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The Possible Role of mRNA Expression Changes of GH/IGF-1/Insulin Axis Components in Subcutaneous Adipose Tissue in Metabolic Disturbances of Patients With Acromegaly

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Summary

We explored the effect of chronically elevated circulating levels of growth hormone (GH)/insulin-like-growth-factor-1 (IGF-1) on mRNA expression of GH/IGF-1/insulin axis components and p85alpha subunit of phosphoinositide-3-kinase (p85alpha) in subcutaneous adipose tissue (SCAT) of patients with active acromegaly and compared these findings with healthy control subjects in order to find its possible relationships with insulin resistance and body composition changes. Acromegaly group had significantly decreased percentage of truncal and whole body fat and increased homeostasis model assessment-insulin resistance (HOMA-IR). In SCAT, patients with acromegaly had significantly increased IGF-1 and IGF-binding protein-3 (IGFBP-3) expression that both positively correlated with serum GH. P85alpha expression in SCAT did not differ from control group. IGF-1 and IGFBP-3 expression in SCAT were not independently associated with percentage of truncal and whole body fat or with HOMA-IR while IGFBP-3 expression in SCAT was an independent predictor of insulin receptor as well as of p85alpha expression in SCAT. Our data suggest that GH overproduction in acromegaly group increases IGF-1 and IGFBP-3 expression in SCAT while it does not affect SCAT p85alpha expression. Increased IGF-1 or IGFBP-3 in SCAT of acromegaly group do not appear to contribute to systemic differences in insulin sensitivity but may have local regulatory effects in SCAT of patients with acromegaly.

Key words

Acromegaly • GH/IGF-1/insulin axis components • Adipose tissue • Insulin resistance • p85alpha subunit of PI3K

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Introduction

Acromegaly is associated with an increased prevalence of glucose metabolism disorders. Clinically confirmed diabetes mellitus is observed in approximately one quarter of all patients with acromegaly (Droste *et al.* 2014). The degree of glucose intolerance has been reported to correlate with serum GH levels, age, duration of the disease and family history of diabetes (Resmini *et al.* 2009). Importantly, patients with acromegaly-associated diabetes display exponentially increased mortality rates, since untreated acromegaly and increased plasma insulin levels/insulin resistance in diabetes are both associated with higher rate of cardiovascular mortality and malignancies (Droste *et al.* 2014). GH-induced insulin resistance, increased hepatic glucose

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production and accentuated lipolysis contribute to the presence of diabetes and impaired glucose tolerance in acromegaly (Rodrigues *et al.* 2011). IGF-1 has rather opposing effects on insulin sensitivity and lipolysis than GH; however, in acromegaly, increased IGF-1 levels are unable to counteract the negative metabolic actions of GH excess (Resmini *et al.* 2009).

We have previously shown that type 2 diabetes and obesity are accompanied by differences not only in serum concentrations but also in mRNA expression of some GH/IGF-1/insulin axis components in subcutaneous adipose tissue and we suggested that these differences might contribute to allover metabolic and adipose tissue metabolism disturbances in type 2 diabetes and to their improvement after dietary intervention (Touskova *et al.* 2012).

Adipose tissue is the major target of growth hormone action (Garten *et al.* 2012) and increased lipolysis and reduced triglyceride accumulation together with inhibited preadipocyte differentiation due to elevated GH levels in acromegaly contribute to the reduction of adipose tissue mass (Plockinger and Reuter 2008, Richelsen 1997). GH may mediate its actions on adipose tissue *via* specific GH receptors on both preadipocytes and mature adipocytes, but some effects are mediated indirectly through the GH-regulated secretion of IGF-1 (Richelsen 1997). Locally produced IGFBP-1-3 have been suggested to play a protective role against obesity among other mechanisms also *via* inhibiting the stimulatory effects of IGF-1 on adipogenesis (Nguyen *et al.* 2015, Wheatcroft *et al.* 2007, Ueda and Ashida 2012), and to also have specific regulatory roles in glucose metabolism (Rajpathak *et al.* 2009, Claudio *et al.* 2010, Chan *et al.* 2005). Subcutaneous adipose tissue appears to be an interesting target tissue in terms of exploring GH effects, since several previous studies demonstrated differences in the amount of subcutanoues adipose tissue in accordance with differences of GH action (Berryman *et al.* 2004, Lin *et al.* 2012, Ibáñez *et al.* 2010).

Numerous studies showed several different mechanisms by which GH affects insulin sensitivity in adipose tissue, including interferences with insulin signaling cascade (Castro *et al.* 2004, Smith *et al.* 1997). Among others, an up-regulation of p85alpha regulatory subunit of PI3K by GH has been considered as a potential explanation for the insulin resistance in white adipose tissue of mice with GH excess (del Rincon *et al.* 2007).

To our knowledge, the local expression of GH/IGF-1/insulin axis components in subcutaneous adipose tissue of patients with acromegaly has been very scarcely studied so far. In the current study, we hypothesized that chronically elevated GH/IGF-1/insulin serum levels in acromegalic patients might induce differences in mRNA expression of GH/IGF-1/insulin axis components in subcutaneous adipose tissue that may in turn contribute to dysregulation of glucose metabolism and reduction of adipose tissue mass. To this end, we measured serum concentrations and mRNA expression of selected components of GH/IGF-1/insulin axis in subcutaneous adipose tissue together with metabolic and anthropometric parameters in acromegalic patients and compared them with the findings in healthy age-matched subjects. To gain further insight into the mechanism of GH-induced insulin resistance in subcutaneous adipose tissue in acromegaly we also explored the mRNA expression of p85alpha regulatory subunit of PI3K.

Methods

Study subjects

Twelve acromegalic patients (AC group, 8 men and 4 women, aged 49.6±8.1 years) and twelve lean healthy subjects (C group, 4 men and 8 women, aged 50.7±5.2 years) were included in the study. Acromegalic patients were examined at the moment of establishing the diagnosis of acromegaly, therefore they had no prior treatment for acromegaly. The diagnostic criteria for acromegaly were increased IGF-1 serum levels above the upper limit of the normal range (according to age) and the non-supresibility of GH serum levels below 0.4 ng/ml in the oral glucose tolerance test. Exclusion criteria were age <18 years old, malignancy, inflammatory disease, type 1 diabetes, current treatment with glucocorticoids. Five out of twelve acromegalic patients were on antihypertensive treatment, three used oral antidiabetic treatment and one was treated with insulin. The treatment remained unchanged for at least three months prior to the start of the study. Control subjects had no history of acromegaly, obesity and/or diabetes mellitus, arterial hypertension, or lipid metabolism disturbances and received no medication. Blood tests confirmed normal blood count, biochemical and hormonal parameters.

During the program all acromegalic patients were hospitalized at the Third Department of Medicine, General University Hospital in Prague. Written informed consent was signed by all participants before the beginning of the study. The study was approved by Human Ethics Review Board, First Faculty of Medicine

and General University Hospital, Prague, Czech Republic and was performed in accordance with the guidelines proposed in the Declaration of Helsinki.

Anthropometric examination, blood and adipose tissue sampling

All participants included in the study were examined only once. All subjects were measured and weighted, and their BMI was calculated. Blood samples for biochemical and hormonal parameters measurement were taken after overnight fasting. Blood samples were separated by centrifugation for 10 min at 1000 x g within 30 min from blood collection. Serum or plasma was subsequently stored in aliquots at –80 °C until further analysis.

Samples of subcutaneous adipose tissue for mRNA expression analysis were obtained from abdominal region with subcutaneous needle aspiration biopsy from all participants. Approximately 100 mg of adipose tissue was collected to 1 ml of RNA stabilization Reagent (RNAlater, Qiagen, Hilden, Germany) and stored at –80 °C until further analysis.

The amount and percentage of whole body fat, truncal body fat and lean body mass was assessed by body composition measurement using Dual-Energy X-Ray Absorptiometry (DEXA, Hologic Discovery, USA).

Hormonal and biochemical assays

Serum levels of total insulin-like growth factor-1 (IGF-1) were measured by IRMA kit (Immunotech, Prague, Czech Republic). LOD (Limit of detection) and LOQ (Limit of quantitation) were 12.0 ng/ml. Serum IGFBP-1, IGFBP-2 and IGFBP-3 levels were measured by ELISA kits (DiaSource ImmunoAssays S.A., Nivelles, Belgium). LOD was 0.4 ng/ml for IGFBP-1, 0.2 ng/ml for IGFBP-2 and 10 ng/ml for IGFBP-3. LOQ was 6.4 ng/ml for IGFBP-1, 4.2 ng/ml for IGFBP-2 and 10 ng/ml for IGFBP-3. Growth hormone (GH) levels were measured by IRMA kits (Immunotech, Prague, Czech Republic). LOD and LOQ were 0.10 mIU/l. Serum C-reactive protein (CRP) levels were measured by high sensitive ELISA (Bender Medsystems, Vienna, Austria) with a LOD of 3 pg/ml and LOQ 1.5 ng/ml. The intra- and interassay variabilities for all methods were less than 5.0 and 10.0 %, respectively.

Biochemical parameters (fasting insulin, fasting blood glucose, HbA1c, total and HDL-cholesterol and triglycerides) were measured in the Department of Biochemistry of General University Hospital, Prague, by standard laboratory methods. The value of LDL-cholesterol was calculated according to Friedewald formula.

Total RNA isolation from adipose tissue

Samples of subcutaneous adipose tissue were homogenized on a MagNA Lyser Instrument using MagNA Lyser Green Beads (Roche Diagnostics GmbH, Germany). Total RNA from homogenized tissue was extracted on MagNA Pure instrument using Magna Pure Compact RNA Isolation kit (tissue) (Roche Diagnostics GmbH, Germany). The integrity of the RNA was checked by visualization of 18S and 28S ribosomal bands on 1 % agarose gel with ethidium bromide. The RNA concentration was determined from absorbance at 260 nm on a NanoPhotometer (Implen, Munchen, Germany).

Determination of mRNA expression by quantitative real-time PCR

Total RNA was used for reverse transcription to synthesize the first strand cDNA. Reverse transcription was performed using 0.25 μg of total RNA to synthesize the first strand cDNA using the random primers as per the instructions of the High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA).

Measurements of mRNA expression were performed on an ABI PRISM 7500 instrument (Applied Biosystems, Foster City, CA, USA) using TaqMan® Universal PCR Master Mix, NO AmpErase® UNG and specific TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, CA, USA). All PCRs for each gene were amplified separately. Controls with no template cDNA were performed with each assay and all samples were run at least in duplicates. The increase in fluorescence was measured in real time and threshold cycle (Ct) values were obtained. To compensate for variations in RNA amount and efficiency of reverse transcription, beta-2-microglobulin was used as endogenous reference and results were normalized to the mean of these values. The formula $2^{-\Delta Ct}$ was used to calculate relative gene expression.

Statistical analysis

Statistical analysis was performed on SigmaStat software Version 3.0 and the graphs were created in Sigma Plot software Version 8.0 (SPSS Inc., Chicago, IL, USA). Prior to analysis, all continuous variables were assessed for normality (Kolmogorov-Smirnov test).

Anthropometric, biochemical and hormonal data are expressed as mean ± standard deviation (SD) or median (interquartile range), according to the normality of data. Comparisons of anthropometric, biochemical, hormonal and other parameters among the two groups studied (AC, C) were evaluated by Unpaired t-test or Mann-Whitney Rank Sum test as appropriate.

The associations between serum and mRNA expression of GH/IGF-1/insulin axis components and p85alpha in SCAT and other variables in a combined group of AC subjects and age-matched controls were estimated by Spearman´s rank order correlation. Further backward stepwise regression analysis calculations were performed to show the independent relationships of GH/IGF-1/insulin axis components, p85alpha in SCAT and other biochemical or anthropometric characteristics. Only the parameters with significant correlation from Spearman correlation test ($p<0.05$) were used for these analyses. In all statistical tests p values <0.05 were considered statistically significant.

Results

Anthropometric, metabolic and hormonal characteristics of study subjects

Anthropometric, metabolic and hormonal characteristics of the study subjects (AC and C groups) are summarized in Table 1. BMI and percentage of lean body mass were significantly increased in AC group compared with C group, while percentage of whole body fat and truncal fat were decreased in AC group relative to C group. Serum levels of GH, total IGF-1 and IGFBP-3, fasting glucose, insulin, HbA1c, HOMA-IR and triglycerides were significantly increased in AC group compared with C group. On the contrary, IGFBP-1, IGFBP-2, HDL and CRP serum levels were decreased in acromegalic patients. Total and LDL cholesterol did not significantly differ from control group.

mRNA expression of GH/IGF-1/insulin axis components and p85alpha in subcutaneous adipose tissue

The summary of mRNA expressions of GH/IGF-1/insulin axis components and p85alpha in subcutaneous adipose tissue of AC and C group is shown in Figure 1. IGF-1 and IGFBP-3 mRNA expression in subcutaneous adipose tissue were significantly increased in patients with acromegaly compared with control group. GH-R, IGF-1R, IGF-2, IGF-2R, IGFBP-2, INS-R, p85alpha mRNA expression did not significantly differ from control group. IGFBP-1 was not detected in adipose tissue.

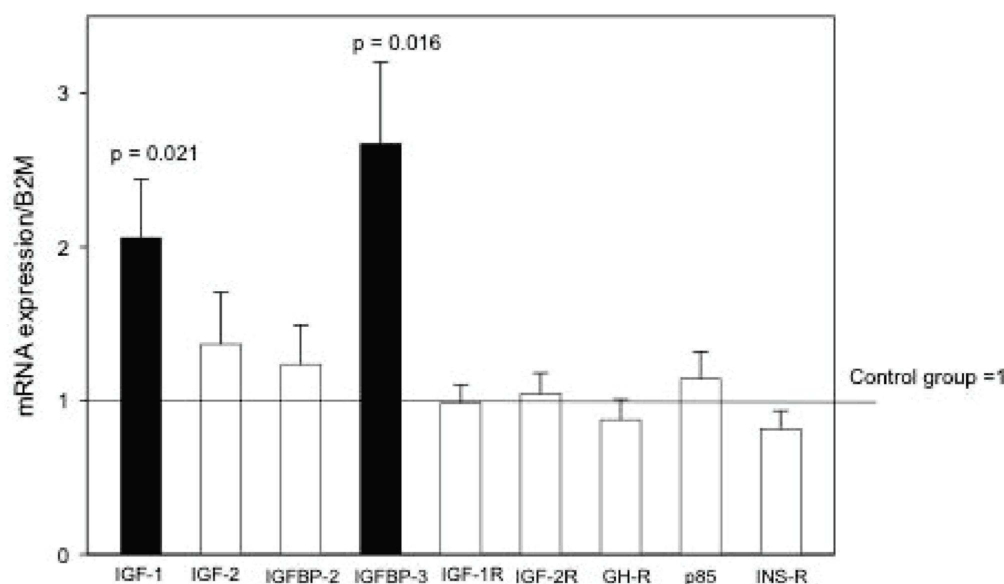

Fig. 1. GH/IGF-1/insulin axis components and p85alpha mRNA expression differences in SCAT of AC group (n=12) relative to control group (n=12). The mean relative mRNA expressions for the parameters of acromegalic group are expressed as relative ratio to the mean mRNA expression of control group that is taken as 1.0 (line-Control group) for every gene separately. Statistical significance is from Unpaired t-test or Mann-Whitney Rank Sum test as appropriate. P value <0.05 indicated statistical significance. Values were adjusted for sex in both AC and C group and for the presence of diabetes in AC group. GH-R, growth hormone receptor; IGF-1, insulin-like growth factor-1; IGF-2, insulin-like growth factor-2; IGFBP, insulin-like growth factor binding protein; IGF-1R, insulin-like growth factor-1 receptor; IGF-2R, insulin-like growth factor-2 receptor; INS-R, insulin receptor; p85, p85alpha subunit of phoshoinositide-3-kinase.

Table 1. Clinical, anthropometric, metabolic and hormonal characteristics of the study groups.

	Control group	AC group	P-value
No. of subjects	12	12	NA
Sex (male/female)	4/8	8/4	NA
Age (years)	50.7 ± 5.2	49.6 ± 8.1	0.701
Body mass index (kg/m^2)	23.5 (22.0-25.2)	31.0 (28.5-33.5)	<0.001
Whole body fat (%)	29.3 ± 5.1	21.4 ± 5.7	0.021
Truncal fat (%)	27.7 ± 4.9	20.3 ± 5.1	0.019
Lean body mass (%)	69.0 ± 5.1	77.1 ± 6.2	0.023
Fasting blood glucose (mmol/l)	4.97 ± 0.37	6.06 ± 0.85	<0.001
Fasting insulin (mIU/l)	19.2 ± 6.8	45.3 ± 25.1	0.006
HbA1c (% IFCC)	3.72 ± 0.84	4.70 ± 0.76	0.014
HOMA-IR index	1.88 (1.35-2.81)	10.01 (8.03-13.69)	0.006
Triglycerides (mmol/l)	1.17 ± 0.41	2.02 ± 0.76	0.004
Total cholesterol (mmol/l)	5.25 ± 0.72	4.73 ± 0.81	0.107
LDL cholesterol (mmol/l)	3.30 ± 0.72	2.77 ± 0.74	0.087
HDL cholesterol (mmol/l)	1.42 ± 0.31	1.04 ± 0.22	0.009
CRP (mg/l)	0.49 (0.23-2.00)	0.09 (0.07-0.22)	0.004
GH (mIU/l)	1.5 (0.6-2.3)	61.1 (9.7-96.1)	0.003
Total IGF-1 (ug/l)	137 (127-154)	1028 (655-1429)	<0.001
IGFBP-1 (ug/l)	6.53 (2.64-10.03)	0.12 (0.05-0.49)	<0.001
IGFBP-2 (ug/l)	293 (232-332)	141 (89-163)	0.006
IGFBP-3 (mg/l)	2.97 ± 0.58	6.89 ± 1.27	<0.001

Normally distributed data are shown as mean ± SD, non-parametric data as median (interquartile range). Statistical significance is from Unpaired t-test or Mann-Whitney Rank Sum test as appropriate. P value <0.05 indicated statistical significance. Values were adjusted for sex in both AC and C group and for the presence of diabetes in AC group. AC, acromegalic group; C, control group; CRP, C-reactive protein; GH, growth hormone; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment – insulin resistance; IFCC, International Federation of Clinical Chemistry; IGF-1, insulin-like growth factor-1; IGFBP, insulin-like growth factor binding protein; LDL, low-density lipoprotein; NA, not applicable.

Relationships of IGF-1 and IGFBP-3 mRNA expression in SCAT with other parameters

The relationships between IGF-1 and IGFBP-3 mRNA expression in SCAT (the only significantly increased parameters in SCAT in AC group compared to C group) with the other studied parameters were explored using Spearman correlation test. The significant associations are summarized in Table 2. Backward stepwise regression analysis was performed in order to explore the independent predictors of IGF-1 and IGFBP-3 mRNA expression in SCAT. The significant relationships are summarized in Table 3. In addition, we explored whether these two parameters might be independent predictors of selected metabolic (HOMA-IR, INS-R, p85alpha mRNA expression in SCAT) and body composition parameters (the percentage of truncal and whole body fat). The significant relationships are summarized in Table 4. The parameters used as independent variables for these selected metabolic and body composition parameters were the parameters with significant correlation in Spearman correlation test (data not reported).

IGF-1 mRNA expression in SCAT was inversely associated with percentage of truncal fat and positively with percentage of LBM. IGF-1 in SCAT correlated positively with serum GH, INS-R, GH-R, p85alpha and IGFBP-3 mRNA expression in SCAT and inversely with serum CRP levels (Table 2). IGF-1 mRNA expression in SCAT could be independently predicted only from IGFBP-3 mRNA expression in SCAT (Table 3).

IGFBP-3 mRNA expression in SCAT was inversely associated with percentage of truncal fat, whole body fat and positively with percentage of LBM. IGFBP-3 mRNA expression in SCAT correlated positively with fasting blood glucose, insulin, HOMA-IR, serum GH, mRNA expression of IGF-1, INS-R, GH-R,

p85alpha in SCAT and was inversely associated with serum CRP (Table 2). In Model 1 (including: fasting glucose, fasting insulin, HOMA-IR, serum CRP, serum GH, IGF-1 mRNA expression in SCAT as independent variables), IGFBP-3 mRNA expression in SCAT could be predicted from a linear combination of five independent variables: fasting glucose, fasting insulin, serum GH, serum CRP levels and IGF-1 mRNA expression in SCAT (Table 3). In Model 2 (including: percentage of truncal fat, whole body fat and LBM, IGF-1 and p85alpha mRNA expression in SCAT as independent variables), IGFBP-3 mRNA expression in SCAT could be predicted only from IGF-1 mRNA expression in SCAT (Table 3).

Table 2. The significant relationships of mRNA expression of IGF-1 and IGFBP-3 in SCAT with anthropometric, metabolic parameters, serum and SCAT GH/IGF-1/insulin axis components and p85alpha in combined population of acromegalic patients and normal-weight healthy subjects.

	SCAT (n=24)			
	IGF-1		IGFBP-3	
	R	p	R	p
Truncal fat (%)	-0.552	0.039	-0.574	0.031
Whole body fat (%)	-0.446	0.105	-0.543	0.043
LBM (%)	0.615	0.024	0.613	0.019
Fasting blood glucose	0.292	0.174	0.453	0.030
Fasting insulin	0.332	0.162	0.511	0.021
HOMA-IR index	0.330	0.151	0.541	0.009
CRP	-0.699	<0.001	-0.449	0.041
Serum GH	0.555	0.009	0.552	0.013
IGF-1 in SCAT	x	x	0.837	<0.001
IGFBP-3 in SCAT	0.837	<0.001	x	x
INS-R in SCAT	0.516	0.014	0.486	0.022
GH-R in SCAT	0.463	0.023	0.437	0.033
p85alpha in SCAT	0.841	<0.001	0.717	<0.001

Statistical significance is from Spearman correlation test. Statistical significance was assigned to p<0.05. CRP, C-reactive protein; GH, growth hormone; GH-R, growth hormone receptor; HOMA-IR, homeostasis model assessment – insulin resistance; IGF-1, insulin-like growth factor-1; IGFBP, insulin-like growth factor binding protein; INS-R, insulin receptor; LBM, lean body mass; p85alpha, p85alpha subunit of phoshoinositide-3-kinase; SCAT, subcutaneous adipose tissue. The non-significant correlations are not reported.

Table 3. The independent predictors of IGF-1 and IGFBP-3 mRNA expression in SCAT.

Dependent	Independent	p	Standardized coefficients beta	AdjR²
IGF-1 in SCAT	*IGFBP-3 in SCAT*	0.010	0.569	0.839
IGFBP-3 in SCAT (Model 1)	*Fasting glucose*	<0.001	0.0539	
	Fasting insulin	0.024	$-6.18*10^{-4}$	
	Serum GH	0.002	$7.71*10^{-4}$	0.971
	Serum CRP	0.016	0.0106	
	IGF-1 in SCAT	<0.001	0.568	
IGFBP-3 in SCAT (Model 2)	*IGF-1 in SCAT*	<0.001	0.541	0.833

Statistical significance is from backward stepwise regression analysis. Statistical significance was assigned to p<0.05. CRP, C-reactive protein; GH, growth hormone; IGF-1, insulin-like growth factor-1; IGFBP, insulin-like growth factor binding protein; SCAT, subcutaneous adipose tissue. The non-significant correlations are not reported.

Table 4. The independent predictors of selected metabolic and body composition parameters.

Dependent	Independent	p	Standardized coefficients beta	AdjR²
HOMA-IR	*Serum IGF-1*	<0.001	0.0162	0.682
INS-R in SCAT	*IGFBP-3 in SCAT*	<0.001	0.109	0.809
	HDL	<0.001	0.0351	
	IGF-1R in SCAT	0.027	4.479	
P85alpha in SCAT	*IGFBP-3 in SCAT*	<0.001	0.166	0.981
	IGFBP-2 in SCAT	<0.001	3.144	
	INS-R in SCAT	0.013	0.318	
	GH-R in SCAT	<0.001	-0.0451	
	LBM (%)	0.006	$-9.72*10^{-4}$	
Truncal fat (%)	*HbA1c*	0.009	-4.866	0.505
Whole body fat (%)	*HbA1c*	0.011	-4.896	0.479

Statistical significance is from backward stepwise regression analysis. Statistical significance was assigned to p<0.05. GH, growth hormone; GH-R, growth hormone receptor; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment – insulin resistance; IGF-1, insulin-like growth factor-1; IGFBP, insulin-like growth factor binding protein; IGF-1R, insulin-like growth factor-1 receptor; INS-R, insulin receptor; LBM, lean body mass; p85alpha, p85alpha subunit of phoshoinositide-3-kinase; SCAT, subcutaneous adipose tissue. The non-significant correlations are not reported.

HOMA-IR could be independently predicted only from serum IGF-1 levels (Table 4). INS-R mRNA expression in SCAT could be predicted from a linear combination of three independent variables: IGFBP-3 in SCAT, serum HDL and IGF-1R in SCAT (Table 4). P85alpha mRNA expression in SCAT could be predicted from a linear combination of five independent variables: SCAT mRNA expression of IGFBP-3, IGFBP-2, INS-R, GH-R and percentage of LBM (Table 4). HbA1c was found to be the independent predictor of percentage of truncal and whole body fat (Table 4).

IGF-1 and IGFBP-3 in SCAT were not found to be among the independent predictors of HOMA-IR and percentage of truncal and whole body fat, while IGFBP-3 in SCAT was found to be an independent predictor of INS-R and p85alpha mRNA expression in SCAT. The other non-significant relationships are not reported.

Discussion

The most important finding of our study is significantly increased mRNA expression of IGF-1 and IGFBP-3 in SCAT in patients with acromegaly relative to healthy lean subjects (Fig. 1). Both IGF-1 and IGFBP-3 mRNA expression in SCAT positively correlated with serum GH levels (Table 2) suggesting its direct stimulatory effect on local IGF-1 and IGFBP-3 production in subcutaneous fat. Previous studies have reported adipose tissue production of IGF-1 in response to GH stimulation in experimental conditions and in healthy individuals (Vikman *et al.* 1991, Peter *et al.* 1993, Wabitsch *et al.* 1996, Jørgensen *et al.* 2006). Peter *et al.* (1993) reported that IGFBPs, including IGFBP-3 mRNA expression, in rat white adipose tissue were all regulated by GH. Other experimental studies showed a stimulatory effect of GH on IGFBP-3 expression in the liver, muscle and skin in GH deficient rats (Lemmey *et al.* 1997), on serum IGFBP-3 (Wester *et al.* 1998) or on its secretion from porcine adipose tissue (Chen *et al.* 1996) or human preadipocytes (Wabitsch *et al.* 2000). Recently, increased IGF-1 and IGFBP-3 mRNA expression was found also in SCAT of subjects with acromegaly (Hochberg *et al.* 2015). In our study, serum GH was one of the independent predictors of IGFBP-3 mRNA expression in SCAT confirming its direct regulatory role (Table 3).

Previous studies demonstrated stimulatory effect of IGF-1 on skin (Lemmey *et al.* 1997) or liver (Gosteli-Peter *et al.* 1994) IGFBP-3 mRNA expression, but to our best knowledge no such data exist to date for the presence of this effect in adipose tissue. Importantly, in our study IGF-1 mRNA expression in SCAT was an independent predictor of IGFBP-3 mRNA expression in SCAT and vice versa (Table 3), suggesting their possible local mutual regulatory interactions.

As expected, we found significantly decreased

percentage of whole body as well as truncal fat and increased percentage of lean body mass in acromegalic patients compared to control group (Table 1), which is in agreement with previous studies (Katznelson 2009). Furthemore, we observed an inverse relationship of IGFBP-3 expression in SCAT with percentage of truncal and whole body fat and of IGF-1 expression in SCAT with percentage of truncal fat (Table 2), suggesting a possible causal relationship. In previous studies, IGF-1 and IGFBP-3 expression have been shown to increase during human preadipocyte differentiation (Baxter *et al.* 2009). While IGF-1 stimulates this process and adipogenesis (Peter *et al.* 1993, Chen *et al.* 1995) IGFBP-3 has the opposite effects (Baxter *et al.* 2009, Chan *et al.* 2009). In our previous study obese diabetic women had decreased IGFBP-3 mRNA expression in subcutaneous fat (Touskova *et al.* 2012). While the concept of local regulatory role of IGF-1 and IGFBP-3 in SCAT is tempting, we did not confirm IGF-1 or IGFBP-3 mRNA expression in SCAT as independent predictors of percentage of truncal and whole body fat in a backward stepwise regression analysis (Table 4), however this concept certainly warrants further investigation.

To gain further insight into its possible regulatory role in systemic metabolic changes in acromegaly we explored the relationships between IGF-1 and IGFBP-3 mRNA expression in SCAT with selected markers of glucose metabolism and insulin resistance (Table 2). In previous studies, the IGF-1 expression in adipose tissue transplants was shown to be associated with anti-inflammatory and favorable metabolic effects in diabetic mice (Gunawardana and Piston 2015). Both, IGF-1 (Kubota *et al.* 2008, Neacsu *et al.* 2013) and IGFBP-3 (Mohanraj *et al.* 2013) exerted anti-inflammatory effects and insulin-sensitizing effects in adipocytes. On the other hand, several studies indicated that IGFBP-3 may decrease insulin sensitivity in adipocytes by various mechanisms (Kim *et al.* 2007, Chan *et al.* 2005). In our current study we did not find an independent association of IGF-1 or IGFBP-3 in SCAT with HOMA-IR. Interestingly, IGFBP-3 in SCAT was an independent predictor of INS-R as well as of p85alpha mRNA expression in SCAT (Table 4), suggesting possible local role of IGFBP-3 in the regulation of insulin sensitivity in adipose tissue of AC.

In acromegaly, the increased insulin resistance is paradoxically often present despite the decreased amount of adipose tissue. For all the studies documenting different local mechanisms of GH-induced insulin resistance in adipose tissue (Castro *et al.* 2004, Smith *et al.* 1997), some experimental studies suggested that GH action in adipose tissue is not crucial in deterioration of the overall insulin resistance (List *et al.* 2013, Johansen *et al.* 2005). In our study, increased HOMA-IR was not significantly associated with the decreased percentage of whole body or truncal fat. These findings may support the hypothesis that in acromegaly, adipose tissue may not be the main site contributing to the whole body insulin resistance and point to the liver or the skeletal muscle as other important contributors.

Numerous previous experimental studies have suggested an important role of GH excess in the stimulation of p85alpha regulatory subunit of PI3K tissue expression and subsequent development of GH-induced insulin resistance (del Rincon *et al.* 2007, de Castro Barbosa *et al.* 2009, Barbour *et al.* 2005). On the other hand, an attenuation of p85alpha expression has been proposed as one of the mechanisms for the treatment of type 2 diabetes (Mauvais-Jarvis *et al.* 2002). Insulin has been shown as another important regulator of p85alpha regulatory subunit. Insulin resistant conditions were previously associated with contrasting results showing increased (Adochio *et al.* 2009, Cornier *et al.* 2006) but also decreased (Anai *et al.* 1998) p85alpha tissue (liver, muscle) expression and also with blunted stimulatory effect of acute hyperinsulinemia on p85alpha tissue expression (Lefai *et al.* 2001). In our study, elevated serum GH/insulin levels in patients with acromegaly failed to increase p85alpha mRNA expression in subcutaneous adipose tissue where its mRNA expression was comparable to that of the control group (Fig. 1), which confirms the result of the recent study on patients with acromegaly (Hochberg *et al.* 2015). No significant relationships were found between serum GH/IGF-1/insulin and p85alpha in SCAT or between p85alpha in SCAT and metabolic parameters (HOMA-IR, fasting blood glucose, insulin, HbA1c) arguing against an involvement of p85alpha regulatory subunit in SCAT in mediating the metabolic effects of systemic GH excess in acromegaly. P85alpha was positively associated with INS-R mRNA expression in SCAT, suggesting a possible parallel regulation of their expressions.

The limitations of our study include the relatively low number of study subjects and the cross-sectional design of the study. We are aware that the correlations found in our study do not necessarily establish a causal connection and that the mRNA expression differences in subcutaneous adipose tissue

might be secondary to insulin resistance. In addition, for the assessment of insulin resistance we have only measured HOMA-IR and did not perform hyperinsulinemic euglycemic clamp that would be more precise for the whole body insulin sensitivity evaluation.

In conclusion, in our study we found increased IGF-1 and IGFBP-3 mRNA expression in subcutaneous adipose tissue of patients with acromegaly. None of these factors independently predicted the changes in body composition or systemic insulin sensitivity. Nevertheless, their local effect on adipogenesis and insulin sensitivity in subcutaneous adipose tissue could be present and further investigation of this possibility is needed. On the contrary, our data do not support an up-regulation of p85alpha subunit of PI3K expression as a mechanism of GH-induced insulin resistance in subcutaneous adipose tissue of acromegalic patients.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

AC, acromegaly group; C, control group; CRP, C-reactive protein; DEXA, dual-energy X-ray absorbtiometry; GH, growth hormone; GH-R, growth hormone receptor; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment – insulin resistance; IFCC, International Federation of Clinical Chemistry; IGF-1, insulin-like growth factor-1; IGF-2, insulin-like growth factor-2; IGFBP-1-3, insulin-like growth factor binding protein-1-3; IGF-1R, insulin-like growth factor-1 receptor; IGF-2R, insulin-like growth factor-2 receptor; INS-R, insulin receptor; LBM, lean body mass; LDL, low-density lipoprotein; p85alpha, p85alpha subunit of phosphoinositide-3-kinase; PI3K, phosphoinositide-3-kinase; qRT-PCR, quantitative real-time PCR; SCAT, subcutaneous adipose tissue.

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