PCR performed using ABI Sequence Detection (ABI 7900HT; Applied Biosystems, Foster City, CA, USA) and SYBR Green. RT-PCR primers were as follows: human decorin forward 5′-GGGAGAAGAC ATTGGTTTGTTGA-3′, reverse 5′-AAGATAGGGAGAGGTAG AAGAAATAGC-3′.

Table 1 Phenotypic characteristics of human subjects from which adipose tissue biopsy was performed

Phenotype	*IS*	*IR*	*T2D*
No. of cases	16	16	6
Age (years)	55 ± 4	52 ± 4	63 ± 3
BMI	25.3 ± 0.7	28.0 ± 1.5	35.9 ± 3.0*
Weight (kg)	71.6 ± 1.2	76.8 ± 4.2	93.9 ± 6.0*
Waist (cm)	89 ± 3	95 ± 4	113 ± 6*
Fasting blood glucose (mM)	4.93 ± 0.13	5.04 ± 0.20	8.45 ± 0.70*
Fasting insulin (pM)	70.8 ± 4.1^	151.4 ± 16.7	145.1 ± 17.4
HOMA	2.2 ± 0.1^	4.8 ± 0.6**	7.9 ± 1.2*

Abbreviations: BMI, body mass index; HOMA, homeostasis model assessment of insulin sensitivity; IR, insulin resistant; IS, insulin sensitive; T2D, type 2 diabetic. Statistical differences in characteristics by group (^$P<0.003$ compared with IR and T2D, **$P=0.004$ compared with IS and T2D, *$P<0.001$ compared with IR and IS).

Human plasma samples

The samples were obtained during a large-scale epidemiological investigation of obesity, type 2 diabetes and the metabolic syndrome in Mauritius. The multiethnic population numbers about 1.2 million and consists of 70% Indian origin, 2% Chinese and the remaining 28% Creoles, being predominantly of African and Malagasy ancestry with some European admixture. This population has a high prevalence of obesity and diabetes.[13] The subjects analysed in this study consisted of 145 NGT and 141 T2D with subjects selected randomly from each group within an age range of 27–77 years. All samples were obtained with informed consent and all protocols were approved by the Inner Eastern Health Care Network Institutional Review Board.

Statistical analysis

Statistical analysis was performed using SPSS (Version 14.0, SPSS Inc., Chicago, IL, USA). Levene's test for homogeneity of variance was used to determine if variance between groups was equal. If homogeneity was not equal, a one-way analysis of variance with a Games–Howell *post hoc* analysis was used to compare group means, and if the variance was equal, a *post hoc* LSD (least significant difference) test was used. Associations between gene expression levels and phenotypic measures were determined using Pearson's correlation for normally distributed data or Spearman's correlation for data

that were not normally distributed. Data that were not normally distributed underwent non-parametric testing and a Mann–Whitney test was used to compare group means. Differences and correlations were considered significant at $P<0.05$.

To compare mean phenotypic values between NGT and T2D groups, independent sample *t*-test was used for normally distributed data or a Mann–Whitney test for variables that were not normally distributed. The decorin ELISA data underwent Mann–Whitney testing, as the data were not normally distributed. Associations between circulating decorin levels and phenotypic measurements were determined using Spearman's correlation. Regression analysis was used to determine independent associations with circulating decorin levels. Data were not corrected for multiple testing, as we consider all of the variables analysed to be interdependent.

Results

Decorin gene expression in P. obesus

Using SST in conjunction with cDNA microarray technology, we demonstrated that decorin, a small leucine-rich proteoglycan often found in the extracellular matrix (ECM), was a secreted protein that was differentially expressed in lean and obese *P. obesus*. Decorin exhibited differential expression in the skeletal muscle of NGT, IGT and T2D *P. obesus* (Supplementary Figure 1). Subsequent analysis of decorin gene expression in a range of *P. obesus* tissues revealed markedly higher expression in adipose tissue depots compared with all other tissues examined (Figure 1). Decorin mRNA was also found to be expressed in other tissues, including the heart, stomach, lung and ovary.

Owing to the striking gene expression observed in adipose tissue of *P. obesus*, decorin gene expression levels were analysed in visceral (mesenteric) and subcutaneous (subscapular) adipose tissues from NGT, IGT and T2D *P. obesus* by RT-PCR (Figure 2a). Decorin gene expression was significantly higher in visceral compared to subcutaneous adipose tissue in NGT and IGT *P. obesus* ($P=0.016$ and $P=0.005$, respectively). In the subcutaneous depots, decorin gene expression was significantly higher in IGT and T2D compared to NGT *P. obesus* ($P=0.029$ and $P=0.003$, respectively) and significantly correlated with body weight ($r^2=0.42$, $P=0.006$), plasma insulin ($r^2=0.39$, $P=0.010$) and blood glucose ($r^2=0.39$, $P=0.009$) concentrations and per cent body fat ($r^2=0.37$, $P=0.017$). In the visceral depots, decorin expression was significantly higher in T2D compared to NGT *P. obesus* ($P=0.018$) and was significantly correlated with body weight ($r^2=0.26$, $P=0.043$) and blood glucose concentration ($r^2=0.32$, $P=0.021$).

Decorin is predominantly expressed within stromal/vascular cells in fractionated adipose tissue. To determine which cells in adipose tissue express decorin, epididymal adipose tissue from IGT *P. obesus* was fractionated into two cellular populations—adipocytes and stromal/vascular cells. Within epididymal adipose tissue, decorin was predominantly expressed in the stromal/vascular fraction ($P<0.001$, gene expression (mean a.u. ± s.e.m.): adipocytes 7 ± 3, stromal/vascular cells 49 ± 6). Successful fractionation was confirmed using adipocyte- and stromal/vascular-specific markers such as leptin and CD68 (Supplementary Figure 2). These findings clearly demonstrate that the expression of decorin within adipose tissue of IGT *P. obesus* was predominantly from the stromal/vascular constituents of adipose tissue.

Decorin is localized to blood vessels and connective tissue in adipose tissue. To determine the location of decorin

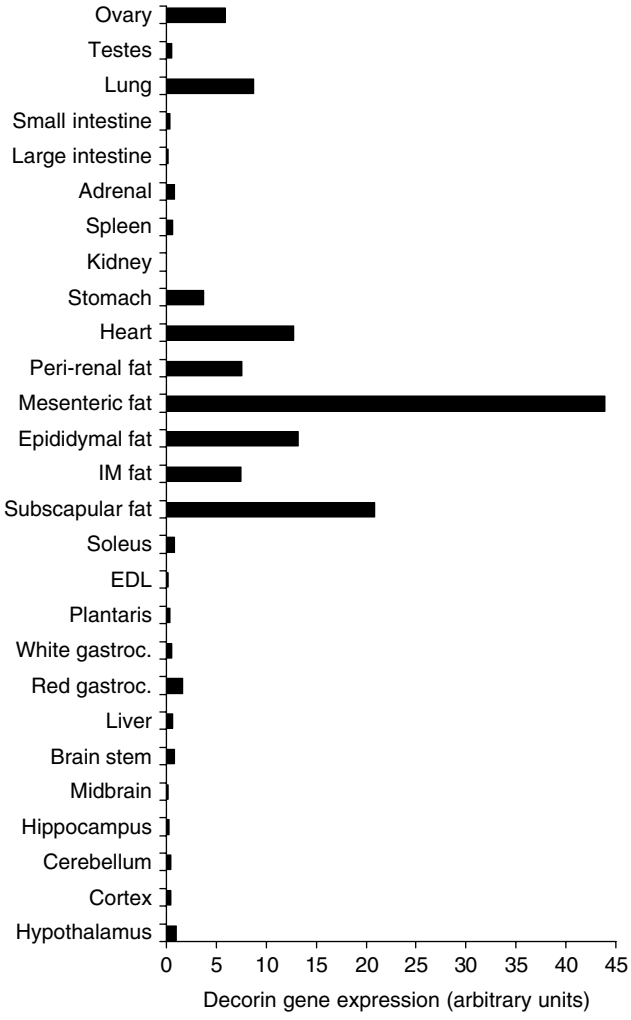

Figure 1 Analysis of decorin gene expression in *P. obesus* by RT-PCR. RNA was extracted from a variety of tissues from T2D *P. obesus* and decorin gene expression measured by RT-PCR. Decorin gene expression is represented relative to hypothalamic expression levels. IM fat, intramuscular fat; EDL, extensor digitorum longus; white gastroc., white gastrocnemius; red gastroc., red gastrocnemius; RT-PCR, real-time PCR; T2D, type 2 diabetic.

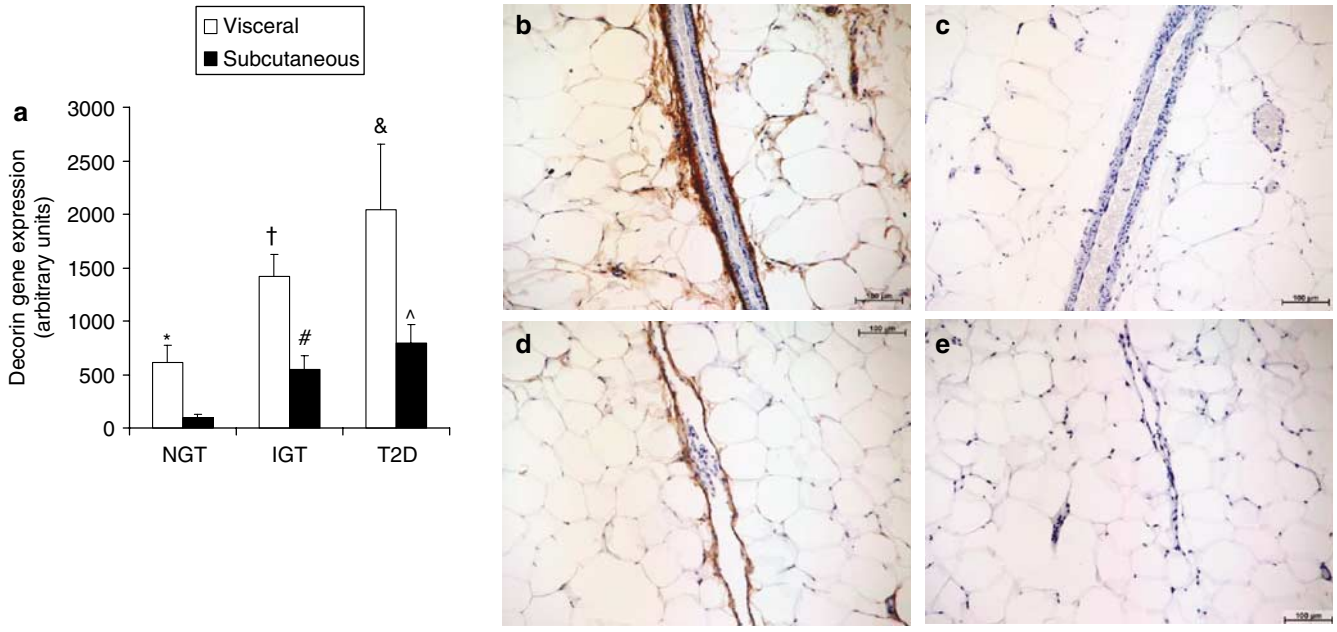

Figure 2 Analysis of decorin gene expression in *P. obesus* visceral and subcutaneous adipose tissue. (**a**) Decorin gene expression measured by RT-PCR using RNA extracted from visceral (mesenteric) and subcutaneous (subscapular) adipose tissue of NGT, IGT and T2D *P. obesus* and data are mean ± s.e.m. ($n=7$–8 per group). Data are represented relative to expression in NGT subcutaneous adipose tissue. Decorin gene expression was significantly increased in visceral NGT compared to subcutaneous NGT ($^{*}P=0.016$) and visceral IGT compared to subcutaneous IGT *P. obesus* ($^{\dagger}P=0.005$). Decorin expression was significantly increased in subcutaneous IGT and T2D compared to subcutaneous NGT ($^{\#}P=0.029$, $^{\wedge}P=0.003$). Decorin gene expression was significantly increased in visceral T2D compared to visceral NGT ($^{\&}P=0.018$). (**b**, **d**) Immunohistochemical localization of decorin counterstained with haematoxylin in visceral (mesenteric) (**b**) and epididymal (**d**) adipose tissue of *P. obesus*, with intense staining surrounding blood vessels. (**c**, **e**) Negative controls, goat IgG, substituted for decorin antibody and counterstained with haematoxylin in visceral (mesenteric) (**c**) and epididymal (**e**) adipose tissue of *P. obesus*. IGT, impaired glucose tolerant; NGT, normal glucose tolerant; RT-PCR, real-time PCR; T2D, type 2 diabetic.

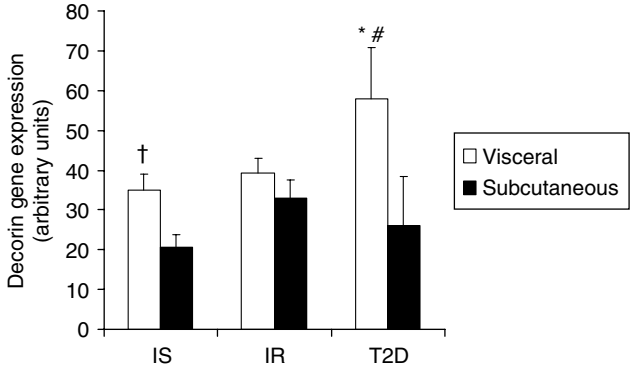

Figure 3 Analysis of decorin gene expression in human visceral and subcutaneous adipose tissue. RNA was extracted from abdominal visceral and subcutaneous adipose tissue of IS, IR and T2D human subjects and decorin gene expression was measured by RT-PCR. Data are mean ± s.e.m. ($n=6$–16 per group). Decorin gene expression was significantly higher in human visceral T2D than that in subcutaneous T2D ($^{*}P=0.004$) and significantly higher in visceral IS than that in subcutaneous IS ($^{\dagger}P=0.001$). Decorin expression was significantly higher in human visceral T2D than that in visceral IS and visceral IR subjects ($^{\#}P=0.02$ and $P=0.04$, respectively). IGT, impaired glucose tolerant; IR, insulin resistant; IS, insulin sensitive; NGT, normal glucose tolerant; RT-PCR, real-time PCR; T2D, type 2 diabetic.

expression in adipose tissue, immunohistochemistry was performed on epididymal, visceral (mesenteric) and subcutaneous (subscapular) adipose tissue from *P. obesus*. A similar pattern of staining was observed in both NGT and IGT *P. obesus* and within all three types of adipose tissue, with the most intense decorin staining surrounding blood vessels within the adipose tissue (Figures 2c and e). No staining was observed in the negative controls (Figures 2c and e). In addition, weak staining was observed within connective tissue surrounding the rim of some adipocytes, and within the septa of the adipose tissue itself (data not shown).

Decorin gene expression within human visceral and subcutaneous adipose tissue. Gene expression of decorin in abdominal visceral and subcutaneous adipose tissue from IS, IR and T2D subjects was measured by RT-PCR. The phenotypic characteristics of each group are presented in Table 1. Decorin gene expression was significantly higher in human visceral adipose tissue than that in subcutaneous adipose tissue in both IS and T2D subjects ($P=0.001$ and $P=0.04$, respectively) (Figure 3). Furthermore, decorin gene expression was significantly higher in visceral adipose tissue from T2D subjects than that from IS and IR subjects ($P=0.02$ and $P=0.04$, respectively). There were no significant differences in decorin expression in subcutaneous adipose tissue samples between IS, IR and T2D groups.

Decorin plasma levels were increased in patients with type 2 diabetes. To determine whether circulating levels of decorin

Table 2 Physical and metabolic characteristics of human subjects for ELISA

Phenotype	*NGT* *Mean ± s.e.m.*	*T2D* *Mean ± s.e.m.*	*Significance (P-value)*
No. of cases	145	141	
Age (years)	46.14 ± 0.93	50.40 ± 0.87	**0.001**
Fasting glucose (mM)	5.30 ± 0.03	8.33 ± 0.23	**<0.001**
Two-hour glucose (mM)	5.92 ± 0.10	14.59 ± 0.37	**<0.001**
Fasting serum insulin (pM)	71.4 ± 3.5	98.4 ± 5.0	**<0.001**
Two-hour serum insulin (pM)	446.6 ± 32.3	536.9 ± 36.9	0.056
homa_b	121.36 ± 3.87	77.37 ± 3.65	**<0.001**
homa_s	51.63 ± 2.85	34.41 ± 2.46	**<0.001**
Height (cm)	161.35 ± 0.79	159.24 ± 0.80	0.061
Weight (kg)	66.40 ± 0.98	68.78 ± 1.10	0.107
BMI (kg/m^2)	25.53 ± 0.34	27.14 ± 0.41	**0.004**
WHR	0.85 ± 0.01	0.89 ± 0.01	**<0.001**
Fat mass (kg)	20.78 ± 0.61	22.48 ± 0.59	**0.046**
Body fat (%)	31.01 ± 0.77	32.75 ± 0.64	0.082
Total cholesterol (mM)	5.02 ± 0.09	5.39 ± 0.14	**0.044**
HDL (mM)	0.94 ± 0.03	0.87 ± 0.03	**0.039**
Triglycerides (mM)	1.42 ± 0.06	2.09 ± 0.15	**<0.001**
Systolic BP	124.12 ± 1.65	136.11 ± 1.68	**<0.001**
Diastolic BP	74.59 ± 1.11	80.56 ± 1.12	**<0.001**
Decorin (ng ml^{-1})	1.80 ± 0.09	2.05 ± 0.13	**0.049**

Abbreviations: BMI, body mass index; BP, blood pressure; ELISA, enzyme-linked immunosorbent assay; HDL, high-density lipoprotein; homa_b, quantitative estimate of β-cell function; homa_s, quantitative estimate of insulin sensitivity; NGT, normal glucose tolerant; T2D, type 2 diabetic; WHR, waist-to-hip ratio. Circulating decorin levels were measured in plasma from 145 NGT and 141 T2D human subjects by ELISA. Significant differences between NGT and T2D groups ($P<0.05$) are shown in bold.

Table 3 Decorin is associated with phenotypes associated with metabolic syndrome development

Phenotype	*All subjects, decorin*	*All subjects, decorin adjusted for age*	*Males, decorin*
Age (years)	$P=0.046$, $r^2=0.014$		$P=0.408$, $r^2=0.004$
Fasting glucose (mM)	$P=0.085$, $r^2=0.010$	$P=0.352$, $r^2=0.003$	**$P=0.027$, $r^2=0.031$**
Two-hour glucose (mM)	$P=0.012$, $r^2=0.022$	$P=0.110$, $r^2=0.009$	**$P=0.001$, $r^2=0.065$**
Fasting serum insulin (pM)	$P=0.459$, $r^2=0.002$	$P=0.376$, $r^2=0.003$	$P=0.582$, $r^2=0.002$
Two-hour serum insulin (pM)	$P=0.571$, $r^2=0.001$	$P=0.803$, $r^2<0.001$	$P=0.456$, $r^2=0.004$
homa_b	$P=0.292$, $r^2=0.004$	$P=0.720$, $r^2<0.001$	$P=0.071$, $r^2=0.021$
homa_s	$P=0.357$, $r^2=0.003$	$P=0.325$, $r^2=0.003$	$P=0.471$, $r^2=0.004$
Height (cm)	$P=0.333$, $r^2=0.003$	$P=0.073$, $r^2=0.011$	$P=0.425$, $r^2=0.004$
Weight (kg)	$P=0.172$, $r^2=0.007$	$P=0.095$, $r^2=0.010$	$P=0.551$, $r^2=0.002$
BMI (kg/m^2)	$P=0.493$, $r^2=0.002$	$P=0.707$, $r^2<0.001$	$P=0.913$, $r^2<0.001$
WHR	**$P=0.005$, $r^2=0.027$**	**$P=0.024$, $r^2=0.018$**	**$P=0.006$, $r^2=0.048$**
Fat mass (kg)	$P=0.388$, $r^2=0.003$	$P=0.556$, $r^2=0.001$	$P=0.623$, $r^2=0.002$
Body fat (%)	$P=0.719$, $r^2<0.001$	$P=0.899$, $r^2<0.001$	$P=0.354$, $r^2=0.005$
Total cholesterol (mM)	$P=0.581$, $r^2=0.001$	$P=0.761$, $r^2<0.001$	$P=0.934$, $r^2<0.001$
HDL (mM)	$P=0.628$, $r^2<0.001$	$P=0.679$, $r^2<0.001$	$P=0.435$, $r^2=0.004$
Triglycerides (mM)	$P=0.160$, $r^2=0.007$	$P=0.265$, $r^2=0.004$	$P=0.132$, $r^2=0.015$
Systolic BP	$P=0.021$, $r^2=0.018$	$P=0.450$, $r^2=0.002$	$P=0.039$, $r^2=0.027$
Diastolic BP	$P=0.092$, $r^2=0.010$	$P=0.205$, $r^2=0.006$	$P=0.232$, $r^2=0.009$

Abbreviations: BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; homa_b, quantitative estimate of β-cell function; homa_s, quantitative estimate of insulin sensitivity; WHR, waist-to-hip ratio. Circulating decorin levels were measured in plasma from 145 NGT and 141 T2D human subjects by ELISA. Associations were performed using Spearman's correlation. Linear regression was performed on overall circulating decorin levels due to an association with age ($P=0.046$). Significant associations between plasma decorin levels and phenotypes ($P<0.05$) are shown in bold.

were associated with type 2 diabetes, an ELISA was developed to measure plasma decorin levels in 141 T2D and 145 matched control NGT subjects. The phenotypic characteristics of each group are presented in Table 2. Circulating levels of decorin were found to be 12% higher in T2D than that in NGT subjects ($P=0.049$). Plasma decorin levels were significantly correlated with age (Spearman's test, $n=286$, $r^2=0.014$, $P=0.046$) (Table 3). After adjusting for age by regression analysis, plasma decorin levels were significantly associated with waist-to-hip ratio (WHR, Spearman's test, $n=286$, $r^2=0.018$, $P=0.024$), a measure of central adiposity.[14] Collectively, these data suggest an association between circulating decorin levels and the metabolic syndrome.

There were no significant differences in circulating levels of decorin between male and female subjects; however, plasma decorin levels were found to be significantly correlated with age in female subjects (Spearman's test, $n=128$, $r^2=0.036$, $P=0.032$). In male subjects, plasma decorin levels were higher in T2D than that in NGT subjects ($n=158$, $P=0.006$) and plasma decorin levels were correlated with WHR, fasting glucose and 2-h glucose levels in an oral glucose tolerance test, and systolic blood pressure (Table 3).

Discussion

We have combined an SST with cDNA microarray analysis[11] of *P. obesus* gene expression to identify genes encoding secreted proteins that had not previously been associated with the development of obesity, insulin resistance and type 2 diabetes. As part of these studies, we have identified the small leucine-rich proteoglycan decorin as a secreted protein that was more highly expressed in adipose tissue depots in *P. obesus* compared with all other tissues analysed. This observation suggested that decorin may play an important role in adipose tissue physiology and prompted us to characterize decorin expression in adipose tissue further.

We have shown that decorin gene expression was higher in visceral adipose tissue than that in subcutaneous adipose tissue in lean and obese *P. obesus*. Although similar to previous studies that have found that decorin mRNA expression is higher in abdominal adipose tissue than that in subcutaneous adipose tissue from cows, pigs and C57BL/6 mice,[15] our findings extend these observations to show that decorin expression is higher in both visceral and subcutaneous adipose tissue from obese IGT and T2D than that from lean NGT *P. obesus*. In addition, this study has shown for the first time that decorin gene expression is higher in visceral adipose tissue than that in subcutaneous adipose tissue in lean IS humans, and that circulating decorin levels are higher in people with T2D and are associated with measures of adiposity and insulin sensitivity. Although these data are associative, our findings suggest that decorin may play an important role in the normal function of adipose tissue and also in the pathophysiology of obesity and associated disorders such as insulin resistance.

Our data indicate that the majority of decorin mRNA expressed by adipose tissue is derived not from the adipocytes themselves but from the stromal/vascular cells within the adipose tissue. This observation was further supported by immunohistochemical analysis of decorin expression in adipose tissue, which demonstrated that decorin protein was predominantly localized to regions adjacent to blood vessels within the adipose tissue. A recent study showed that decorin expression was higher in adipocytes and stromal/vascular cells isolated from obese Zucker rats compared with lean animals, however any differences in the relative expression of decorin between the two cell populations was not reported.[16] The stromal/vascular cells found in adipose tissue comprise a range of cell types, including endothelial and smooth muscle cells associated with blood vessels, preadipocytes and inflammatory cells such as macrophages. As some of these cell types, such as endothelial cells, are known to express decorin in other tissues,[17,18] it is tempting to speculate that these cells are a major source of decorin expression in adipose tissue. Further studies using flow cytometry or immunohistochemistry with cell population-specific markers will be required to conclusively determine the source of decorin expression in adipose tissue.

Prolonged positive energy balance induces a number of changes in adipose tissue, including mature adipocyte expansion, new adipocyte formation, the accumulation of inflammatory cells within adipose tissue and neovascularization to provide blood to the expanding adipose tissue.[19,20] To accommodate these changes, remodelling of the ECM occurs by degradation of the existing ECM and production of new ECM. As decorin is required for the correct folding of other ECM components such as collagen,[21] the elevated levels of decorin expression in adipose tissue from obese IGT and T2D *P. obesus* raises the possibility that decorin expression may be induced in adipose tissue in the obese state to facilitate new ECM structures forming within the expanding adipose tissue.

The intense decorin staining surrounding blood vessels from *P. obesus* combined with the predominant expression of decorin mRNA by stromal/vascular constituents of adipose tissue suggests that decorin may also be involved in regulating angiogenesis or vasculature homeostasis within the adipose tissue. As angiogenesis is markedly upregulated within the adipose tissue during obesity,[20,22] the increased decorin gene expression in adipose tissue from obese *P. obesus* is consistent with a role for decorin in angiogenesis. Several studies have supported a proangiogenic role for decorin and, in particular, in angiogenesis associated with inflammatory conditions.[18,23–26] This is noteworthy as obesity has been associated with inflammation of the adipose tissue.[27] Decorin may, therefore, have a proangiogenic role during adipose tissue expansion in times of positive energy balance. In contrast, decorin may also exhibit antiangiogenic effects under certain circumstances.[28–32] Clearly, further experiments are required to substantiate the role of decorin in adipose tissue angiogenesis. The effect of decorin deficiency on the vascularization of adipose depots in decorin-knockout mice fed a high-fat diet will be a key experiment in determining the role of decorin in obesity-associated angiogenesis within the adipose tissue.

Obesity is associated with a state of inflammation characterized by an accumulation of activated macrophages within the adipose tissue.[33–36] It is therefore noteworthy that decorin has been shown to act as a proinflammatory factor and is produced at sites of inflammation.[18,37–41] In contrast,

decorin has also been reported to bind and inhibit the proinflammatory activity of the complement protein C1q[42] and has been hypothesized to antagonize C1q-induced insulin resistance in adipose tissue that is associated with obesity.[16] These observations indicate that decorin may play a role in inflammation associated with obesity in adipose tissue; however, this is likely to be a complex interaction between pro- and anti-inflammatory activities.

In summary, we have shown that decorin was highly expressed in adipose tissue with expression higher in obese IGT and T2D than that in lean NGT *P. obesus*. We have also shown that decorin is predominantly expressed by non-adipocytes in adipose tissue and that the protein expression is adjacent to blood vessels in the adipose tissue. These findings suggest that decorin may play a role in adipose tissue homeostasis in both the lean and obese states.

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Disclosure

PZ is chairman of the scientific advisor board of ChemGenex Pharmaceuticals. KW and GC are employees of ChemGenex Pharmaceuticals.

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