PAPP-A, IGFBP-4 and IGF-II are secreted by human adipose tissue cultures in a depot-specific manner

Mette Faurholdt Gude1, Rikke Hjortebjerg1,2, Claus Oxvig3, Anne Anker Thyø4, Nils Erik Magnusson1, Mette Bjerre1, Steen Bønløkke Pedersen1,5 and Jan Frystyk1,5

1Medical Research Laboratory, Department of Clinical Medicine, Faculty of Health, Aarhus University, Aarhus C, Denmark, 2Danish Diabetes Academy, Odense, Denmark, 3Department of Molecular Biology and Genetics, Faculty of Science and Technology, Aarhus University, Aarhus C, Denmark, 4Departments of Abdominal Surgery, and 5Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus C, Denmark

Abstract

Objective: Adipose tissue secretes pregnancy-associated plasma protein-A (PAPP-A), which may increase local IGF action through cleavage of IGF-binding protein-4 (IGFBP-4). We tested whether this mechanism was operational in human visceral and subcutaneous adipose tissue (i.e. VAT and SAT).

Design: Explants of VAT and SAT from 26 obese subjects (hereof 17 women, BMI 39.5 (37.2; 42.8) kg/m² (median (25%; 75% confidence interval) and SAT from eight lean, age-matched women (BMI 23.6 (22.4; 24.9) kg/m²) were incubated with or without GH (100 µg/L) and the media were harvested.

Methods: Media were assessed for concentrations of PAPP-A, intact and PAPP-A-cleaved IGFBP-4, IGF-I and IGF-II, and IGF-I receptor (IGF-IR) activation by bioassay.

Results: In obese subjects, VAT media contained higher concentrations than SAT of PAPP-A (4.4-fold) and both PAPP-A-generated IGFBP-4 fragments (C-terminal: 3.3-fold, N-terminal: 1.5-fold) (all \( P < 0.0005 \)). Intact IGFBP-4 levels were similar in SAT and VAT. VAT media contained elevated IGF-II (1.4-fold; \( P < 0.005 \)), but similar IGF-I concentrations compared with SAT. Still, VAT media contained a 1.8-fold increased ability to stimulate the IGF-IR (\( P < 0.005 \)). IGF-I protein concentration and IGF-IR activation increased more in VAT media than SAT media following GH stimulation (both \( P < 0.05 \)). At baseline, SAT media protein levels from lean and obese women were similar, with the exception of PAPP-A being 1.8-fold elevated in VAT media (\( P < 0.05 \)). GH induced a similar increase in IGF-I media levels in SAT from obese and lean women.

Conclusion: Human adipose tissue cultures secrete enzymatically active PAPP-A, IGFBP-4 and IGF-II in a depot-specific manner, suggesting differential regulation of IGF activity. Further, IGF-II appears to be more prominent than IGF-I. Finally, VAT appears more GH responsive than SAT.

Introduction

Adipose tissue synthesizes proteins belonging to the insulin-like growth factor (IGF) system. This includes the IGF-I receptor (IGF-IR), its two ligands, IGF-I and IGF-II, and many of the IGF-binding proteins (IGFBPs) (1, 2, 3). The role of the IGF system in adipose tissue remains to be defined, but the expression and secretion of IGF-I appear to be important for adipocyte homeostasis. In vitro, IGF-I stimulates adipocyte growth and lipogenesis.
induce a higher IGF-I production in adipose tissue from obese than lean women.

Subjects and methods

Study participants

VAT and SAT were collected peri-operatively during elective bariatric surgery from 26 obese subjects (age 38.0 (33.5; 43.5) (median (25%; 75% confidence interval)); BMI 39.5 (37.2; 42.8) kg/m²), with no differences in BMI and age between women (n=17, age 38.0 (29.0; 42.0) years, BMI 40.1 (35.4; 44.7) kg/m²) and men (n=9, age 40.0 (35.5; 46.0) years, BMI 39.3 (38.2; 41.8) kg/m²). SAT was collected from eight lean women (age 40.0 (30.0; 52.3) years, BMI 23.6 (22.4; 24.9) kg/m²), whose age was matched with that of the obese women. The latter tissue samples originated from cosmetic liposuctions performed on the abdomen and/or the hips.

All lean subjects were healthy and took no medication influencing adipose tissue metabolism. Among obese subjects some received anti-hypertensives (n=7), oral anti-diabetics (n=5), anti-psychotics (n=2), proton-pump inhibitors (n=4), thyroxine (n=4), anti-arrhythmics (n=3), anti-estrogen (n=1) or anti-thrombotics (n=1), or were treated with medications for asthma (n=1), osteoporosis (n=1) or depression (n=6). The project was approved by the local Ethical Committee and performed in accordance with the Helsinki Declaration.

Adipose tissue culturing

Immediately after collection, adipose tissue fragments were rinsed in sterile saline. SAT from lean women was incubated in fragments of 500mg, floating freely in 5mL incubation medium (consisting of Medium 199 (Sigma) supplemented with 25 mM HEPES, pH 7.4 and 1% human serum albumin, 1 mM insulin, antipain and leupeptin (each 20 μg/mL/100 mL medium) and penicillin (100 IU/mL) and streptomycin (100 IU/mL) (each 1 mL/100 mL medium)) at 37°C, 5% CO₂. Due to practical circumstances, the SAT and VAT fragments obtained during bariatric surgery only resulted in 200mg tissue fragments. These fragments were incubated in 2mL incubation medium and consequently, the ratio between tissue and medium was similar for all fragments. After an equilibration period of 24h, tissues were incubated for 48 h at 37°C before harvest of cell media. SAT from lean women was incubated with various concentrations of GH (0 (i.e. baseline), 1, 10 and 100 μg/L; Novo Nordisk A/S), whereas tissue samples from obese men and women were incubated

(4) as well as the differentiation of pre-adipocytes into mature adipocytes, and IGF-I also controls anti-apoptotic signaling (5, 6, 7, 8).

The vast majority of the IGFs are complexed to IGFBPs, which block the growth factors from activating their primary target, the IGF-IR (9). Accordingly, to become receptor accessible the IGFs need to be liberated. This is achieved by enzymatic cleavage of the IGFBPs, causing release of bound IGF. Pregnancy-associated plasma protein-A (PAPP-A) is one of the most well-characterized IGFBP-degrading enzymes. The primary substrate for PAPP-A is IGFBP-4, which is cleaved in an IGF-dependent manner, and by this mechanism PAPP-A has been demonstrated to control growth, visceral fat accumulation and longevity in experimental animal models (10, 11, 12).

Adipose tissue from different regions is inherently distinct in regard to expression and secretion of pro- and anti-inflammatory proteins (13, 14). The regional differences may explain that accumulation of visceral adipose tissue (VAT) associates more strongly with an unfavorable metabolic risk profile than does subcutaneous adipose tissue (SAT) (15). Also PAPP-A, which holds pro-atherosclerotic properties (11), appears to be secreted in a site-specific manner. Studies in experimental animals have demonstrated that PAPP-A is highly expressed in and secreted from pre-adipocytes of visceral origin compared with subcutaneous pre-adipocytes, and the expression and secretion of IGFBP-4 follow the same regional distribution (10, 16, 17, 18). At present, human data are scanty and it remains unknown whether the site-specific secretion of PAPP-A and IGFBP-4 translates into regional differences in local IGF action.

Insulin stimulates the hepatic growth hormone (GH) sensitivity by increasing the GH receptor (GHR) synthesis (19). This explains why obese subjects, despite a reduced GH secretion (20), are able to maintain serum IGF-I levels within the normal range (21, 22, 23, 24), and it also explains why obese subjects respond with a higher increase in serum IGF-I following GH administration than lean subjects (25). Whether obesity also augments the sensitivity to GH in human adipose tissue remains unknown.

This study aimed to compare the expression and secretion of components of the IGF system in media from cultures of SAT and VAT from obese subjects, focusing on media levels of PAPP-A, intact IGFBP-4 and PAPP-A-generated IGFBP-4 fragments and their impact on IGF bioactivity. Furthermore, by stimulating SAT with GH in vitro, we tested the hypothesis that GH was able to
without GH (baseline) and with GH (100 µg/L). After harvest, the media were frozen at −20°C for later analysis of proteins, whereas tissue fragments were frozen in liquid nitrogen and kept at −80°C for mRNA extraction.

**Bioactive IGF by kinase receptor activation assay (KIRA)**

The ability of IGFs present in cell media to stimulate the IGF-IR in vitro (i.e. IGF bioactivity) was measured by an in-house kinase receptor activation (KIRA) assay (26) with modifications (27). Media were diluted 1:2 before assay and all samples from the same patient were measured in the same assay. IGF-IR phosphorylation was compared with that of a serial dilution of recombinant human IGF-I (WHO 02/254; the National Institute for Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire, UK). Samples with readings below detection limit (zero calibrator (NSB) plus 3 s.d. of the NSB signal) were given the concentration of zero. As the IGF-IR may be stimulated by IGF-I as well as IGF-II, the output of the assay has been designated ‘bioactive IGF’.

**IGF-immunoassays**

Media concentrations of total, immunoreactive IGF-I were measured by in-house time-resolved immunofluorometric assay (TR-IFMA) following sample acidification and IGF-II blockage of IGFBPs as previously outlined (3) with modifications (28). Media were acidified to pH <3 by a 1:10 dilution in acetic acid (200 mM, 0.5 g/L HSA, 9 g/L NaCl and 5 mL/L Tween 20) containing an excess of IGF-II (200 µg/L), and incubated for 2 h at 5°C. The samples (100 µL) were added directly to a 96-well microtiter plate, which before use had been coated with IGF-I antibody (MAb41, generously provided by Novo Nordisk A/S), blocked (10 mL/L Tween 20, 0.5 g/L NaN₃ in 40 mM PBS) and added 100 µL Tris buffer (400 mM, pH 8.5: 17.68 g/L Tris–HCl, 34.88 g/L Tris-base, 2.5 µM EDTA, 2 mL/L Tween 20, 9 g/L NaCl and 1 g/L HSA), which neutralized the acetic acid. After overnight incubation at 5°C, the wells were washed (50 mM Tris–HCl pH 8.0 with 9 g/L NaCl, 5 mL/L Tween 20 and 0.5 g/L NaN₃) and 200 µL of biotinylated IGF-I antibody (catalog # I-8773, Sigma) containing europium-labeled streptavidin (1:1000; Perkin Elmer Life Science) were added to the wells and read using time-resolved immunofluorometry. A serial dilution of rhIGF-I (WHO 02/254) served as calibrator and was treated as cell media. All samples were assayed in duplicates.

Cell media levels of immunoreactive, total IGF-II were assayed in a similar manner. However, IGFBPs were blocked with 200 µg/L IGF-I and different antibodies were used for coating (clone S1F2, catalog # 05-166, Merck Millipore) and detection (polyclonal antibody # I-7276, Sigma, tagged with europium), and rhIGF-II (WHO 96/538, obtained from NIBSC) served as calibrator. Otherwise, the procedures were as described for IGF-I.

**IGFBP-4**

We originally assessed IGFBP-4 by a commercial assay, which had been validated for measurements in cell culture media (catalog # DY804, Bio-Techne, Abingdon, UK), whereas its ability to recognize IGFBP-4 fragments generated by PAPP-A had not been explored. However, after having finalized all tissue incubations, we had the opportunity to establish three novel in-house IGFBP-4 assays that specifically targeted intact IGFBP-4, the PAPP-A-generated C-terminal (CT) IGFBP-4 fragment and the PAPP-A-generated N-terminal (NT) IGFBP-4 fragment respectively. The three assays were based on antibodies and calibrators generously donated by HyTest Ltd. (Turku, Finland) and they have recently been described in detail (29). In brief, culture media levels of IGFBP-4, CT-IGFBP-4 and NT-IGFBP-4 were measured in duplicate using TR-IFMAs based on monoclonal antibodies and recombinant human calibrators generously provided by HyTest Ltd. In each fragment assay, one of the antibodies specifically recognizes the proteolytic neo-epitope generated upon degradation of IGFBP-4 by PAPP-A. In all three assays, intra- and inter-assay CVs were below 10% and 15% respectively. Detection limits were 0.5 µg/L for IGFBP-4, 0.4 µg/L for CT-IGFBP-4 and 0.9 µg/L for NT-IGFBP-4. As the assays for intact and PAPP-A-fragmented IGFBP-4 are relatively new, these assays were the last to be performed. Hence, as some media vials were used, we were only able to measure intact and degraded IGFBP-4 in a limited number of samples.

**PAPP-A assay**

Media levels of PAPP-A were determined using a commercial ELISA, generously provided by Ansh Labs (Webster, TX, USA). The assay was performed according to protocol procedures. All samples were determined in single determination.
Clinical Study

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PAPP-A and IGFBP-4 in human adipose tissue

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This was performed as outlined previously (30). Total RNA was extracted from frozen adipose tissue using TRIzol reagents (Gibco BRL, Life Technologies). The RNA concentration was determined by absorbance at 260 nm, and purity and quality were checked by 260/280 ratio and by gel electrophoresis respectively. Reverse transcription polymerase chain reaction (RT-PCR) was performed with random hexamer primers as described by the GeneAmp PCR Kit (Perkin Elmer Cetus). Real-time PCR was performed with a SYBR Green real-time PCR assay using an iCycler PCR machine from Bio-Rad Laboratories. The primers for target genes are shown in Table 1. Quantitative results obtained by real-time RT-PCR were expressed as cycle threshold (CT). The relative gene expression of target gene to β-actin was calculated by the formula 1/((CT target − CT β-actin)^2) as described previously (30).

Results

Protein levels in culture media

GH stimulation of SAT from lean women

SAT from lean women were available in large amounts and therefore used to study the effect of increasing doses of GH, comparing baseline (zero) vs 1, 10 and 100 µg/L. GH stimulation of SAT dose-dependently increased concentrations of IGF-I (P=0.003; Fig. 1A), IGF-II (P=0.003; Fig. 1B) and bioactive IGF (P=0.003; Fig. 1C). IGFBP-4 also increased following GH stimulation (P=0.002; Fig. 1D), whereas its effects on PAPP-A were less clear-cut, as we only observed a difference when comparing GH concentrations of 1 and 100 µg/L (P=0.02; Fig. 1E).

SAT media from lean vs obese women

SAT media from lean women were compared with SAT media from obese women, focusing on the conditions at baseline and after GH stimulation (100 µg/L). The most notable differences included IGFBP-4 (Fig. 1D) and PAPP-A (Fig. 1E), which at baseline were 2.0-fold and 1.8-fold higher, respectively, in SAT media from obese than those from lean women (P<0.001). Upon GH treatment, the difference in IGFBP-4 persisted (Fig. 1D), whereas media levels of PAPP-A remained numerically, but insignificantly, higher in obese SAT media (Fig. 1E).

GH stimulation increased media concentrations of IGF-I (P<0.05; Fig. 1A) similarly in SAT from lean and obese women. By contrast, IGF-II decreased (P<0.05) in obese SAT media, but increased (P<0.05) in lean SAT media (Fig. 1B). The opposing responses of IGF-II to GH stimulation may explain that bioactive IGF increased less in obese than in lean SAT media (P<0.05; Fig. 1C). A few other differences in the response to GH stimulation were noticed. GH stimulation caused a small, but significant

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primers used in the study.</th>
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<tr>
<td><strong>Target</strong></td>
<td><strong>Sense</strong></td>
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<tr>
<td>IGF-I</td>
<td>GACAGGGGCTTTTATTTCAAC</td>
</tr>
<tr>
<td>IGF-II</td>
<td>AGCCGTGCGCATCCTTGGAG</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>CATTCAGCTGCTCAACGGA</td>
</tr>
<tr>
<td>GH receptor</td>
<td>ATTTTCAAAACAGCAAACAGA</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>拥有</td>
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</table>

Statistics

The vast majority of data did not comply with a parametric distribution, and log transformation did not change this. Therefore, we used Mann–Whitney rank sum test, Wilcoxon signed rank sum test or Friedman repeated-measures analysis of variance on ranks as appropriate. A significant Friedman test was followed by the Student–Newman–Keul post hoc test, which is suitable for multiple comparisons. The only exception was IGFBP-4 data from obese subjects (when comparing SAT and VAT before and after GH stimulation), which complied with a normal distribution. In this case we used repeated-measures ANOVA followed by Student–Newman–Keul test. Spearman rank order correlation was used to study the associations between variables. P-values <0.05 were considered significant. All data are stated as median and interquartile range.

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increase in IGFBP-4 levels in SAT media from lean but not obese women ($P<0.05$; Fig. 1D), whereas GH stimulation decreased PAPP-A levels in SAT media from obese but not lean women ($P<0.05$; Fig. 1E).

**VAT media vs SAT media from obese subjects**

The paired experiment compared SAT and VAT media before and after GH stimulation (100 µg/L). As we did not observe clear gender differences (data not shown), it was decided to pool data. At baseline, IGF-I concentrations (Fig. 2A) did not differ between unstimulated VAT and SAT media, whereas bioactive IGF levels were 1.8-fold higher in VAT than those in SAT media ($P<0.005$). Stimulation by GH increased IGF-I concentrations (Fig. 2A) and bioactive IGF (Fig. 2C) in both media ($P<0.05$), with both levels being higher in VAT than those in SAT ($P<0.05$). In contrast to IGF-I, GH altered neither IGF-II concentrations in SAT nor VAT media. Noteworthy, VAT media contained significantly higher IGF-II concentrations than SAT media at baseline (1.4-fold) as well as after GH stimulation (2.1-fold) ($P<0.005$) (Fig. 2B). This observation may explain that VAT media contained significantly higher levels of bioactive IGF (Fig. 2C) than SAT media at baseline ($P<0.05$) as well as after GH stimulation ($P<0.05$).

Another marked difference between SAT and VAT was their secretion of IGFBP-4 and PAPP-A. When measured with the commercial IGFBP-4 assay, VAT media contained approximately twice as much IGFBP-4 (Fig. 2D) and four times as much PAPP-A ($P<0.001$) as SAT at baseline, and this difference remained unchanged after GH stimulation (Fig. 2E). GH did not change PAPP-A levels in media from VAT, but caused a small yet significant reduction in PAPP-A levels from SAT.

IGFBP-4 normally inhibits IGF-mediated IGF-IR activation in vitro. Hence, the combination of high levels of IGFBP-4 and bioactive IGF in VAT media was counterintuitive compared with SAT media. However, we learned that the commercial IGFBP-4 assay detected both intact IGFBP-4 and NT-IGFBP-4 generated upon PAPP-A cleavage (data not shown). Accordingly, baseline media IGFBP-4 levels were re-measured using our IGFBP-4 fragment-specific assays (29). By these assays we could demonstrate that intact IGFBP-4 levels were identical in VAT and SAT media, whereas levels of NT and CT fragments were 1.5-fold and 3.3-fold respectively higher in VAT than in SAT ($P<0.001$; Fig. 3A). Based on these findings, we returned to SAT media from lean women. We had only access to media from SAT exposed to 1.0 µg/L GH, but still these measurements (Fig. 3B) showed similar
levels of intact as well as NT- and CT-IGFBP-4, i.e. as observed in SAT media from obese women (Fig. 3A).

Correlations between levels of bioactive IGF, IGFBP-4, and PAPP-A are shown in Table 2. The three distinct measures of IGFBP-4 were positively correlated in both SAT and VAT media. In VAT, bioactive IGF correlated positively with PAPP-A, and inversely with NT-IGFBP-4. No such correlations were observed in SAT.

**Correlations between BMI and GH induced increments in bioactive IGF and IGF-I**

To study the impact of BMI on the GH responsiveness of SAT, data from lean and obese women were analyzed using Spearman rank order correlations between BMI and increments in bioactive IGF and IGF-I concentrations, following GH stimulation (i.e. concentrations after GH stimulation minus concentrations at baseline). BMI correlated inversely with the increase in bioactive IGF ($r=-0.54; \ p=0.005; \ n=25$), but not with the increase in IGF-I concentration ($r=-0.16; \ p=\text { NS}; \ n=25$).

**mRNA levels in adipose tissue cultures**

**GH stimulation of SAT from lean women**

Upon GH treatment, the expression of IGF-I increased, but only at the highest GH dose ($p<0.05$; Table 3). Otherwise, no significant effects of GH were detected.

**SAT from lean vs obese women**

SAT from obese women contained higher baseline expression of IGF-IR, IGF-IIR, IGF-II and IGFBP-4 and lower expression of GHR and PAPP-A, compared with SAT from lean women, whereas the expression of IGF-I was similar (all $P<0.05$; Table 3). The differences in expression patterns were slightly altered following GH stimulation, as GHR and IGF-II were no longer different.
VAT vs SAT from obese subjects

At baseline, SAT showed a higher expression of GHR and a lower expression of PAPP-A than VAT (P<0.05; Table 4), but apart from this no differences were observed. GH stimulation increased IGF-I expression similarly in SAT and VAT, whereas the expression of GHR was no longer different between the two tissues. Otherwise, GH stimulation caused no changes in the expression pattern observed at baseline.

Discussion

This study compared the expression, secretion and GH responsiveness of proteins belonging to the IGF system in cultures of VAT and SAT from lean and obese subjects. A central observation was that adipose tissue cultures secrete substantial amounts of enzymatically active PAPP-A, IGFBP-4 and IGF-II in a depot-specific manner. Thus, VAT media contained substantially more IGF-II, PAPP-A and PAPP-A-degraded IGFBP-4 fragments than SAT media. Consequently, VAT media possessed an increased IGF-IR activation potential in vitro. Another central observation was that in obese subjects, GH stimulation resulted in a higher secretion of IGF-I from VAT than SAT, whereas SAT from obese and lean women responded with similar increases in IGF-I following GH stimulation.

SAT from obese women secreted almost twice as much PAPP-A as SAT from lean women. Furthermore, VAT from obese subjects secreted more than four times as much PAPP-A as SAT, which confirm in a human setting what has previously been documented pre-clinically (17).

In addition, IGFBP-4 was highly abundant in culture media. However, using highly specific IGFBP-4 fragment assays between SAT from obese and lean women, whereas the other differences observed at baseline persisted (P<0.05; Table 3). Finally, SAT from lean and obese women responded similarly to GH as only the expression of IGF-I increased (P<0.05).

Figure 3

Culture media levels of intact IGFBP-4 and IGFBP-4 fragments. Intact IGFBP-4 and IGFBP-4 fragments were measured in culture media from VAT and SAT from obese women (A) and SAT from lean women (B). The two fragments were determined by assays that are specific for the PAPP-A-generated neo-epitopes on the IGFBP-4 fragments. As we had only limited media available for the assays, measurements on obese women (A) were restricted to the baseline condition only. In lean women (B), measurements were performed on SAT media following stimulation with the lowest GH dose (1 µg/L). No difference between the concentrations of intact IGFBP-4 and the two IGFBP-4 fragments was observed. All data are stated as median (interquartile range). Differences between protein levels were performed using the Friedman repeated-measures analysis of variance on ranks, which if significant was followed by the Student–Newman–Keul method. *P < 0.05.

Table 2

Spearman rank order correlation between media levels of bioactive IGF, intact IGFBP-4, N-terminal IGFBP-4, C-terminal IGFBP-4 and PAPP-A. All correlations are based on 26 VAT and SAT media, analyzed separately. Only data from unstimulated tissues (baseline condition) are available. Data are stated as r-values and P-values. For clarity, only data with correlations with P-values below 0.05 have been included.

<table>
<thead>
<tr>
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<th>VAT</th>
<th>SAT</th>
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<tr>
<td></td>
<td>IGF bioactivity</td>
<td>Intact IGFBP-4</td>
</tr>
<tr>
<td>IGF bioactivity</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Intact IGFBP-4</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>N-terminal IGFBP-4</td>
<td>NS</td>
<td>0.50; P &lt; 0.01</td>
</tr>
<tr>
<td>C-terminal IGFBP-4</td>
<td>NS</td>
<td>0.84; P &lt; 0.0001</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

All significant correlations are in bold. NS, not significant.
Table 3  SAT mRNA densities of GH and IGF-related peptides and receptors. The four left-sided data columns show mRNA levels in SAT obtained from eight lean women. The two right-sided data columns show mRNA levels in SAT from 17 obese women. Each SAT from lean women was incubated with either 0 (baseline), 1, 10 or 100 µg/L, whereas SAT from obese women was only stimulated with 0 (baseline) and 100 µg/L GH. Lean and obese women were matched according to age. All data are stated as median (interquartile range). Among lean women, the effect of SAT stimulation with increasing concentrations of GH was tested using the Friedman repeated-measures analysis of variance on ranks, which if significant was followed by the Student–Newman–Keul test. Differences between SAT from lean and obese women were tested using the Mann–Whitney test, and differences between SAT among obese women were tested using the Wilcoxon test.

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Lean baseline</th>
<th>Lean GH stimulation (1 µg/L)</th>
<th>Lean GH stimulation (10 µg/L)</th>
<th>Lean GH stimulation (100 µg/L)</th>
<th>Obese baseline</th>
<th>Obese GH stimulation (100 µg/L)</th>
</tr>
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<tbody>
<tr>
<td>GHR</td>
<td>0.087 (0.044–0.111)</td>
<td>0.048 (0.020–0.079)</td>
<td>0.025 (0.019–0.042)</td>
<td>0.051 (0.027–0.069)</td>
<td>0.032 (0.020–0.075)</td>
<td>0.031 (0.021–0.049)</td>
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<tr>
<td>IGF-IR</td>
<td>0.003 (0.001–0.004)</td>
<td>0.003 (0.002–0.004)</td>
<td>0.001 (0.000–0.004)</td>
<td>0.003 (0.002–0.006)</td>
<td>0.003 (0.002–0.006)</td>
<td>0.024 (0.020–0.042)</td>
</tr>
<tr>
<td>IGF-I</td>
<td>0.156 (0.053–0.207)</td>
<td>0.067 (0.047–0.090)</td>
<td>0.056 (0.035–0.071)</td>
<td>0.073 (0.045–0.091)</td>
<td>0.151 (0.076–0.209)</td>
<td>0.265 (0.143–0.595)</td>
</tr>
<tr>
<td>IGF-II</td>
<td>0.043 (0.025–0.110)</td>
<td>0.071 (0.034–0.107)</td>
<td>0.065 (0.034–0.102)</td>
<td>0.151 (0.076–0.209)</td>
<td>0.151 (0.076–0.209)</td>
<td>0.304 (0.018–0.078)</td>
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<tr>
<td>IGFBP-4</td>
<td>0.335 (0.245–0.624)</td>
<td>0.251 (0.142–0.512)</td>
<td>0.221 (0.151–0.423)</td>
<td>0.301 (0.240–0.447)</td>
<td>0.301 (0.240–0.447)</td>
<td>0.572 (0.392–2.281)</td>
</tr>
<tr>
<td>PAPP-A**</td>
<td>0.009 (0.007–0.013)</td>
<td>0.008 (0.005–0.013)</td>
<td>0.007 (0.005–0.014)</td>
<td>0.011 (0.009–0.014)</td>
<td>0.011 (0.009–0.014)</td>
<td>0.321 (0.126–0.587)</td>
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<td>0.072 (0.051–0.098)</td>
<td>0.047 (0.036–0.100)</td>
<td>0.053 (0.033–0.070)</td>
<td>0.077 (0.059–0.089)</td>
<td>0.077 (0.059–0.089)</td>
<td>0.007 (0.054–0.093)</td>
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</table>

SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

**A p < 0.05 compared with baseline media levels in SAT tissues from the same subject; **b p < 0.05 compared with media from lean SAT tissue stimulated by 1 µg/L GH; **c p < 0.05 compared with media from lean SAT tissue stimulated by 10 µg/L GH; **d p < 0.05 when comparing baseline SAT media levels from lean and obese women; **e p < 0.05 when comparing 100 µg/L GH-stimulated SAT media levels from lean and obese women.

**Data are multiplied by 10.

SAT mRNA densities of GH and IGF-related peptides and receptors. The four left-sided data columns show mRNA levels in SAT obtained from eight lean women. The two right-sided data columns show mRNA levels in SAT from 17 obese women. Each SAT from lean women was incubated with either 0 (baseline), 1, 10 or 100 µg/L, whereas SAT from obese women was only stimulated with 0 (baseline) and 100 µg/L GH. Lean and obese women were matched according to age. All data are stated as median (interquartile range). Among lean women, the effect of SAT stimulation with increasing concentrations of GH was tested using the Friedman repeated-measures analysis of variance on ranks, which if significant was followed by the Student–Newman–Keul test. Differences between SAT from lean and obese women were tested using the Mann–Whitney test, and differences between SAT among obese women were tested using the Wilcoxon test.
Table 4 Adipose tissue mRNA densities of GH and IGF-related peptides and receptors in SAT and VAT. Each group consists of 26 mRNA measurements. Each study participant served as a donor of VAT and SAT. Thus, data are based on paired observations. All data are stated as median (interquartile range). Differences between mRNA levels were performed using the Friedman repeated-measures analysis of variance on ranks, which if significant was followed by the Student–Newman–Keul method.

<table>
<thead>
<tr>
<th>mRNA</th>
<th>VAT baseline</th>
<th>VAT GH stimulation (100 µg/L)</th>
<th>SAT baseline</th>
<th>SAT GH stimulation (100 µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHR*</td>
<td>0.014 (0.001–0.045)</td>
<td>0.014 (0.011–0.053)</td>
<td>0.036 (0.020–0.087)*</td>
<td>0.031 (0.018–0.050)</td>
</tr>
<tr>
<td>IGF-IR</td>
<td>0.031 (0.024–0.084)</td>
<td>0.030 (0.025–0.079)</td>
<td>0.028 (0.021–0.050)</td>
<td>0.027 (0.017–0.046)</td>
</tr>
<tr>
<td>IGF-IIR</td>
<td>0.264 (0.107–0.668)</td>
<td>0.256 (0.138–1.026)</td>
<td>0.311 (0.158–0.786)</td>
<td>0.313 (0.190–0.557)</td>
</tr>
<tr>
<td>IGF-I</td>
<td>0.051 (0.025–0.114)</td>
<td>0.106 (0.036–0.283)*</td>
<td>0.031 (0.019–0.100)</td>
<td>0.108 (0.083–0.174)*</td>
</tr>
<tr>
<td>IGF-II</td>
<td>1.122 (0.423–3.242)</td>
<td>0.933 (0.399–5.596)</td>
<td>0.683 (0.420–2.769)</td>
<td>0.647 (0.341–1.810)</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>0.300 (0.150–0.925)</td>
<td>0.289 (0.194–1.292)</td>
<td>0.358 (0.180–0.724)</td>
<td>0.289 (0.126–0.551)</td>
</tr>
<tr>
<td>PAPP-A**</td>
<td>0.032 (0.010–0.071)</td>
<td>0.021 (0.011–0.244)</td>
<td>0.007 (0.004–0.039)b</td>
<td>0.013 (0.006–0.031)c</td>
</tr>
</tbody>
</table>

R, receptor; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

aP < 0.05 when comparing baseline and GH-stimulated tissue of similar localization; bP < 0.05 when comparing baseline SAT and VAT; cP < 0.05 when comparing GH-stimulated SAT and VAT.

*Friedman's test was significant, but the post hoc test could not identify differences between groups. However, a paired test (Wilcoxon) showed that levels of GHR was significantly higher in SAT than those in VAT at baseline (P < 0.02), but not after GH stimulation (P = NS); **Data are multiplied by 10.

of PAPP-A protein, despite a lower mRNA expression. In obese subjects, on the other hand, the mRNA expression followed the secretion, being high in VAT and low in SAT. In summary, this indicates that transcriptional as well as post-translational mechanisms may be in play.

In this study we focused on IGFBP-4, but adipose tissue is indeed reported to express all IGFBPs (2). We were able to detect the mRNA expression of IGFBP-2, -3, -5 and -6 (data not shown), but the incubation media contained no detectable IGFBP-2 or -3 protein, as previously reported by us (3), and we had no assay for IGFBP-5. Therefore these data were not included. The only other IGFBP present in the incubation media in detectable concentrations was IGFBP-6 (data not shown). Similar to IGFBP-4, levels of IGFBP-6 were higher in VAT media than SAT media (median levels being ≈12 and ≈6 µg/L respectively), with no difference between SAT from lean and obese women. Thus, IGFBP-6 may also play a role in adipose tissue and indeed this tissue may be used as a model to improve our incomplete understanding of the role of IGFBP-6 in human biology.

Both VAT and SAT responded to GH stimulation by increasing IGF-I production, but VAT appeared to be more GH sensitive than SAT. This is in line with clinical studies showing that treatment of GH-deficient patients results in a relatively more pronounced reduction in VAT than that in SAT (34, 35, 36). On the other hand, neither the expression of IGF-IR nor the expression of IGF-I differed between the two fat depots; if anything, the expression of GHR was lower in VAT than that in SAT. Thus, the ability of VAT to secrete more IGF-I than SAT may be caused by post-transcriptional mechanisms. In this context it is tempting to focus on insulin, which shares overlapping intercellular signaling cascades with GH (37). However, our tissue culture system requires insulin to be added to the incubation medium; therefore, it is not possible to study the distinct effects of GH in the absence of insulin.

The liver is the major source of circulating IGF-I (28) and obese subjects respond with larger increases in IGF-I following GH stimulation than lean subjects (25), indicative of an augmented hepatic GH sensitivity (24). On this basis, we found it of interest to study whether the increased GH responsiveness in obesity also included adipose tissue. However, data suggested the opposite as the baseline expression of GHR was lower in SAT from obese than that from lean women. Nevertheless, the two tissues responded with similar increases in IGF-I mRNA expression and protein secretion following GH stimulation, whereas the levels of bioactive IGF correlated inversely with BMI. Thus, obesity does not appear to increase the SAT synthesis of IGF-I following GH stimulation; indeed, the overall IGF bioactivity may be lower following GH exposure.

Our study has limitations. We compared whole tissue fragments and accordingly, differences in cellular composition and number of adipocytes in VAT and SAT were not taken into consideration (14, 36). We acknowledge that visceral adipocytes are in general smaller than subcutaneous adipocytes, and that the observed differences in GH response may in part relate to adipocyte numbers. However, from a biological point of view, we and others believe that studies of whole tissue fragments (including all cell types and extracellular matrix) are more representative of the physiology of adipose tissue in vivo than the studies of isolated adipocytes (36). It is also a limitation that we for practical reasons had to compare 200 mg of SAT and VAT from obese women vs 500 mg of...
SAT from lean women, as a comparison of similar tissue sizes would have been ideal. Further, depending on where the liposuction was performed, the lean SAT originated from hips as well as abdomen; although we do not believe so, this may have influenced our results. In addition, our study did not include details about diseases among the obese tissue donors. Drug lists from the participants revealed that some of the obese donors received anti-diabetic medication and no doubt, it would be of interest to investigate the consequences of diabetes and its treatment on the IGF system in adipose tissue. Finally, as our data are obtained in vitro, one has to be cautious when extending these findings to the in vivo situation.

In this study we present novel data on the production of IGF components in human cultures of VAT and SAT. Given the demonstrated pro-atherogenic role of PAPP-A (11, 12, 38), our findings are compatible with the notion that VAT via a dysfunctional IGF system may be pathogenically linked to the development of cardiovascular disease, which is frequent in subjects suffering from visceral obesity (39).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This work was supported by the Danish Medical Research Council and the Department of Clinical Medicine at Aarhus University, Denmark. R H was funded by a research grant from the Danish Diabetes Academy supported by the Novo Nordisk Foundation.

Acknowledgements
The authors thankfully acknowledge the technicians at the Medical Research Laboratory for technical assistance. The authors are grateful to HyTest Ltd. for providing antibodies and for the IGFBP-4 and the two IGFBP-3 fragment assays. The authors also would like to thank Ansh Labs for providing kits for PAPP-A.

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PAPP-A and IGFBP-4 in human adipose tissue

M F Gude and others

European Journal of Endocrinology


Received 1 July 2016
Revised version received 16 August 2016
Accepted 1 September 2016