Inflammatory adipokines contribute to insulin resistance in active acromegaly and respond differently to different treatment modalities

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Abstract

Background: Active acromegaly is associated with insulin resistance, but it is uncertain whether inflammation in adipose tissue is a contributing factor.

Aim: To test if GH/IGF1 promotes inflammation in adipocytes, and if this is relevant for systemic insulin resistance in acromegaly. Furthermore, to investigate the effect of treatment modalities (transsphenoidal surgery (TS), somatostatin analogs (SAs), and pegvisomant (PGV)) on glucose metabolism and inflammatory biomarkers in acromegaly.

Methods: The *in vitro* effects of GH/IGF1 on gene expression of adipokines in human adipocytes were investigated. Body composition, glucose metabolism, and circulating adipokines (adiponectin (AD), high-molecular weight AD (HMWAD), leptin, vascular endothelial growth factor-A (VEGF-A), monocyte chemotactic protein 1 (MCP1), and thioredoxin (TRX)) were measured in 37 patients with active acromegaly before and after treatment.

Results: *In vitro* GH, but not IGF1, increased VEGF and MCP1 in human adipocytes. In all treatment groups, body fat increased and IGF1 decreased to the same extent. Fasting glucose decreased in the TS ($P=0.016$) and PGV ($P=0.042$) groups, but tended to increase in the SA group ($P=0.078$). Insulin and HOMA-IR decreased in both TS and SA groups, while the PGV group showed no changes. Serum VEGF and MCP1 decreased significantly in the TS group only ($P=0.010$, $P=0.002$), while HMWAD increased with PGV treatment only ($P=0.018$). A multivariate analysis model identified the changes in GH and VEGF as predictors of improvement in HOMA-IR after treatment ($R^2=0.39$, $P=0.002$).

Conclusions: i) GH directly promotes inflammation of human adipocytes by increasing VEGF and MCP1 levels; ii) glucose metabolism and inflammation (VEGF and MCP1) improve to some extent after treatment, despite an increase in adipose tissue mass; and iii) the decrease in insulin resistance after therapy in acromegaly depends, to some extent, on treatment modalities.

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Introduction

Elevated fasting glucose levels, insulin resistance, and overt type 2 diabetes are common features of patients with active acromegaly (1). Lipolysis and increased levels of free fatty acids (FFAs), impairment of insulin signaling, and changes in adipokines and inflammation in adipose tissue are considered as potential underlying mechanisms (2, 3).

The most reproducible direct metabolic effect of growth hormone (GH) is stimulation of lipolysis and release of FFAs. The increased FFAs may affect insulin sensitivity by competition with glucose for substrate oxidation, impairment of insulin signaling, altering β -cell function, or triggering monocyte–macrophage

accumulation in adipose tissue (2, 4, 5). Further, GH directly alters the insulin signal cascade activity in mice, but studies on humans have not supported these observations (6, 7, 8). Data regarding the effects of GH on circulating adipokines in human subjects are sparse and the results often contrast those obtained from animal models (9). Dysfunctional adipose tissue is recognized as a substantial contributor to general insulin resistance either by its own insulin-resistant state or, more significantly, by endocrine cross-talk with the tissues involved in glucose metabolism (10). Thus, several adipocyte-derived mediators play a central role in the regulation of insulin sensitivity, via their impact on inflammation and immunity (11, 12).

We have recently shown that serum levels of NAMPT – a known inflammatory cytokine – are elevated in acromegaly and that GH increases NAMPT expression in mature human adipocytes. Further, we suggested a pathogenic role of this adipokine in adipose tissue inflammation (3).

Different treatment modalities may impact glucose metabolism differently in acromegaly (13). For example, in addition to the central effect of somatostatin analogs (SAs), a direct effect was described in the periphery on key organs for glucose homeostasis (14, 15, 16, 17). Moreover, pegvisomant (PGV) acts as a specific GH antagonist, thus being considered an insulin sensitizer (18).

Based on the relationship between inflammation in adipose tissue and insulin resistance, we hypothesized that GH induces production of inflammatory adipokines, which relates to a systemic insulin resistance present in active acromegaly. Moreover, we speculated that glucose metabolism and circulating inflammatory markers change after therapy in a manner that depends on the treatment modality. We aimed to: i) quantify the *in vitro* effects of GH/insulin-like growth factor 1 (IGF1) on selected inflammatory adipokines in human mature adipocytes; ii) evaluate the association of these adipokines with glucose metabolism parameters in patients with acromegaly; and iii) examine the effect of different treatment modalities on inflammation and insulin resistance in active acromegaly.

Subjects and methods

Subjects

Thirty-seven patients diagnosed with active acromegaly (18 females and 19 males) were investigated prospectively. The diagnosis of acromegaly was based on clinical

evaluation, elevated IGF1 levels, and a failure to suppress GH during an oral glucose tolerance test, in accordance with international guidelines (19). The patients were treated with either transsphenoidal surgery (TS; $n=14$), SA (Sandostatin LAR; $n=16$), or PGV ($n=7$). The TS and SA patients were examined at the Section of Endocrinology, Rikshospitalet, Oslo University Hospital (Norway) as participants in a prospective randomized study (ClinicalTrials.gov Identifier: NCT00521300) (20), while the PGV patients were investigated in an open, non-randomized trial at Department of Internal Medicine and Endocrinology, Aarhus University Hospital (Denmark) (21), as previously described. The TS and SA patients were treatment naïve. Five of the PGV patients received treatment before inclusion (four had previous surgery and one patient was treated with PGV). A good response to treatment was arbitrarily defined as a 50% decrease in IGF1 levels.

The study was approved by the Local Ethical Committee and was conducted according to the Declaration of Helsinki. Informed consent was obtained from all patients.

Blood sampling and biochemical measurements

Blood samples were drawn in the morning and after an overnight fast, and serum was collected and stored at -80°C . GH was measured by immunoassay and serum IGF1 was measured by RIA as previously described (22, 23). GH day curve represents the mean of five GH values measured during 1 day, every 2 h. Insulin was analyzed by RIA (Millipore Corporation, MO, USA). Serum levels of leptin, adiponectin (AD), high-molecular weight AD (HMWAD), and vascular endothelial growth factor-A (VEGF-A) were measured by enzyme immune-assays (EIA) (R&D systems, Minneapolis, MN, USA). Thioredoxin (TRX, Abnova, Taipei City, Taiwan) and monocyte chemotactic protein 1 (MCP1; Peprotech, Rocky Hill, NJ, USA) were measured by EIA. Intra- and inter-assay coefficients of variation were $<10\%$ for all assays. Insulin resistance (HOMA-IR) and insulin secretion (HOMA- β) were calculated based on fasting insulin and glucose levels using the HOMA2 Calculator (24).

Body composition

Bone mineral density and body composition were measured by dual-energy X-ray absorptiometry as described previously (22). Bioelectrical impedance analysis (BIA) was performed in PGV patients as described previously (21).

Cell cultures

Human subcutaneous (SC; donor no. 16344 and lot no. 7F4245) and visceral (VA; donor no. 14324 and lot no. 6F3501) preadipocytes culture media and differentiation factors were purchased from Lonza Walkersville, Inc. (MD, USA). Preadipocytes were differentiated to mature adipocytes using differentiation media (PBM-2 with 10% FCS, 2 mM glutamine, 100 UI/ml penicillin, 100 µg/ml streptomycin, 0.5 µM insulin, 0.1 µM dexamethasone, 50 µM indomethacin, and 0.5 mM isobutyl-1-methylxanthine) for 14 days, as described previously (3). Differentiated adipocytes were washed twice with PBS, cultured overnight without FCS, and then stimulated with GH and IGF1 (100 and 500 ng/ml, R&D systems) for 3 and 12 h respectively.

In a separate set of experiments, mature SC adipocytes were stimulated for 3 h with GH (100 ng/ml) with or without the addition of the GH receptor antibody (GHRAb) (10 µg/ml, R&D systems).

In all experiments, culture media was collected and cells were harvested at the indicated time points using QIAzol lysis reagent (Qiagen).

Real-time quantitative PCR

Total RNA was isolated as described (3). For real-time PCR analyses, sequence-specific oligonucleotide primers that cross the exon–exon junction of the cDNA for VEGF (forward primer (FP), TCATCACGAAGTGGTGAAGTTCAT; reverse primer (RP), ATCAGGGTACTCCTGGAAGATGTC), MCP1 (FP, AAGCTGTGATCTTCAAGACCATTGT; RP, TGGAATCCTGAACCCACTTCTG), β -actin (FP, AGGCAC-CAGGGCGTGAT; RP, TCGTCCCAGTTGGTGACGAT), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; FP, CCAAGGTCATCCATGACAACCT; RP, AGGGGCCATC-CACAGTCTT) were designed using Primer Express Software, version 2.0 (Applied Biosystems). Gene expression for IL1 β , IL1Ra, PAI1, TNF α , IL6, IL8, TWEAK, Resistin, CCL3, CCL4, CCL5, CXCL1, CXCL12, and CX3CL were also measured. The sequence-specific primers are available upon request. A SYBR Green assay was performed using the qPCR Master Mix for SYBR Green I (Eurogentec, Seraing, Belgium). mRNA was quantified using the standard curve method of the ABI Prism 7500 (Applied Biosystems). Data were normalized to β -actin or GAPDH (reference genes).

Statistical analysis

One-way ANOVA was performed to assess the total variability of an *in vitro* group data. Further, unpaired

student's sample *t*-test (two-tailed) was used to evaluate the differences between control and stimulated cells.

All the measurements were checked for normal distribution by visual methods (histograms and Q–Q plots) and further log-transformed if necessary. Data are presented as mean $\pm$ s.d. or median (IQR).

To compare the variables before and after treatment (within-group differences), the Wilcoxon' match-pair test was performed. The Mann–Whitney *U* test was used to compare the changes between good responders and poor responders in the same group. The Kruskal–Wallis test with adjustment for multiple comparisons was used to compare the changes between three different treatment groups (between group differences).

Univariate correlation analysis was used to determine independent associations between variables. In addition, to determine the independent predictors of HOMA-IR change during the different treatment modalities, a subsequent multiple linear regression analysis was performed with inclusion of the variables that had a *P* value below 0.10 in the univariate analysis. All the statistical calculations were performed with SPSS, version 18. *P* values were two-tailed and considered significant when <0.05 .

Results

In vitro effect of GH/IGF1 on human adipocytes

Based on the hypothesis that GH promotes inflammation in the adipocytes, we investigated the *in vitro* effect of GH and IGF1 on gene expression of selected adipokines. We identified that GH increased VEGF and MCP1 expression in human mature adipocytes, while neither GH nor IGF1 regulated the expression of IL1 β , IL1Ra, PAI1, TNF α , IL6, IL8, TWEAK, Resistin, CCL3, CCL4, CCL5, CXCL1, CXCL12, and CX3CL in SC adipocytes (data not shown). As shown in Fig. 1, GH increased VEGF and MCP1 expression levels at 3 and 12 h respectively, both in the SC and in VA adipocytes. In contrast to GH, no consistent effect was observed with IGF1. Further, when SC adipocytes were stimulated for 3 h with GH in the presence of GHRAb, the increase in VEGF was partially abrogated (Fig. 1C) and the increase in MCP1 was totally prevented (Fig. 1F), suggesting a direct effect of GH on induction of these cytokines.

Study population

Table 1 shows the main baseline demographics, body composition, and biochemical variables of the study

Figure 1

Effects of GH/IGF1 on VEGF (A, B and C) and MCP1 (D, E and F) expression in human SC (A, C, D and F) and VA (B and E) adipocytes. SC and VA were differentiated for 14 days, overnight FCS starved, and stimulated with GH (100 and 500 ng/ml), IGF1 (100 and 500 ng/ml) for 3 and 12 h and with GH (100 ng/ml) and GHRab (10 μ g/ml) for 3 h. GH dose dependently increased VEGF (A and B) and MCP1 (D and E) expression in SC (A and D)

and VA (B and E) at 3 and 12 h. IGF1 presented no consistent effect. Further, the incubation of SC with GH and GHRAb prevented the GH-induced VEGF and MCP1 gene expression (C and F). Data are mean $\pm$ S.E.M. ($n=4$; US, unstimulated; * $P<0.05$, ** $P<0.01$, and *** $P<0.001$ vs unstimulated control, unpaired Student's sample t -test (two-tailed)).

populations, as a whole and stratified by the different treatments. At baseline, total body fat mass correlated positively with VEGF ($r=0.38$, $P=0.026$), leptin ($r=0.86$, $P<0.001$), insulin ($r=0.59$, $P<0.001$), and HOMA-IR ($r=0.60$, $P<0.001$) and negatively with GH ($r=-0.60$, $P<0.001$). Total body lean mass was correlated negatively with HMWAD ($r=-0.61$, $P=0.001$), AD ($r=-0.43$, $P=0.034$), and leptin ($r=-0.50$, $P=0.007$).

In the entire cohort of patients, total body fat mass increased (3.5 (5.6) kg, $P \leq 0.001$) while total body lean mass decreased (3.2 (3.8) kg, $P \leq 0.001$) following treatment. Glucose metabolism variables (insulin, HOMA-IR, and HOMA- β) improved, together with a significant increase in the HMWAD:AD ratio and a significant decrease in MCP1 and TRX levels (Table 1). No significant changes were observed in the fasting glucose, AD, HMWAD, leptin, or VEGF levels (Table 1).

Regardless of treatment modality, IGF1 decreased and total body fat increased to the same extent in all the groups (Fig. 2A and B). However, fasting glucose decreased in the TS and PGV groups, and tended to

increase in the SA group (Table 1). Insulin, HOMA-IR, and HOMA- β decreased in both the TS and SA groups, but not in the PGV group (Table 1, Fig. 2C and D). In the TS group, AD, VEGF, MCP1, and TRX decreased significantly. In the SA group leptin increased, while in the PGV group HMWAD increased significantly (Table 1).

Disease activity after treatment predicts improvement in glucose and inflammatory markers

The next step was to identify if the treatment response correlated with changes in selected biomarkers of inflammation and glucose metabolism. The patients were divided into good and poor responders based on the decrease in IGF1 levels following treatment (Fig. 3A). A normalization of IGF1 levels is expected when the optimal PGV treatment is used. However, one patient in the PGV group was considered as a poor responder based on a decrease in IGF1 by only 46.9%. Consequently, we did not perform statistical analysis between good and poor responders in the PGV group. The increase in body fat

Table 1 Clinical and biochemical characteristics of patients before and after treatment.

	All			TS			SA			PGV		
	Basal	After	P	Basal	After	P	Basal	After	P	Basal	After	P
n	37			14			16			7		
Age (years)	48±11			47±10			49±11			48±14		
Sex, male/female (n)	19/18			5/9			9/7			5/2		
BMI (kg/m ²)	27.9±3.8	28.0±4.2	0.360	29.0±4.8	29.6±5.2	0.046	27.0±3.1	27.0±3.2	0.347	27.3 (7.1)	26.7 (6.7)	0.018
Body composition												
Total body fat (kg)	19.5±10.1	23.5±11.2	<0.001	20.6 (18.9)	26.7 (19.5)	0.002	18.5±10.3	21.8±10.1	0.001	16.8 (22.7) ^a	19.9 (13.6) ^a	0.091
Total body lean (kg)	61.0±13.6	57.1±12.3	<0.001	57.0 (19.7)	54.2±12.0	0.001	63.1±13.8	60.6 (21.5)	<0.001			
Biochemistry												
GH (μg/l) ^b	26.3 (34.3)	3.6 (10.3)	<0.001	20.3 (34.3)	1.7 (9.9)	0.004	33.5 (42.8)	5.1 (24.6)	<0.001	ND	ND	
GH-OGTT (μg/l) ^c	6.5 (8.6)	0.49 (2.4)	<0.001	6.0 (9.4)	0.37 (2.3)	0.016	7.6 (9.6)	1.0 (6.6)	0.001	ND	ND	
IGF1 (nM/l)	87.8±26.6	41.8±23.6	<0.001	80.5±22.1	31.5 (22)	0.001	85.8±22.4	48.2±30.9	0.001	104.0 (74)	44.0 (22)	0.018
IGF1 (% ULN)	216 (95)	88 (81)	<0.001	207 (95)	70 (62)	0.001	213 (111)	92 (113)	0.001	336±140	129 (68)	0.018
Good response ^d		24 (65%)			10 (71%)			8 (50%)			6 (86%)	
Glucose metabolism												
Glucose (mM/l)	5.8±0.7	5.7±0.9	0.364	5.7±0.7	5.0 (0.9)	0.016	5.6 (1.3)	6.2±1.0	0.078	5.5 (0.6)	5.4 (0.6)	0.042
Insulin (μU/ml)	26.7 (15.8)	13.9 (12.8)	<0.001	31.1 (28.8)	12.7 (13.4)	0.002	27.2±9.2	20.3±12.4	0.020	17.3 (17.1)	15.7 (22.7)	1.00
HOMA-IR	3.1 (1.7)	1.6 (1.5)	<0.001	3.4 (3.2)	1.4 (1.4)	0.002	3.1 (1.5)	2.4±1.5	0.020	2.0 (1.9)	1.7 (2.4)	1.00
HOMA-β	149 (105)	116 (52)	<0.001	216 (130)	120 (60)	0.003	140 (81)	105.3±30.3	0.002	130.8±58.4	140 (109)	0.499
Adipokines												
AD (μg/ml)	11.9±4.5	11.0±4.8	0.270	12.3 (8.9)	6.4 (11.1)	0.037	12.1 (7.5)	12.5 (7.6)	1.00	8.3 (6.5)	10.4 (8.0)	0.310
HMWAD (μg/ml)	6.2±3.4	6.4±3.0	0.376	6.3 (5.5)	3.7 (5.0)	0.136	5.8 (4.8)	7.4±2.9	0.215	4.9±2.1	5.7 (4.4)	0.018
HMWAD:AD ratio	0.54±0.16	0.61±0.15	0.030	0.51±0.21	0.63±0.20	0.139	0.56±0.15	0.62±0.13	0.112	0.52±0.14	0.55±0.10	0.499
Leptin (μg/ml)	8.0 (21.6)	9.1 (23.5)	0.318	21.5 (20.6)	19.2 (27.0)	0.754	3.7 (18.6)	7.3 (15.4)	0.017	5.3 (37.6)	5.0 (33.9)	0.128
VEGF (pg/ml)	218 (309)	224 (242)	0.266	391 (258)	253 (198)	0.010	213 (276)	236 (321)	0.289	104 (167)	156±104	0.799
MCP1 (pg/ml)	171±80	136±56	0.001	185 (100)	125 (56)	0.002	182±94	135 (84)	0.121	105 (45)	91 (23)	0.396
TRX (ng/ml)	23.1 (27.7)	22.0 (14.8)	0.009	45.1±26.7	21.9 (19.7)	0.019	23.3 (29.3)	25.5 (11.2)	0.683	23.0 (9.1)	15.7 (9.7)	0.063

BIA, bioelectrical impedance analysis; ND, not determined. Data are given as mean ± s.d. or median (IQR); P values are within-group differences (Wilcoxon^a match-pair test).

^aTotal body fat is measured by BIA.

^bGH is presented as a mean of five values measured 1 day, every 2 h.

^cGH is presented as nadir value during OGTT.

^dGood response is defined as an IGF1 decrease by at least 50%.

Figure 2

Changes in biochemical variables and total body fat during treatments (TS (white square), SA (dark gray square), and PGV (light gray square)). IGF1 decreased (A) and total body fat increased (B) to the same extent in all the groups. Glucose decreased in the TS and PGV groups, and tended to increase in the SA group (C). HOMA-IR decreased in both the TS and SA groups, but not in the PGV group (D). *P* values are between-group differences in change from baseline to 12 months (TS), 6 months (SA), and 1 month (PGV) (Kruskal–Wallis test with multiple comparison adjustment). Data are presented as mean $\pm$ min/max values.

and the changes in MCP1 were similar between good responders and poor responders (Fig. 3B and E). Insulin (data not shown) and HOMA-IR (Fig. 3C) were improved in the TS group independent of treatment response and in good responders SA, and remained unchanged in poor responders SA. Moreover, in poor responders SA, an increase in serum VEGF was observed (Fig. 3D).

Determinants of insulin resistance (HOMA-IR) change during treatment for acromegaly

In addition to a positive correlation with total body fat mass, HOMA-IR was also positively correlated with leptin ($r=0.43$, $P=0.009$) and VEGF ($r=0.43$,

$P=0.011$) in the whole population study before treatment. HOMA-IR decreased significantly following treatment both with TS (55.7 (26) %, $P=0.002$) and SA (31.9 (56) %, $P=0.02$). HOMA-IR did not change significantly in the PGV group (Fig. 2D). We performed a univariate correlation analysis to determine independent associations between the changes in HOMA-IR, body composition, and circulating biomarkers in relation to treatment in acromegaly (Table 2). As expected, a decrease in disease activity as estimated by a decrease in GH levels and of total lean body mass was correlated with changes in HOMA-IR. The change in circulating VEGF was correlated with the change in HOMA-IR.

Furthermore, a multiple regression analysis model identified the changes in GH and VEGF as positive predictors for the improvement of HOMA-IR after treatment (Table 2).

Discussion

In this study, we adopted an exploratory approach measuring gene expression of multiple cytokines expressed in human mature adipocytes and considered to potentially induce insulin resistance. We demonstrated that GH, but not IGF1, increased the gene expression of VEGF and MCP1, whereas the expression of several other cytokines was unaffected by both GH and IGF1. VEGF is the master regulator of angiogenesis, while MCP1 is a known cytokine that recruits monocytes, T cells, and dendritic cells at the site of inflammation (25, 26). Accumulation of an increased number of adipose tissue macrophages (and subsequently a chronic state of tissue inflammation) is an important cause of insulin resistance (11). Our *in vitro* results showing that GH directly increases VEGF and MCP1 in mature adipocytes support the hypothesis that by increasing the inflammatory potential of mature adipocytes, GH could promote insulin resistance in adipose tissue in active acromegaly. Sustained increases in VEGF levels have been shown to induce islet hypervascularization, fibrosis, and inflammation, resulting in β -cell death and hyperglycaemia indicating a direct role of VEGF in deteriorating glucose metabolism (27). Indeed, adipose tissue levels of VEGF have been demonstrated to correlate with the degree of insulin resistance in obesity (28). In accordance, our *in vivo* findings, showing a correlation between circulating VEGF and insulin resistance at baseline as well as following treatment in active acromegaly, suggest that this association is not necessarily dependent on fat mass but on the degree of inflammation in adipose tissue. This is supported by our data showing

Figure 3

Different response between good and poor responders during treatments (TS (white square), SA (dark gray square), and PGV (light gray square)). The patients were divided as good responders (a decrease of $\geq 50\%$ in IGF1) or poor responders (a decrease of $< 50\%$ in IGF1). (A). Total body fat (B) and MCP1 (E) changed similar between good responders and poor responders in the TS and SA groups. Changes in HOMA-IR (C), VEGF (D) and TRX (F) differed between good responders and poor responders in the SA group. TS group, good responders ($n = 10$) and poor responders ($n = 4$); SA group, good responders

($n = 8$) and poor responders ($n = 8$); PGV group, good responders ($n = 6$) and poor responders ($n = 1$). Data are presented as mean $\pm$ min/max values. P values are between-group differences in change from baseline to 12 months (TS), 6 months (SA), and 1 month PGV (Kruskal–Wallis test with multiple comparison adjustment). P values ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$) are within-group differences in change between good and poor responders (Mann–Whitney U test). Statistical measurement of within-group differences for PGV group was not performed.

a significant decrease in insulin resistance following treatment of active acromegaly, despite an increase in total fat mass. Thus, the expansion of adipose tissue may not necessarily be regarded as a factor that deteriorate the insulin sensitivity, because normal functional adipose tissue protects against diabetes by acting, first and foremost, as a storage depot for FFA (29).

Previous studies have shown that some of the mechanisms involved in GH-induced impairment of glucose metabolism include increased lipolysis, FFAs and a downstream impairment of insulin signaling in rodent models (2). In addition, GH may directly enhance the inflammatory trait of adipose tissue that contributes and potentiates the development of insulin resistance (3). In acromegaly, macrophages and other immune cell types in adipose tissue are exposed to high local concentrations of FFAs released by GH-induced lipolysis. FFAs can potentially act as signals themselves and activate inflammation in macrophages by interacting with TLR4–NF- κ B/JNK inflammatory signaling pathways (11). In this way, the adipose tissue's macrophages and other immune cells may express and exacerbate the response to the inflammatory signals in acromegaly.

We previously described that GH increases MCP1 in subcutaneous adipocytes through a mechanism dependent of NAMPT enzymatic activity (3). This study presented confirmatory and new data, which showed that visceral adipocytes also respond to GH stimulation in a similar way.

The most evident improvement in glucose/inflammatory parameters was observed in the TS group. In the SA group, fasting glucose showed an increasing tendency, together with the improvement in insulin, HOMA-IR, and HOMA- β . Previously, it has been shown that SAs may lower glucose tolerance due to suppression of pancreatic insulin secretion, but improve insulin sensitivity by lowering GH levels (13, 30, 31, 32). Indeed, the improvement in glucose metabolism in the SA group in our study seems to be associated with a good response to treatment. A surprising finding was that in poor responders SA, but not TS, an increase in circulating VEGF levels was observed. This is probably not to be explained by a direct effect of SAs on peripheral tissues as different studies showed that somatostatin analogs present anti-VEGF properties in different tumor models (33). Rather, the observed increase in VEGF levels in these patients may

Table 2 Determinants of HOMA-IR change after treatment of acromegaly.

	Univariate <i>R</i> (<i>P</i> if trend)	Multiple linear regression β / <i>P</i>
Age	0.15	
Total fat BM	−0.06	
Total lean BM	0.42 (0.024)	0.30/0.079
GH	0.58 (0.001)	0.37/0.036
IGF1	0.33 (0.054)	
AD	0.33 (0.063)	
HMWAD	0.21	
Leptin	0.08	
VEGF	0.41 (0.016)	0.35/0.032
MCP1	0.28	
TRX	0.13	
Total		$R^2=0.39/0.002$

BM, body mass; R^2 , adjusted.

represent an indirect effect, due to a persistence of increased levels of GH and active disease.

TRX is induced and secreted by a variety of oxidative stresses and shows protective functions against oxidative stress. Serum/plasma TRX levels are good markers for oxidative stresses in various disorders. TRX levels are increased in different acute inflammatory states (myocarditis, viral infection, and HIV) or cancer (34). Serum TRX levels decreased significantly following TS and showed a decreasing trend in the PGV group, probably because of a decrease in the oxidative stress and consecutively due to a decreased demand for an anti-oxidative protein, but remained unchanged in the SA group. Taken together, our results showed that despite an improvement in insulin resistance and an increase in leptin levels (probably as a consequence of a partial control of disease activity), the patients in the SA group exhibited unchanged circulating levels of additional adipokines.

In this study, we did not find an amelioration of insulin sensitivity, estimated by HOMA-IR in the PGV group, although we previously showed that treatment with PGV improved insulin sensitivity in these patients as measured by euglycemic–hyperinsulinemic clamp (21). HOMA-IR is derived from fasting glucose and insulin levels and reflects primarily basal hepatic insulin sensitivity (24). By contrast, the hyperinsulinemic clamp reflects insulin-stimulated glucose disposal predominantly into skeletal muscle (35). It is therefore likely that four weeks' treatment with PGV mainly improved insulin sensitivity in the skeletal muscle (21). Alternatively, the lack of a significant improvement in HOMA-IR in our study could be due to a type 2 error.

An increase in HMWAD levels, independent of changes in glucose metabolism, was observed in the PGV

group. This suggests that the increase in HMWAD is a direct effect of blocking the GH receptor and not a secondary result due to the changes in insulin sensitivity. Our findings are supported by a study that describes the increased levels of HMWAD in mouse lacking GH receptor (36). Given our results, it may be interesting to study if GH modulates disulfide bond A oxidoreductase-like protein (DsbA-L), a recently described protein that promotes AD multimerization (37). HMWAD is thought to be the most biologically active form of AD in terms of glucose homeostasis. High levels of total AD and HMWAD were associated with a lower incidence of diabetes and peripheral artery disease (38, 39). Furthermore, we observed that the addition of GHRAb prevented the GH increase in VEGF and MCP1 in mature adipocytes. This indicates that the decrease of inflammation in adipose tissue may represent an additional mechanism to explain the insulin sensitizer properties of PGV.

The main limitation of our study is the limited number of patients in the different treatment modalities and different duration of treatment in these groups. However, the duration of treatment seems of minor concern as the decrease in IGF1 levels and the increase in body fat were similar between different treatment modalities. The peculiar increase in VEGF in poor responders treated with SA is difficult to explain, and further studies with a higher number of patients are needed before a definitive conclusion is made. The *in vitro* studies are limited by the use of preadipocytes from different donors and the possible associated variability.

In summary, this study together with our previous results demonstrate that GH directly increases the inflammatory phenotype of human mature adipocytes, indicating a possible contribution of adipose tissue to the systemic insulin resistance in active acromegaly. The improvement of systemic insulin resistance depended to some extent upon treatment modality and was predicted by a decrease in GH and VEGF circulating levels.

Declaration of interest

N C Olarescu, T Ueland, K Godang, and R Lindberg-Larsen have nothing to declare. J Bollerslev received unrestricted research grants from Pfizer and Novartis and served as an advisory board member for Pfizer. J O L Jørgensen received unrestricted research grants from Pfizer, Novartis, and IPSEN and serves as an advisory board member for Pfizer. None of the authors have conflicts of interest.

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