Insulin regulation of gene expression and concentrations of white adipose tissue-derived proteins in vivo in healthy men: relation to adiponutrin

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Abstract

Adiponutrin is a newly described white adipose tissue (WAT)-derived protein whose function and regulation remain widely unclear in humans though it is suggested to be related to insulin sensitivity. Recently, we found that adiponutrin expression is reduced in type 2 diabetic subjects in basal and insulin-stimulated states. To examine adiponutrin regulation by the insulin pathway in relation to other WAT-related proteins with well-known relation to insulin signaling and action, we examined in healthy young men (1) the association of adiponutrin with p85\(\alpha\) PI3K and HKII, leptin, adiponectin, and acylation-stimulating protein (ASP) and (2) the regulation of adiponutrin and WAT-derived proteins by 3-h hyperinsulinemic euglycemic clamp (HIEG). At baseline (N=20), adiponutrin expressions were positively correlated with those of p85\(\alpha\) PI3K (R=0.54, P=0.017), HKII (R=0.58, P=0.010), and serum leptin (R=0.51, P=0.036), but not with any other parameter measured including insulin sensitivity. Hyperinsulinemia (N=10, +236.5% above baseline) significantly increased the expression of adiponutrin (+770%, P=0.002), p85\(\alpha\) PI3K (+150%, P=0.033), HKII (+147%, P=0.007), and serum leptin (+11%, P=0.031), while it decreased serum adiponectin (−15%, P=0.001). In the insulin-stimulated state, adiponutrin mRNA expression levels correlated with basal p85\(\alpha\) PI3K (R=0.76, P=0.018) and HKII (R=0.86, P=0.003) expression levels, with percentage increase in insulin (R=0.73, P=0.040), and with insulin-stimulated state HKII (R=0.82, P=0.007), leptin (R=0.84, P=0.005), and adiponectin (R=0.85, P=0.004) mRNA levels. In healthy young men, adiponutrin expression is unregulated by hyperinsulinemia and is related to basal and/or insulin-stimulated p85\(\alpha\) PI3K, HKII, adiponectin, and leptin expression levels. We hypothesize that insulin-mediated regulation of adiponutrin expression is under the PI3K pathway. The relevance of the present findings to reduced adiponutrin expression in type 2 diabetes is discussed.


Introduction

Adiponutrin is a newly described non-secreted protein mainly expressed in brown and white adipose tissues (WAT; Baulande et al. 2001, Kershaw et al. 2006). To date, the physiological function of adiponutrin is unknown though it is suggested that adiponutrin may facilitate lipid storage in WAT (Jenkins et al. 2004, Lake et al. 2005, Kershaw et al. 2006). Similarly, the physiological regulation of adiponutrin expression is still being explored but several lines of evidence, mainly in rodents, suggest that adiponutrin may be regulated by energy balances and particularly by insulin.

In vitro, adiponutrin expression increases with differentiation of murine pre-adipocytes and is induced by glucose and insulin treatments of these cells (Baulande et al. 2001, Kershaw et al. 2006). Accordingly, adiponutrin expression in rodent’s WAT decreases with fasting (Baulande et al. 2001, Bertile & Raclot 2004, Wiesner et al. 2004, Kershaw et al. 2006).
and increases with refeeding (Baulande et al. 2001, Kershaw et al. 2006) and with the ingestion of high-carbohydrate and high-protein meals (Polson & Thompson 2003, 2004). Moreover, WAT adiponutrin expression is decreased in two mice models of altered insulin action: the insulin-receptor knockout mice and the insulin-deficient mice (streptozotocin-induced diabetes), a defect that is corrected upon insulin replacement in this later mouse model (Kershaw et al. 2006). Finally, as adiponutrin expression is increased in obese fa/ob Zucker rats (having increased ob gene expression) (Guerre-Millo 1997, Baulande et al. 2001), while it is reduced in leptin–deficient obese ob/ob mice (Lake et al. 2005), leptin pathway may be implicated in the regulation of adiponutrin expression.

Little is known about adiponutrin regulation in human. To date only two studies were reported, results of which are partially inconsistent with each other and murine data. S.c. WAT adiponutrin expression was not increased in obese subjects and was associated with insulin sensitivity in one study (Liu et al. 2004) but not in the other (where insulin sensitivity was associated with adiponutrin expression in visceral WAT only; Johansson et al. 2006). However, both human studies suggest that adiponutrin expression may be regulated by insulin and glucose. In one study, adiponutrin expression in WAT was decreased after energy restriction and was increased upon refeeding (Liu et al. 2004) and in the other, adiponutrin expression in cultured human adipocytes was induced by insulin in a glucose-dependent manner (Johansson et al. 2006).

Clearly much remains to be explored regarding the regulation of adiponutrin in human, particularly given the possible role of adiponutrin in insulin sensitivity. Recently, we examined the regulation of adiponutrin expression by hyperinsulinemia in type 2 diabetic obese subjects. Despite their obese state and the origin of adiponutrin being WAT, fasting adiponutrin expression as well as insulin-stimulated increase in adiponutrin expression were reduced in obese diabetic subjects when compared with controls (Moldes et al. 2006).

Insulin sensitivity as well as the regulation of many WAT-derived proteins involved in insulin action are defective in type 2 diabetes and may affect the regulation of adiponutrin expression. To better understand the in vivo regulation of adiponutrin by the insulin pathway and explain its reduced expression in type 2 diabetes, we explored (1) the association of adiponutrin with insulin sensitivity and WAT-derived proteins of known relation to insulin signaling and action and (2) the concomitant regulation of adiponutrin with the WAT-derived proteins by insulin infusion. The WAT-derived proteins examined were hexokinase II (HK II), the regulatory subunit p85α of phosphatidylinositol-3 kinase (PI3K), leptin, adiponectin, and acylation-stimulating protein (ASP). These proteins have been well characterized and described in relation to insulin action in healthy and in reduced insulin sensitivity state-like type 2 diabetes (Lefai et al. 2001, Saltiel & Kahn 2001, Faraj et al. 2004).

Materials and Methods

Subjects and study design

Twenty lean, healthy Caucasian young men participated in the study. Subjects underwent routine medical examination and biological testing. None had familial or personal history of diabetes, obesity, dyslipidemia, or hypertension or was on any medication. All subjects were sedentary, having <2 h-structured exercise per week, and were instructed not to engage in any exercise for the previous 48 h before the study. The study was conducted after 12 h fast. All subjects gave written consent after being informed of the nature, purpose, and possible risks of the study. The experimental protocol was approved by the ethics committee of the University of Montreal.

Analytical procedures

Hyperinsulinemic euglycemic clamp (HIEG) technique To examine the effect of hyperinsulinemia alone on adiponutrin expression in WAT in vivo, ten men underwent a 3-h HIEG clamp where hyperinsulinemia was coupled to euglycemia as previously described (DeFronzo et al. 1979). Briefly, plasma insulin was elevated to a plateau concentration using primed-constant exogenous insulin infusion at 75 mU/m² per min. Plasma glucose was maintained at fasting levels using 20% dextrose infusion adjusted every 5–10 min according to repeated plasma glucose measurements. Insulin sensitivity measured by the clamp (Mclamp) represents glucose infusion rate at the last 30 min of the clamp at the steady state and is expressed as mg/kg per min (DeFronzo et al. 1979, Karelis et al. 2005). Insulin-stimulated data represent data analyzed at the last 30 min of the clamp.

Analysis of serum parameters Fasting serum glucose concentrations were measured with a glucose analyzer (Beckman Glucose analyzer, Mississauga, ON, Canada). Serum triglyceride (TG) and total cholesterol were measured by an automated analyzer (Beckman–Coulter, Brea, CA, USA). Serum insulin, adiponectin, and leptin levels were measured in duplicate with a commercial RIA kit (Linco Research, St Charles, MO, USA) procedure using 125I-labeled bioactive human insulin, adiponectin, and leptin respectively as tracers and a rabbit polyclonal antibody raised against full-length peptides. Serum ASP was assayed by an in-house enzyme-linked immunosorbant assay using a monoclonal antibody as capture antibody and a polyclonal antibody as detecting antibody as described previously in detail (Maslowska et al. 1999). Serum leptin, adiponectin, and ASP were not measured for three subjects for lack of sufficient serum samples. Insulin sensitivity in the fasting state was calculated using quantitative insulin sensitivity check index (QUICKI) as 1/(log10 glucose + log10 insulin), where glucose is in mg/dl and insulin is in μU/ml (Rabasa-Lhoret et al. 2003).
White adipose tissue biopsy S.c. abdominal WAT samples at the periumbilical level were obtained as previously described by needle biopsy under local anesthesia (Xylocaine 20 mg/ml, AstraZeneca; Vidal et al. 1996). Basal tissue samples were collected 1 h before the initiation of the clamp. Insulin-stimulated tissue samples were collected at the end of the 3 h clamp during which insulin infusion rate was maintained at 75 mU/m² per min and plasma glucose concentration was maintained at fasting levels. Adipose tissue biopsies were immediately frozen in liquid nitrogen and stored at −80 °C for extraction of mRNA later.

Analysis of white adipose tissue mRNA expression levels The WAT mRNA expression levels of adiponutrin, leptin, adiponectin, complement protein C3 (ASP precursor), p85α PI3K, and HKII were measured in the basal and the insulin-stimulated states. The total RNA was extracted from frozen WAT samples using the RNeasy Total RNA Minikit (Qiagen). First-strand cDNAs were synthesized from 250 ng total RNA in the presence of 100 U Superscript II (Invitrogen) using both random hexamers and oligo (dT) primers (Promega). Real-time PCR was performed using a LightCycler (Roche Diagnostics) in a final volume of 20 μl containing 5 μl of a 100-fold dilution of the reverse transcriptase (RT) reaction and 15 μl reaction buffer from the FastStart DNA Master SYBR Green kit (Roche Diagnostics) with 3 mM MgCl₂ and the specific primers.

After amplification, melting curve analysis was performed to verify the specificity of the reactions. For quantification, a standard curve was systematically generated with six different concentrations accessible in the GenBank database. The expression levels of target genes were normalized according to a reference gene hypoxanthine phosphoribosyl-transferase (HPRT). The sequence of HPRT forward primer was 5′-TTG-CTG-ACC-TGC-TGG-ATT-AC-3′ and the reverse primer was 5′-AGT-TGA-GAG-ATC-ATC-TCC-AC-3′.

Statistical analysis Data are expressed as mean ± s.d. The correlation was analyzed by Pearson Product Moment correlation. In the correlation analysis with adiponutrin, one data point of high adiponutrin mRNA expression level was eliminated from both basal and insulin-stimulated states as they presented outliers/influential data that we believe created many false positives. The differences between subjects who did or did not undergo the HIEG clamp were examined with a two-tailed t-test, while the effect of insulin on the various measured serum and WAT parameters were analyzed by two-tailed paired t-test. Statistical analysis was performed using SigmaStat (Jandel, San Rafael, CA, USA) with significance set at P ≤ 0.05.

Results

Basal state

Basal anthropometric and metabolic characteristics of the 20 healthy, lean young men are presented in Table 1. As presented, adiponutrin is a relatively scarce protein as its fasting mRNA expression level was the least among the examined WAT-derived proteins, while adiponectin expression was the most abundant. At baseline, adiponutrin mRNA expression was positively correlated with the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Basal anthropometric and metabolic characteristics of the 20 lean young men</th>
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<tbody>
<tr>
<td></td>
<td><strong>Mean ± s.d.</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>23.1 ± 2.4</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>69.0 ± 6.7</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>22.3 ± 1.5</td>
</tr>
<tr>
<td><strong>Serum concentrations</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Glucose (mmol/l)</strong></td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td><strong>Insulin (μU/ml)</strong></td>
<td>66.9 ± 30.6</td>
</tr>
<tr>
<td><strong>QUICKI</strong></td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/l)</strong></td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/l)</strong></td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td><strong>Leptin (ng/ml)</strong></td>
<td>2.8 ± 2.2</td>
</tr>
<tr>
<td><strong>Adiponectin (μg/ml)</strong></td>
<td>9.6 ± 3.7</td>
</tr>
<tr>
<td><strong>ASP (nM)</strong></td>
<td>25.1 ± 9.6</td>
</tr>
<tr>
<td><strong>WAT mRNA expression levels</strong></td>
<td></td>
</tr>
<tr>
<td><strong>HPRT</strong></td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td><strong>Adiponutrin/HPRT</strong></td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td><strong>Leptin/HPRT</strong></td>
<td>13.6 ± 1.4</td>
</tr>
<tr>
<td><strong>Adiponectin/HPRT</strong></td>
<td>83.4 ± 43.6</td>
</tr>
<tr>
<td><strong>C3/HPRT</strong></td>
<td>13.7 ± 4.7</td>
</tr>
<tr>
<td><strong>PI3K/HPRT</strong></td>
<td>16.7 ± 14.6</td>
</tr>
<tr>
<td><strong>HKII/HPRT</strong></td>
<td>20.9 ± 17.9</td>
</tr>
</tbody>
</table>

n = 20 for all presented parameters except serum leptin, adiponectin, and ASP (n = 17).
mRNA expression levels of p85α PI3K (R=0·54, P=0·017; Fig. 1) and HKII (R=0·58, P=0·010), and with serum leptin (R=0·51, P=0·036). There was no correlation between basal adiponutrin mRNA with any other WAT-derived protein or with age, weight, body mass index (BMI), serum lipids, insulin, glucose, or insulin sensitivity (QUICKI).

To better understand the network of WAT signals from which adiponutrin is derived, we also examined the interaction of the sensitivity (QUICKI). mRNA expression levels of the gene of reference with serum leptin (R=0·47, P=0·038 and QUICKI: R=0·62, P=0·004). The expression levels of HKII mRNA and p85α PI3K mRNA were also highly correlated (R=0·86, P<0·0001; Fig. 1). Similar to that with adiponutrin, basal insulin or QUICKI did not correlate with leptin, adiponectin, or C3 mRNA expression levels.

Insulin-stimulated state

Out of the 20 subjects examined in the basal state, ten underwent HIEG clamps. There was no difference in all measured characteristics between the subjects who did versus those who did not do the HIEG clamp except for lower TG and ASP concentrations in the clamp subjects (clamp subjects TG=0·93±0·19 mM versus non-clamp subjects TG=1·33±0·43 mM and clamp subjects ASP=16·34±7·86 mM versus non-clamp subjects ASP=30·42±5·98 mM, P<0·05 for TG and ASP).

The effect of HIEG clamp on the expression levels and the serum concentrations of the WAT-derived proteins examined in ten lean, healthy men is presented in Table 2 and Fig. 2. Considering the range of fasting plasma insulin in healthy subjects which is 36–179 pM (Pagana & Pagana 1997), the increase in serum insulin with the HIEG clamp was within physiological post-prandial concentrations (five to six times higher than that during fasting; English et al. 2003). The expression levels of the gene of reference HPRT were not affected by the HIEG clamp (Table 2). Hyperinsulinemia significantly increased serum concentrations of leptin and decreased that of adiponectin (Table 2), while it had no effect

Table 2 Effect of the HIEG clamp on the serum concentrations and mRNA expression levels of measured WAT-derived proteins in ten lean young men

<table>
<thead>
<tr>
<th>Variables</th>
<th>Basal</th>
<th>Insulin stimulated</th>
<th>Percentage change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Clamp (mg glucose/kg/min)</td>
<td>–</td>
<td>11·8±1·8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>WAT mRNA expression levels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>5·0±0·6</td>
<td>5·2±0·4</td>
<td>5·7</td>
<td>0·374</td>
</tr>
<tr>
<td>Insulin (pM)</td>
<td>67·5±40·7</td>
<td>110·2±177·1</td>
<td>2364±6</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>3·8±2·9</td>
<td>4·2±3·2</td>
<td>10·8</td>
<td>0·031</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>11·6±4·8</td>
<td>10·0±4·5</td>
<td>−15·0</td>
<td>0·001</td>
</tr>
<tr>
<td>ASP (nM)</td>
<td>16·3±7·9</td>
<td>13·5±13·5</td>
<td>−15·0</td>
<td>0·304</td>
</tr>
</tbody>
</table>

n=10 for all presented parameters except serum leptin, adiponectin, and ASP (n=7).

Figure 1 Pearson correlation of WAT mRNA expression levels of p85α PI3K to those of adiponutrin (R=0·54, P=0·017) and HKII (R=0·58, P=0·010) in healthy, lean young men at baseline.

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Figure 2. The expression levels of WAT-derived proteins at the basal and the insulin-stimulated states induced by HIEG clamps in healthy, lean young men. * P < 0.05, † P < 0.01, and ‡ P < 0.001.

on the mRNA expression levels of these hormones (which may indicate an insulin effect at the post-transcription level only). In addition, hyperinsulinemia significantly increased the mRNA expression levels of adiponutrin, p85α PI3K, and HKII. There was no effect of 3 h hyperinsulinemia on the concentration of ASP or the expression levels of its precursor protein C3.

Insulin-stimulated adiponutrin remained correlated to basal p85α PI3K mRNA ($R = 0.76$, $P = 0.018$) and basal HKII mRNA ($R = 0.86$, $P = 0.003$), and was correlated with percentage increase in insulin during the clamp ($R = 0.73$, $P = 0.040$). The correlation of adiponutrin expression with the examined WAT-derived proteins in the insulin-stimulated state is presented in Fig. 3. Insulin-stimulated adiponutrin mRNA levels correlated with those of HKII ($R = 0.82$, $P = 0.007$), leptin ($R = 0.84$, $P = 0.005$), and adiponectin ($R = 0.85$, $P = 0.004$; Fig. 3). Insulin sensitivity measured by the clamp ($M_{clamp}$) did not correlate with any measured WAT-derived proteins, including adiponutrin, HKII, and p85α PI3K mRNA. Of note, despite high correlation of HKII and p85α PI3K mRNA at baseline, their mRNA expression levels were not correlated in the insulin-stimulated state.

**Figure 3** Pearson correlation of adiponutrin with insulin sensitivity and WAT-derived proteins at the insulin-stimulated state induced by HIIEG clamps in healthy, lean young men.
Insulin regulation of adiponutrin in human · M Faraj and others

Discussion

Recent findings by our group and others indicate that, despite being a WAT-derived protein, adiponutrin expression is not elevated in obese subjects (Liu et al. 2004, Moldes et al. 2006). Furthermore, fasting adiponutrin expression as well as insulin-stimulated increase in adiponutrin expression were reduced in obese type 2 diabetic subjects when compared with controls (Moldes et al. 2006). Therefore, the regulation of adiponutrin expression in human may be altered by reduced insulin sensitivity and altered regulation of WAT-derived proteins in relation to insulin signaling and action in states like type 2 diabetes. In this study, we examined the association of adiponutrin with insulin sensitivity and insulin-related WAT-derived proteins in both the basal and the insulin-stimulated states in healthy subjects to bypass the confounding effect of obesity and diabetes on the regulation of these WAT-derived proteins, including adiponutrin (Lefai et al. 2001, Johansson et al. 2006, Moldes et al. 2006).

The major findings of this study in healthy young men are that: (1) fasting adiponutrin expression level is correlated with that of p85α, PI3K and HKII, and with serum leptin; (2) acute hyperinsulinemia simultaneously induces mRNA levels of adiponutrin, HKII, and PI3K, increases leptin concentration, and decreases adiponectin concentration; (3) insulin-stimulated adiponutrin expression is highly correlated to that of insulin-stimulated HKII, adiponectin, and leptin.

Novel findings in our study indicate that 3 h infusion of physiological doses of insulin induced an eightfold increase in adiponutrin expression, which was correlated to the percentage increase in insulin concentrations. Moreover, basal adiponutrin mRNA levels were highly correlated with those of p85α, PI3K. PI3K has a pivotal role in the propagation of insulin signaling (Saltiel & Kahn 2001) and the regulatory subunit p85α examined in this study is the most abundant variant of the regulatory subunits of PI3K in human WAT (Lefai et al. 2001). Many genes involved in the insulin signaling and action are under the PI3K pathway, among which HKII is examined here (Pendergrass et al. 1998). In our study, adiponutrin expression profile mirrored that of HKII. Both adiponutrin and HKII expression levels were: (1) highly correlated to each other and to p85α, PI3K in the basal state, (2) induced by insulin infusion, and (3) highly correlated in the insulin-stimulated state. Therefore, we hypothesize that, like HKII, insulin-stimulation of adiponutrin expression in human WAT may be under the PI3K pathway. Although in our study, adiponutrin expression did not correlate with that of p85α, PI3K in the insulin-stimulated state, the same observation also applies to HKII, despite the previously documented regulation of HKII expression by the activity of PI3K pathway (Pendergrass et al. 1998).

Type 2 diabetic subjects have reduced insulin action, insulin-mediated upregulation of p85α, PI3K in WAT (Ducluzeau et al. 2001), and expression of genes under the PI3K-dependent pathway like HKII (Pendergrass et al. 1998). Our hypothesis that insulin-mediated regulation of adiponutrin is under the PI3K-dependent pathway may therefore explain reduced adiponutrin expression in type 2 diabetes. However, it should be noted that the lack of correlation between adiponutrin expression and insulin sensitivity at fasting (QUICKI) and insulin-stimulated state (M_Lamp), does not oppose our belief that the degree of insulin resistance may modulate adiponutrin expression. In the present study, the narrow ranges of insulin concentrations and sensitivity in our homogenous and healthy population may have hindered correlation analysis. However, type 2 diabetic subjects exhibit far less insulin sensitivity than our subjects which would drive reduced adiponutrin expression. Of note, the consistent correlation of adiponutrin expression to that of HKII, a major enzyme for the progress of glucose metabolism (Pendergrass et al. 1998) may also suggest that insulin-mediated adiponutrin expression may be glucose-dependent. In fact, this is supported by (1) our data that adiponutrin expression is only reduced in impaired insulin-sensitive and hyperglycemic (or glucose intolerant) type 2 diabetic subjects and not obese subjects (Moldes et al. 2006) and (2) in vitro findings in human adipocytes where insulin failed to induce adiponutrin expression at sub-physiological glucose concentrations (1 mM) as it did at physiological or supra-physiological concentrations of glucose (5 and 25 mM; Johansson et al. 2006). Therefore, insulin and glucose are both important regulators of adiponutrin expression.

Another novel finding in our study is that adiponutrin expression was correlated with leptin concentration and expression at the basal and the insulin-stimulated states respectively. Leptin is a WAT-derived factor that is documented to increase insulin sensitivity by various mechanisms in central and peripheral tissues (Faraj et al. 2004). Therefore, leptin pathway may either facilitate insulin regulation of adiponutrin or be directly involved in adiponutrin regulation. Supporting the later mechanism is the finding that adiponutrin expression is reduced in WAT of ob/ob mice, deficient for leptin gene (Lake et al. 2005), while is increased in fa/fa rats (Baulande et al. 2001), deficient for leptin receptor not leptin gene which is in fact increased in these rats (Guerre-Millo 1997). Despite hyperleptinemia in type 2 diabetes, leptin resistance is suggested to be a defect in these subjects (Zimmet et al. 1999). Accordingly, leptin resistance in type 2 diabetic would further explain our observation of a defective adiponutrin expression in these subjects (Moldes et al. 2006).

Similar to leptin, adiponectin increases the insulin sensitivity in peripheral tissue (Faraj et al. 2004). However, adiponectin is the only known WAT-derived protein whose expression and plasma concentration are consistently reduced in both murine and human obesity (Scherer et al. 1995, Arita et al. 1999, Statnick et al. 2000). Furthermore, in our study, hyperinsulinemia reduced adiponectin concentration as was documented by others (Yu et al. 2002). Adiponectin expression and concentration are reduced in obese type 2 diabetic subjects being driven by both their obesity and their...
chronic hyperinsulinemia states (Statnick et al. 2000). Therefore, high correlation of adiponutrin expression with that of adiponectin in the insulin-stimulated states would further predict reduced adiponutrin expression in obese type 2 diabetic subjects.

Adiponutrin mRNA levels in our study did not correlate with ASP concentrations or the expression of its precursor protein C3. Moreover, in contrast to adiponutrin, hyperinsulinemia did not affect ASP concentration or C3 expression in our study as was previously reported for C3 (Koistinen et al. 2001). Although ASP concentration and C3 expression are elevated in obese type 2 diabetic subjects (Koistinen et al. 2001, Yang et al. 2005), this was linked to their obesity rather than their insulin resistance in one study (Koistinen et al. 2001). Therefore, adiponutrin and ASP may be differentially regulated in human. In fact, the production of ASP from human abdominal VAT increases with ingestion of a high-fat meal (Saleh et al. 1998) and chylomicrons are the most potent regulators of ASP production from human adipocytes (Maslowska et al. 1997, Scantlebury et al. 1998, 2001). On the contrary, adiponutrin expression in mice VAT is neither stimulated with high-fat meal (Polson & Thompson 2001) nor affected by treatments of cultured human adipocytes with fatty acids (Johansson et al. 2006). Therefore, ASP pathway and its regulators (lipid-related signals) are unlikely to contribute to the regulation of adiponutrin in human (Liu et al. 2004).

In conclusion, adiponutrin expression in s.c. VAT of healthy, lean young men is strongly induced by acute hyperinsulinemia and is highly related to the expression levels of HKII and p85α PI3K. We hypothesize that like HKII, insulin-mediated regulation of adiponutrin expression in human VAT may be through a PI3K-dependent pathway. Accordingly, we believe that our recent finding of reduced adiponutrin expression in obese type 2 diabetic subjects may be driven by reduced insulin sensitivity, defective insulin-mediated regulation of PI3K pathway, and glucose metabolism (implying defective regulation of genes under the PI3K pathway like HKII), as well as an impaired leptin sensitivity and adiponectin expression in these subjects.

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