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# Glucose transporter 4 and insulin receptor substrate–1 messenger RNA expression in omental and subcutaneous adipose tissue in women

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**Abstract**

Insulin receptor substrate–1 (IRS-1) and glucose transporter 4 (GLUT4) expression may provide an indirect reflection of the capacity of adipocytes to respond to insulin stimulation. We examined messenger RNA (mRNA) expression of these genes in omental and subcutaneous adipose tissue of women. Paired omental and subcutaneous adipose tissue samples were obtained from 36 women (age, 47 ± 5 years; body mass index, 28.0 ± 5.4 kg/m$^2$) undergoing gynecologic surgeries. Total adiposity and visceral adiposity were assessed by dual-energy x-ray absorptiometry and computed tomography. The GLUT4 and IRS-1 mRNA expression levels were both significantly higher in subcutaneous compared with omental adipose tissue. A negative correlation was observed between body fat percentage and subcutaneous adipose tissue GLUT4 ($r = -0.39$, $P < .05$) and IRS-1 ($r = -0.30$, $P < .08$) mRNA abundance. However, in omental fat, only GLUT4 mRNA was inversely associated with body fat percentage ($r = -0.53$, $P < .001$). Moreover, the homeostasis model assessment of insulin resistance index was associated with mRNA expression of subcutaneous GLUT4 ($r = -0.56$, $P < .001$), subcutaneous IRS-1 ($r = -0.51$, $P < .01$), and omental GLUT4 ($r = -0.54$, $P < .001$), but not omental IRS-1. Interestingly, plasma adiponectin was only associated with subcutaneous GLUT4 ($r = 0.48$, $P < .01$) and IRS-1 ($r = 0.48$, $P < .05$) mRNA expression. The GLUT4 protein, unlike mRNA expression, was higher in omental than in subcutaneous adipose tissue. However, abdominal obesity–related differences in protein or mRNA expression were similar. Omental IRS-1 expression was low and unaffected by visceral obesity. In contrast, omental and subcutaneous GLUT4 as well as subcutaneous IRS-1 were reduced in visceral obesity. This divergent pattern of expression may reflect a lower capacity of omental adipose tissue to respond to insulin stimulation at all adiposity levels.


## 1. Introduction

Increased fat accumulation in the visceral compartment strongly correlates with the presence of metabolic disorders such as insulin resistance [1]. Adipose tissue located inside the abdominal cavity, which includes omental, retroperitoneal, and mesenteric fat, has been shown to have distinct cellularity, metabolism, and gene expression compared with subcutaneous adipose tissue [2-4]. Insulin plays an important role in fat deposition through the regulation of adipose tissue lipolysis, adipogenesis, lipogenesis, and glucose uptake [5]. Glucose transporter 4 (GLUT4) is essential for insulin-induced glucose uptake, whereas maximal insulin-induced antilipolysis, lipogenesis, and glucose uptake require insulin receptor substrate–1 (IRS-1) signaling [6,7]. Expression of these genes can provide an indirect reflection of the metabolic capacity of adipocytes to respond to insulin stimulation [8]. Indeed, reduced IRS-1 expression in human adipose tissue impairs downstream insulin signaling through the phosphatidylinositol 3-kinase and Akt, leading to decreased insulin-induced glucose uptake [9].

Although an increasing number of studies reveal regional differences in adipose tissue metabolism [2,8,10-13], little is

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known regarding regional differences in markers of adipose tissue insulin sensitivity and glucose uptake. Some studies have previously observed such differences [8,11-13] and found that, although basal glucose uptake was higher in omental adipocytes without obvious differences in insulin-induced glucose uptake [11,12], omental adipocytes were more resistant to the antilipolytic effect of insulin [8,13]. These studies have also compared expression of insulin-signaling genes in abdominal adipose tissue depots. Results on IRS-1 protein expression remain inconsistent, as MacLaren et al [14] and Zierath et al [8] reported a lower IRS-1 content in omental vs subcutaneous adipose tissue and the opposite was observed by Lundgren et al [12] and Bashan et al [15]. Human data on depot differences in GLUT4 expression are scarce. Glucose transporter 4 mRNA expression appears to be higher in subcutaneous adipose tissue [11,16], although protein content was higher in omental and round ligament adipose tissue of these patients [11]. However, some of these results were obtained in massively obese subjects [11,13-15]; and only 1 study attempted to evaluate regional differences in insulin-mediated metabolism across the spectrum of adiposity values [8].

In the present study, we aimed to examine associations between GLUT4 and IRS-1 mRNA and protein expression in omental and subcutaneous adipose tissue of women covering a wide range of visceral adipose tissue accumulation. We tested the hypothesis that the capacity of the omental fat depot to respond to insulin, as indirectly assessed by GLUT4 and IRS-1 mRNA and protein expression, would be lower than that of subcutaneous fat and would be further decreased in visceral obesity.

## 2. Materials and methods

### 2.1. Subject recruitment

The study included 36 women aged 40 to 62 years recruited through the elective surgery schedule of the Gynecology Unit of the Laval University Medical Center. Informed consent was obtained from patients, and this project was approved by the ethical committee of the Laval University Medical Research Center. Women of the study were undergoing abdominal gynecologic surgery for total (n = 35) or subtotal (n = 1) abdominal hysterectomies, some with salpingo-oophorectomy of 1 (n = 4) or 2 (n = 14) ovaries. Reasons for surgery included one or more of the following: menorrhagia/menometrorrhagia (n = 18), myoma/fibroids (n = 27), incapacitating dysmenorrhea (n = 8), pelvic pain (n = 1), benign cyst (n = 8), endometriosis (n = 4), adenomyosis (n = 1), pelvic adhesions (n = 4), endometrial hyperplasia (n = 3), polyp (n = 1), and/or ovarian thecoma (n = 1). Based on menstrual history questionnaires, medical files, and blood follicle-stimulating hormone, 8 women were identified as postmenopausal, 4 of whom were using hormonal replacement therapy. The remaining women (n = 28) were identified as pre- and/or perimenopausal.

### 2.2. Body fatness and body fat distribution measurements

These measurements were performed on the morning of or within a few days before or after the surgery. Total body fat mass and fat percentages were determined by dual-energy x-ray absorptiometry. Abdominal subcutaneous and visceral adipose tissue cross-sectional area measurements were performed at the L4-5 vertebrae level by computed tomography as previously described [17]. The quantification of visceral adipose tissue area was done by delineating the intraabdominal cavity at the internal-most aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body using the ImageJ 1.33u software (National Institutes of Health, Bethesda, MD). Intraobserver coefficients of variation between 2 adipose tissue area measures (n = 10) were less than 0.5%.

### 2.3. Blood glucose, insulin, and adiponectin

Overnight fasting plasma glycemia and insulinemia were monitored on the morning of the surgery. Glucose was measured using the glucose oxidase method, and insulin was quantified by radioimmunoassay (Linco Research, St Charles, MO). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using the following formula: fasting insulin (in microunits per milliliter) × fasting glucose (in millimoles per liter) ÷ 22.5 [18]. Initially, none of the women recruited for the study had previously obtained a diagnosis of diabetes or took antidiabetic drugs. However, fasting glycemia monitoring on the morning of the surgery revealed that 6 women had impaired fasting glycemia (≥6.1 mmol/L). Concentrations of adiponectin in plasma were measured by enzyme-linked immunosorbent assay (B-Bridge International, Sunnyvale, CA).

### 2.4. Adipose tissue sampling

During the surgical procedure, subcutaneous and omental adipose tissue samples were, respectively, collected at the site of surgical incision (lower abdomen) and at the distal portion of the greater omentum. Samples were immediately carried to the laboratory in 0.9% saline preheated at 37°C. A portion of the tissue sample was used to perform adipocyte isolation, and the remaining tissue was immediately snap frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

### 2.5. Adipocyte isolation

Adipose tissue samples were digested for 45 minutes at 37°C in the presence of collagenase type I (350 U/mL) in Krebs-Ringer-Henseleit buffer (25 mmol/L HEPES, 125 mmol/L NaCl, 3.7 mmol/L KCl, 5 mmol/L $CaCl_2$, 2.5 mmol/L $MgCl_2$, and 1 mmol/L $K_2HPO_4$ [pH 7.4])

supplemented with 5 mmol/L glucose, 0.1 μmol/L adenosine, 0.1 mg/mL ascorbic acid, and 4% electrophoresis-grade bovine serum albumin (Sigma-Aldrich, St. Louis, MO) according to a modified version of the Rodbell [19] method. After digestion, the suspension was filtered through nylon mesh; and adipocytes were washed 3 times with the buffer. Pictures of cell suspensions were taken using a contrast-phase microscope to assess cell size. The Scion Image software was used to measure the diameter of 250 adipocytes for each tissue sample (Scion Corp, Frederick, MD).

### 2.6. Real time quantitative polymerase chain reaction mRNA measurements

Omental and subcutaneous adipose tissue total RNA was isolated using RNeasy lipid tissue extraction kit (Qiagen, Valencia, CA) following the manufacturer's recommendations. Trace DNA was removed by on-column digestion (Qiagen) before assessment of RNA quantity and quality using an Agilent Technologies 2100 Bioanalyzer and RNA 6000 Nano LabChip kit (Agilent, Mountain View, CA). Total RNA was denatured with random hexamers (Invitrogen, Carlsbad, CA) and dNTPs (Amersham Biosciences, Piscataway, NJ), mixed with Superscript II mixture, and incubated for 120 minutes at 42°C to generate complementary DNA. Amplification of complementary DNA was run in triplicate using 10 nmol/L Z-tailed forward primer (ACTGAACCTGACCGTACA), 100 nmol/L of reverse primer, and 100 nmol/L Amplifluor Uniprimer probe (Chemicon, Temecula, CA) in Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The mixture was incubated using the Applied Biosystems Prism 7900 Sequence Detector for 2 minutes at 50°C and for 4 minutes at 95°C, and cycled 55 times for 15 seconds at 95°C and 40 seconds at 55°C. According to a standard curve, target gene amplification efficiency was normalized to 18S ribosomal RNA amplification and expressed in arbitrary quantity. No significant correlation was observed between 18S ribosomal RNA expression level and adiposity-related variables such as body mass index (BMI), visceral adipose tissue area, or the HOMA-IR index. Primer sequences for GLUT4 (NM_001042, F:5-Z-AACAGATAGGCTCCGAAGATGG-3, R:5-TCCCAGTCACTCACTCGCTGCTG) and IRS-1 (NM_005544, F:5-Z-TCACAGCAGAATGAAGACCTAAATG-3, R:5-TGAGTTAGAAGAGGATTTGCTGAGG-3) were designed using Primer Express 2.0 (Applied Biosystems).

### 2.7. Western blot

Omental and subcutaneous adipose tissue samples were homogenized in sucrose buffer (20 mmol/L HEPES [pH 7.4], 250 mmol/L sucrose, and 4 mmol/L EDTA) containing protease inhibitors. Lipids were removed by centrifugation at 12 000*g* for 10 minutes, and insoluble materials were removed by centrifugation at 150 000 g for 90 minutes. For immunoblotting, 20 μg of protein homogenate diluted in sodium dodecyl sulfate (SDS) buffer 4× (0.8 mmol/L Tris [pH 6.8], 8% SDS, 5 mmol/L EDTA, 40% glycerol, and 0.2 mmol/L dithiothreitol) was heated at 37°C for 30 minutes and separated on a 10% SDS–polyacrylamide gel. Proteins were transferred to nitrocellulose membranes (1 hour at 100 V), and unspecific sites were blocked with 5% nonfat milk diluted in wash solution (50 mmol/L Tris [pH 7.4], 150 mmol/L NaCl, and 0.1% Tween 20) for 1 hour. Membranes were then incubated overnight at 4°C with the primary antibody against IRS-1 (Upstate Biotechnology, Temecula, CA) or GLUT4 (Cedarlane, Temecula, CA), washed 3 × 10 minutes, and incubated for 1 hour with antirabbit immunoglobulin G conjugated to horseradish peroxidase. Finally, membranes were washed 3 × 10 minutes; and proteins were visualized by chemiluminescence. Densitometric analysis was performed with ImageQuant TL software (GE Healthcare, Piscataway, NJ).

### 2.8. Statistical analyses

Omental vs subcutaneous differences in mRNA expression in the entire sample were tested using paired *t* tests. A repeated-measure analysis of variance was used to compare regional differences in mRNA expression between visceral adipose tissue area tertiles. Pearson rank correlation coefficients were computed to quantify associations between GLUT4 or IRS-1 mRNA expression and adipose tissue or adipocyte measures. Variables that were not normally distributed based on a significant Shapiro-Wilk test ($P < .05$) were $\log_{10}$- or Box-Cox–transformed in statistical analyses. All statistical analyses were performed using the JMP statistical software (SAS, Cary, NC).

Table 1
Physical and metabolic characteristics of the 36 women of the study

| Variable | Mean ± SD | Range (minimum-maximum) |
|---|---|---|
| Anthropometrics | | |
| Age (y) | 47 ± 5 | 40-62 |
| Weight (kg) | 74 ± 17 | 52-111 |
| BMI (kg/m$^2$) | 28.0 ± 5.4 | 19.6-41.3 |
| Fat mass (kg) | 27.1 ± 10.3 | 11.2-50.2 |
| Body fat percentage (%) | 35.6 ± 6.5 | 19.6-47.5 |
| Abdominal adipose tissue (cm$^2$) | | |
| Total | 454 ± 200 | 137-991 |
| Visceral | 102 ± 50 | 34-233 |
| Subcutaneous | 352 ± 158 | 103-759 |
| Lipid profile (mmol/L) | | |
| Total cholesterol | 4.9 ± 0.7 | 3.6-6.2 |
| Triglycerides | 1.3 ± 0.7 | 0.5-2.9 |
| VLDL cholesterol | 0.5 ± 0.3 | 0.1-1.2 |
| HDL cholesterol | 1.4 ± 0.3 | 0.8-2.0 |
| Glucose homeostasis | | |
| Fasting glycemia (mmol/L) | 5.6 ± 0.6 | 4.8-7.8 |
| Fasting insulin (pmol/L) | 11.5 ± 6.0 | 2.4-27.6 |
| HOMA-IR index | 3.0 ± 1.8 | 0.8-8.8 |

VLDL indicates very low-density lipoprotein; HDL, high-density lipoprotein.

## 3. Results

Glucose transporter 4 and IRS-1 mRNA expressions in adipose tissue depots were measured in 36 healthy women approximately 47 years old as shown in Table 1. According to the mean BMI of 28.0 kg/m$^2$, women were overweight but covered a wide range of adiposity, with body fat percentages ranging from 19.6% to 47.5%. Moreover, computed tomography measures revealed a 7-fold interindividual variation in visceral and subcutaneous adipose tissue areas.

Regional differences in GLUT4 and IRS-1 mRNA expression levels were found between subcutaneous and visceral adipose tissue (data not shown). Glucose transporter 4 and IRS-1 mRNA expressions were, respectively, 15% and 22% lower in omental adipose tissue compared with subcutaneous adipose tissue (subcutaneous: 34.8 ± 1.9, omental: 29.4 ± 2.3, $P < .01$ for GLUT4 and subcutaneous: 57.1 ± 4.6, omental: 40.2 ± 2.8, $P < .001$ for IRS-1, relative to 18S expression).

Table 2 shows correlations between adiposity measures as well as glucose homeostasis variables and GLUT4 or IRS-1 mRNA expression levels in omental and subcutaneous adipose tissue. Negative correlations were found between omental GLUT4 mRNA expression and adiposity measures including BMI, body fat percentage, and visceral adipose tissue area. Moreover, subcutaneous GLUT4 mRNA expression was negatively correlated with BMI, body fat percentage, and visceral adipose tissue area. Similarly, subcutaneous IRS-1 mRNA expression was negatively correlated with visceral adipose tissue area and showed a trend for a negative correlation with other adiposity measures such as BMI and body fat percentage. No significant correlation was found between omental IRS-1 mRNA expression and adiposity measures. Similar correlation patterns were also found with glucose homeostasis measures and diameters of adipocytes from both adipose tissue depots. Finally, we observed that serum adiponectin was positively correlated with subcutaneous mRNA expression of GLUT4 and IRS-1, whereas no correlation was observed with omental mRNA expression of these genes.

Table 2
Pearson correlation coefficients between characteristic of the women and the omental or subcutaneous adipose tissue GLUT4 and IRS-1 mRNA expression level

| Variable | GLUT4 | | IRS-1 | |
|---|---|---|---|---|
| | OM[a] | SC | OM[a] | SC[a] |
| Anthropometric | | | | |
| Weight[a] | −0.58‡ | −0.38* | NS | NS |
| BMI | −0.54‡ | −0.37* | NS | −0.28 |
| Fat mass[a] | −0.58‡ | −0.42* | NS | −0.28 |
| Body fat percentage | −0.50† | −0.38* | NS | −0.30 |
| Abdominal adipose tissue | | | | |
| Total | −0.54‡ | −0.36* | NS | −0.27 |
| Visceral[a] | −0.60‡ | −0.47† | NS | −0.37* |
| Subcutaneous | −0.49† | −0.32* | NS | NS |
| Glucose homeostasis | | | | |
| HOMA-IR index[a] | −0.57‡ | −0.55‡ | −0.32 | −0.54‡ |
| Fasting glycemia[b] | NS | −0.35* | NS | −0.33* |
| Fasting insulin[a] | −0.60‡ | −0.56‡ | −0.35* | −0.55‡ |
| Adipocytes diameter | | | | |
| Omental | −0.57‡ | −0.54‡ | NS | −0.37* |
| Subcutaneous | −0.64‡ | −0.50† | NS | −0.39* |
| Adiponectin | | | | |
| Serum concentration[b] | +0.29 | +0.51† | NS | +0.53‡ |

N = 36 (n = 35 for subcutaneous IRS-1). OM indicates omental; SC, subcutaneous; NS, not significant.
[a] $Log_{10}$-transformed data.
[b] Box-Cox–transformed data.
* $P \leq .05$.
† $P \leq .01$.
‡ $P \leq .001$.

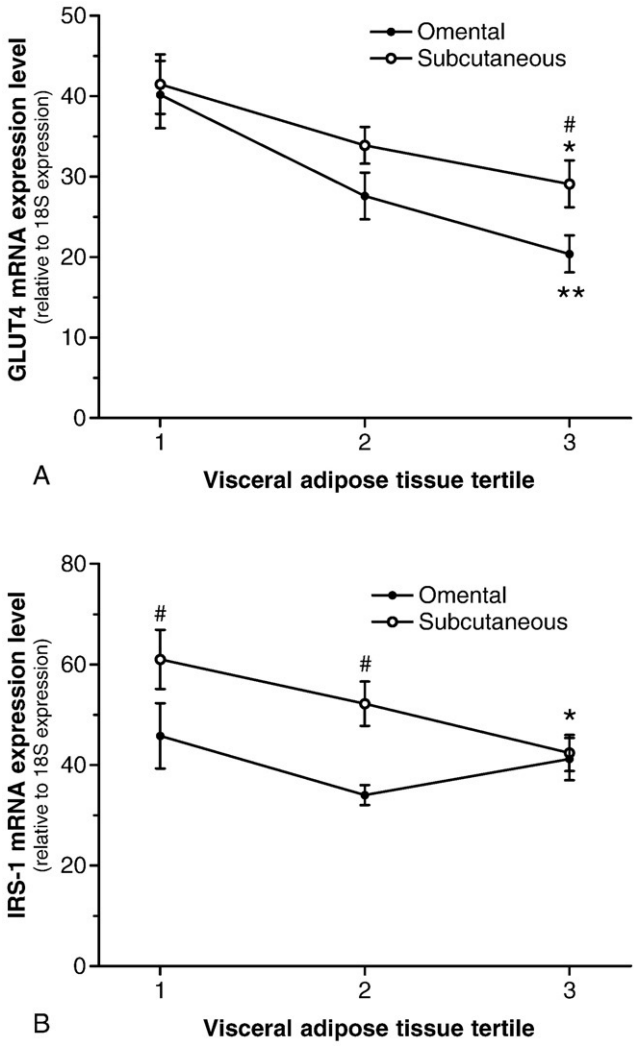


Fig. 1. Regional differences in GLUT4 (A) and IRS-1 (B) mRNA expression in omental and subcutaneous adipose tissue according to tertiles of visceral adipose tissue area. Mean visceral adipose tissue area of each tertile was as follows: (1) 53.5 ± 3.5 cm$^2$, n = 12; (2) 92.0 ± 4.3 cm$^2$, n = 12; and (3) 160.9 ± 10.7 cm$^2$, n = 12. Differences from tertile 1 mRNA expression level in a given adipose tissue compartment are shown (*$P < .05$, **$P < .01$). Means ± SEM are shown. Depot differences determined by repeated-measure analysis of variance in each tertile are indicated ($^{\#}P < .01$).

Regional differences in GLUT4 and IRS-1 mRNA expression according to tertiles of visceral adipose tissue area are shown in Fig. 1. Glucose transporter 4 mRNA

expression in omental and in subcutaneous adipose tissue was reduced in the middle and the upper tertile compared with the lowest tertile of visceral adipose tissue area (Fig. 1A). Subcutaneous GLUT4 mRNA expression was significantly higher than omental GLUT4 mRNA expression in women with high visceral adipose tissue area (Fig. 1A). On the other hand, omental IRS-1 mRNA expression level was similar in all tertiles, whereas subcutaneous IRS-1 mRNA expression was reduced in the upper compared with the lower tertile of visceral adipose tissue area (Fig. 1B). Insulin receptor substrate–1 mRNA expression was significantly higher in subcutaneous adipose tissue compared with omental adipose tissue in the lowest and median tertiles but was identical in the highest tertile of visceral adipose tissue area (Fig. 1B).

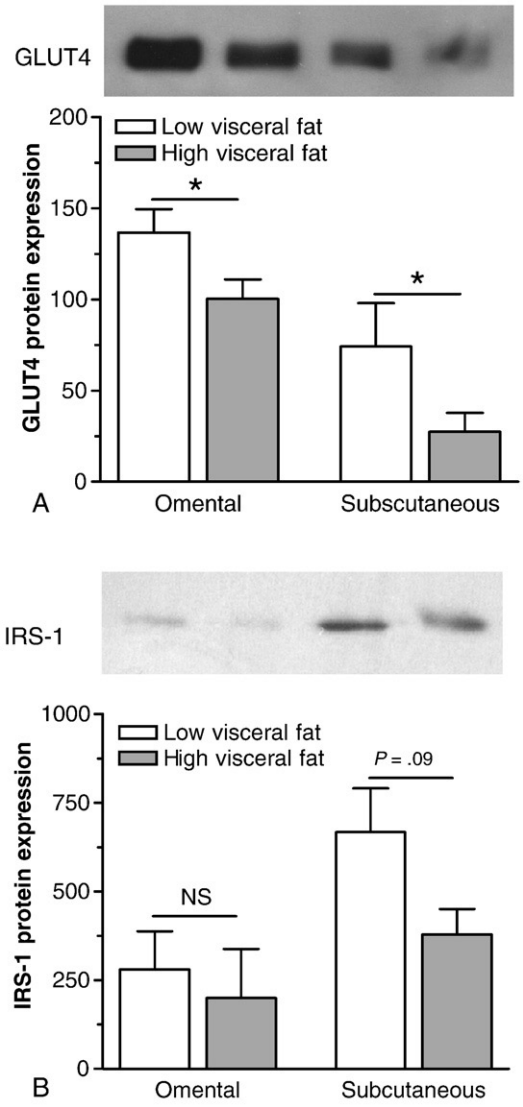


Fig. 2. Regional differences in GLUT4 and IRS-1 protein expression in omental and subcutaneous adipose tissue of women with low (<100 $cm^2$, n = 4) and high (>100 $cm^2$, n = 4) visceral fat accumulation. Representative Western blot and quantification (means ± SEM) are shown. *$P$ less than .05. NS indicates not significant.

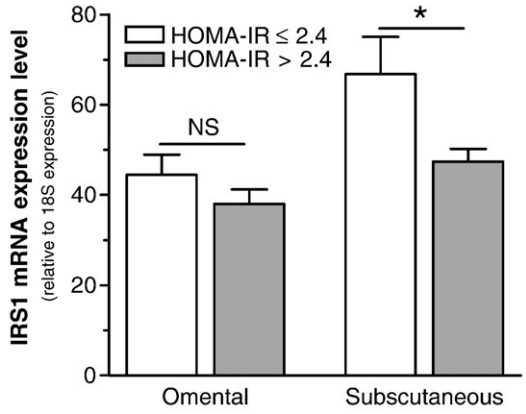


Fig. 3. Comparison of GLUT4 (A) and IRS-1 (B) mRNA expression level in omental or subcutaneous adipose tissue in women with a low (≤2.4, n = 18) or high (>2.4, n = 18) HOMA-IR index. Means ± SEM are shown. *$P$ less than .05; **$P$ less than .01.

Western blot analyses of GLUT4 and IRS-1 protein were performed in subcutaneous and omental adipose tissue of a small representative sample of women with either low (<100 $cm^2$) or high (>100 $cm^2$) visceral adipose tissue area. Representative Western blots and quantification of GLUT4 and IRS-1 protein content are shown in Fig. 2. Interestingly, unlike mRNA expression, GLUT4 protein content was higher in omental adipose tissue (+133%, $P < .05$) (Fig. 2A). However, as seen with mRNA, GLUT4 protein content was reduced in both omental and subcutaneous adipose tissues of women with high visceral adipose tissue areas (−27% and −63%, respectively; $P < .05$) (Fig. 2A). Similarly, IRS-1 protein expression in subcutaneous adipose tissue tended to be significantly lower in women with high visceral fat accumulation (−44%, $P = .08$) (Fig. 2B). On the other hand, omental adipose tissue IRS-1 protein content was similarly low in women with low and high visceral fat accumulation (Fig. 2B).

To further examine the association of GLUT4 and IRS-1 mRNA expression in subcutaneous and omental adipose tissue and insulin sensitivity, we subdivided our sample according to the HOMA-IR index median as shown in Fig. 3. Women with a high HOMA-IR index were characterized by a lower subcutaneous adipose tissue mRNA expression of both GLUT4 (−23%, $P < .01$) and IRS-1 (−29%, $P < .05$) (Fig. 3A, B). Omental adipose tissue expression of GLUT4 was also reduced in women with elevated HOMA-IR index (−35%, $P < .01$) (Fig. 3A), whereas omental IRS-1 mRNA expression was not ($P = .25$) (Fig. 3B).

## 4. Discussion

The aim of the present study was to assess the expression profile of the insulin-signaling molecule IRS-1 and the glucose transporter GLUT4 in subcutaneous and visceral abdominal adipose tissue of healthy women covering a wide range of adiposity. Results of this study show that the

expression of both GLUT4 and IRS-1 mRNA is reduced in the subcutaneous fat compartment of abdominally obese women. Omental expression of GLUT4 was also reduced in women with abdominal obesity. However, IRS-1 mRNA expression in omental adipose tissue was significantly lower than that of subcutaneous adipose tissue and was unaffected by abdominal obesity. Glucose transporter 4 expression in both depots and IRS-1 expression in subcutaneous adipose tissue were clearly correlated with adiposity, insulin resistance index, and adipocyte size. Correlations between IRS-1 mRNA expression in omental adipose tissue and adiposity, insulin resistance index, and adipocyte size were either absent or very weak. As mRNA expression, protein levels of IRS-1 and GLUT4 were reduced in both adipose tissue depots of abdominally obese women, except for IRS-1 in omental adipose tissue. This study shows for the first time that IRS-1 and GLUT4 have divergent expression patterns in abdominal adipose tissues of women, which may reflect a lower capacity of omental adipose tissue to respond to insulin stimulation at all adiposity levels.

Few studies have previously observed regional differences in the expression of IRS-1 [8,12,14,16], GLUT4 [11,12,14,16], and other insulin-signaling components [12,16]. Conflicting results have been previously reported on IRS-1 protein expression. Indeed, MacLaren et al [14] and Zierath et al [8] observed higher protein expression level in subcutaneous adipose tissue; but opposite results were published by Lundgren et al [12] and Bashan et al [15]. Moreover, Lefebvre et al [16] found no significant regional difference in IRS-1 mRNA expression. The lower IRS-1 mRNA and protein expression in omental adipose tissue of the present study confirms previous observations [8,14]. Interestingly, higher subcutaneous adipose tissue mRNA expression of GLUT4 was observed by others [11,16], despite the fact that protein content was higher in omental adipose tissue [11,12]. We confirm these results, as we also observed this discrepancy between GLUT4 mRNA and protein expression in our study.

One aim of this study was to examine the effect of adiposity and especially visceral adipose tissue accumulation on the expression profile of these proteins in subcutaneous and omental adipose tissue. We found that adiposity and especially visceral fat accumulation are associated with important differences in the expression profile of the insulin-signaling components examined. Lefebvre et al [16] had examined the association between GLUT4 and IRS-1 expression and obesity. They reported no significant effect of adiposity on GLUT4 and IRS-1 mRNA expression in either fat depot. MacLaren et al [14] observed that IRS-1 and GLUT4 mRNA expression was significantly increased in insulin-resistant, but not in insulin-sensitive, obese subjects. These discrepancies with our study could be due to differences in study design. Accordingly, their samples included both morbidly obese men and women, which could have confounded the analysis [14,16]. Garvey et al [20] showed that GLUT4 protein content in subcutaneous adipose tissue was severely reduced in obese men and also in men with type 2 diabetes mellitus, which is consistent with our observation.

Our results suggest that IRS-1 expression in omental fat is low compared with subcutaneous fat and is unaffected by abdominal obesity. The origin and the consequences of such a divergent expression pattern remain unclear. As IRS-1 is a key component of the insulin-signaling cascade, lower IRS-1 expression could lead to a lower insulin sensitivity [9]. Because IRS-1 expression was reduced only in subcutaneous adipose tissue and not in omental adipose tissue of women with high visceral adipose tissue accumulation, we speculate that subcutaneous adipose tissue may proportionally become more insulin resistant in these obese women. In consequence, insulin-induced glucose uptake, lipogenesis, and antilipolytic effect may be more profoundly altered in this depot with increasing adiposity [9]. Indeed, as subcutaneous adipocytes reach maximal lipid storage, resistance to insulin action may begin to slow down metabolic flux to this tissue. In parallel, omental adipose tissue insulin sensitivity would be low, even in the normal weight range, and would remain unaltered with higher visceral fat accumulation. We may speculate that these modifications in IRS-1 expression pattern with abdominal obesity could reflect decreased insulin signaling and reduced storage of excess substrates in both the subcutaneous and omental depots and thus favor storage in ectopic sites.

Insulin receptor substrate−1 and GLUT4 mRNA expression in subcutaneous adipose tissue was strongly associated to serum adiponectin level in the women examined. On the other hand, no significant association was found with omental mRNA expression of GLUT4 and IRS-1 mRNA. Thus, decreased plasma adiponectin levels appear to be mainly associated with the capacity of subcutaneous adipose tissue to respond to insulin stimulation as assessed indirectly by IRS-1 and GLUT4 expression. With the present study design, we are not in a position to conclude on the direction of this association. However, Fu et al [21] have shown in 3T3-L1 adipocytes that adiponectin could act as an autocrine factor by promoting adipocyte differentiation and by increasing GLUT4 mRNA expression. Low serum adiponectin in obese women could be the initial step leading to lower GLUT4 and possibly IRS-1 mRNA expression in subcutaneous adipose tissue [21]. Nonetheless, the mechanism underlying the specific association with subcutaneous and not the omental adipose tissue remains to be clarified.

We have further investigated the impact of obesity on GLUT4 and IRS-1 expression by measuring protein content in a subsample of the study. As shown previously, decreases in GLUT4 and IRS-1 could lead to a defect in insulin signaling and glucose uptake [9]. However, we did not evaluate the implication of posttranslational mechanisms such as phosphorylation of IRS-1 on either tyrosine or serine residues [22] and alterations in GLUT4 translocation to the

plasma membrane [23]. Such phenomena are altered in adipose tissues of obese individuals and could reduce normal insulin signaling [22,23]. Because we did not have access to insulin-treated adipose tissue in this study, it was not possible to assess direct measures of insulin sensitivity at the tissue level. Measures performed in this study only provide an indirect view of insulin-signaling capacity.

Because whole adipose tissue samples were used, the amount of mature adipocytes as well as nonadipocyte cells may have influenced our results. Unlike GLUT4, which is only expressed in mature adipocytes, IRS-1 is expressed in mature adipocytes and in nonadipocyte cells. Through IRS-1, insulin can trigger the differentiation of preadipocytes to mature adipocytes and can also modulate glucose uptake and lipolysis rates in mature adipocytes. Because IRS-1 expression was assessed only in frozen samples of whole tissue, we were not able to discriminate the contribution of each cell fraction. This factor may have contributed to the divergent expression patterns observed for GLUT4 and IRS-1.

In conclusion, mRNA expression of GLUT4 and IRS-1 reveals a divergent expression pattern in subcutaneous and omental adipose tissue of obese women because no association was observed between omental IRS-1 mRNA expression and adiposity measures. This dissociation suggests that the metabolic effects of insulin could be differentially altered in each fat depot with increasing visceral adiposity. Decreased GLUT4 expression in both depots with increasing visceral adipose tissue area indirectly suggests that adipocytes from both depots may have a reduced capacity to transport glucose. On the other hand, the fact that omental IRS-1 mRNA and protein expressions were low and unaffected by visceral adiposity could be a signal of low fat storage capacity through lipid uptake in omental adipose tissue of obese women leading to proportionally increased fat storage in ectopic sites.

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