Identification of Novel GH-Regulated Pathway of Lipid Metabolism in Adipose Tissue: A Gene Expression Study in Hypopituitary Men

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Context: Adipose tissue is a major target of GH action. GH stimulates lipolysis and reduces fat mass. The molecular mechanism underlying cellular and metabolic effects of GH in adipose tissue is not well understood.

Objective: The aim of this study is to identify GH-responsive genes that regulate lipid metabolism in adipose tissue.

Design: Eight men with GH deficiency underwent measurement of plasma free fatty acid (FFA), whole-body lipid oxidation, and fat biopsies before and after 1 month of GH treatment (0.5 mg/d). Gene expression profiling was performed using Agilent 44K G4112F arrays using a two-color design. Differentially expressed genes were identified using an empirical Bayes, moderated *t* test, with a false discovery rate under 5%. Target genes were validated by quantitative RT-PCR.

Results: GH increased circulating IGF-I and FFA and stimulated fat oxidation. A total of 246 genes were differentially expressed, of which 135 were up-regulated and 111 down-regulated. GH enhanced adipose tissue expression of *IGF-I* and *SOCS3*. GH increased expression of patatin-like phospholipase domain containing 3 (*PNPLA3*), a novel triglyceride (TG) hydrolase, but not hormone-sensitive lipase (*HSL*), a classical TG hydrolase. GH repressed cell death-inducing DFFA-like effector A (*CIDEA*), a novel lipid droplets-associated protein, promoting TG storage. GH differentially regulated genes promoting diacylglycerol synthesis. GH suppressed hydroxysteroid (11β) dehydrogenase 1, which activates local cortisol production and genes encoding components of extracellular matrix and TGF-β signaling pathway.

Conclusion: GH stimulates the TG/FFA cycle by regulating the expression of novel genes that enhance TG hydrolysis, reduce TG storage, and promote diacylglycerol synthesis. GH represses adipocyte growth, differentiation and inflammation. **(*J Clin Endocrinol Metab* 96: E1188–E1196, 2011)**

Growth hormone is an important regulator of substrate metabolism and body composition. GH exerts its actions directly or indirectly via IGF-I. GH deficiency in adults causes visceral obesity, reduced lean body mass, and impaired physical function, which are reversed with GH replacement therapy. Placebo-controlled trials have shown GH treatment reduced fat mass by 10–20% in GH-deficient adults (1). The cellular mechanisms underlying the effect of GH on fat metabolism, however, are not well understood. The immediate increase in serum free

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Abbreviations: ALR, Agilent log ratio; *CIDEA*, cell death-inducing DFFA-like effector A; CoA, coenzyme A; DAG, diacylglycerol; ECM, extracellular matrix; ES, enrichment score; FDR, false discovery rate; FA-CoA; fatty acid CoA; FFA, free fatty acids; Gly-3-P, glycerol-3-phosphate; HSD11β1, hydroxysteroid 11-β dehydrogenase 1; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; *PNPLA3*, patatin-like phospholipase domain containing 3; qRT-PCR, quantitative RT-PCR; SOCS, suppressor of cytokine signaling; TG, triglyceride.

fatty acids (FFA) after GH exposure indicates that lipolysis is a major effect *in vivo*. Hormone-sensitive lipase (HSL) is one of the key enzymes responsible for the release of FFA from adipose tissue. Inhibition of lipolysis through pharmacological suppression of HSL abrogates the effect of GH on FFA (2, 3), suggesting that HSL would be a target of GH action. There is evidence from animal studies that GH stimulates lipolysis by increasing adipose tissue HSL activity (4, 5), but this has not been confirmed in humans. HSL mRNA in adipose tissue did not change significantly after GH therapy for 1 yr in GH-deficient adults (6).

Increase of fat mass can arise from hypertrophy or hyperplasia of adipocytes. Hypertrophy results from excess triglyceride (TG) accumulation in preexisting mature adipocytes. Recruitment of multipotent stem cells to the adipocyte lineage along with mitotic clonal expansion of existing preadipocytes during adipogenesis contributes to hyperplasia. GH appears to exert differential effects on differentiation and proliferation. Studies of primary culture from human adipose tissue have observed an inhibitory effect of GH on adipocyte differentiation but a stimulatory effect on mitogenesis (7, 8). The mechanism by which GH inhibits the differentiation of primary preadipocytes to adipoctyes is not well understood.

To investigate the mechanism by which GH regulates adipose tissue function at the molecular level, we studied gene expression profiles in adipose tissue and correlated the transcript profiles of adipose tissue to whole-body metabolic changes in hypopituitary men before and after GH replacement therapy. This is a first human study to investigate the molecular effect of GH on adipose tissue using whole-genome microarray analysis.

Subjects and Methods

Study design

Eight hypopituitary men (age 31–66 yr) with GH deficiency and hypogonadism were recruited from the Endocrine Outpatient Clinic at St. Vincent's Hospital (Sydney, Australia). GH deficiency was confirmed by a peak GH response to insulin-induced hypoglycemia of less than 3 ng/ml, with duration of hypopituitarism for at least 1 yr. All subjects received stable hormone replacement for thyroid and adrenal deficiencies throughout the study. The Human Research Ethics Committee of St. Vincent's Hospital approved the study. Written informed consent was obtained from all subjects.

All subjects went through a run-in period when GH and testosterone replacements were withdrawn for 4 wk. During this time and throughout the studies, subjects were instructed to follow their usual diet and habitual activity. Subjects were studied at baseline and at the end of a 4-wk treatment with GH (0.5 mg/d; Lilly Australia, Sydney, New South Wales, Australia) in an open-labeled study. During the GH administration period, all subjects remained hypogonadal. GH was administered in the evenings before retiring. All subjects presented after an overnight fast at 0800 h to the Clinical Research Facility, the Garvan Institute of Medical Research, for the following procedures: 1) blood samples for measurement of IGF-I and FFA, 2) indirect calorimetry for quantification of resting energy expenditure and fat oxidation, and 3) biopsy of sc fat depot from the right thigh.

Serum assays and indirect calorimetry

Serum assays for IGF-I and indirect calorimetry procedures were previously described (9). Serum FFA was measured using the NEFA (nonesterified FFA) C kit by the ACS (acyl-CoA synthetase)-ACOD (acyl CoA oxidase) method (Wako Pure Chemical Industries Ltd., Osaka, Japan).

Adipose tissue biopsy

Local anesthetic (1% lidocaine) was injected approximately 2 cm above the patella over the vastus lateralis. A small piece of sc adipose tissue was obtained with a 6-mm university hospital needle with suction assistance. On the average, 80–100 mg fat tissue was obtained. Specimens were immediately blotted for blood, snap frozen in liquid nitrogen, and stored at −80 C.

Preparation of RNA and microarray hybridization

Total RNA was isolated from the frozen adipose tissue biopsies with RNeasy lipid tissue kit (QIAGEN, Doncaster, Australia) according to the manufacturer's instructions and further purified with ethanol precipitations. RNA quality and concentration were assessed by NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE). On the average, 0.5–1 μg total RNA with OD 260 around 1.8–2.0 was obtained.

RNA samples were labeled with fluorescence Cy3 or Cy5 using the Agilent Quick Amp labeling kit (Agilent Technologies, Santa Clara, CA). Microarray gene expression studies were performed using Agilent G4112F whole human genome arrays, which contain approximately 41,000 unique noncontrol 60-mer probes. Baseline and GH treatment samples were labeled with Cy3 and Cy5, respectively, and hybridized to the same arrays. A dye swap was performed for five subjects (three subjects had insufficient RNA to perform a dye swap) to remove any bias from the labeling dyes. Hybridization, washing, and scanning were carried out at the Ramaciotti center for Gene Function Analysis, University of New South Wales, Sydney, following the standard procedures recommended by Agilent Technologies.

Statistical analysis of microarray data

Our analytical approach follows that in Mitchell *et al.* (10) with the addition of a batch effect and removal of data points that were not detected on the microarray. Briefly, we used the Agilent log ratio (ALR), the log ratio of the processed red/green channel for each probe, to obtain the relative expression level of the two samples hybridized on each array. Probes with ALR of 0.0 and *P* value log ratio = 1.0 correspond to probes that were not detected above background in either the red or green channel and were thus set to NA. ALRs were transformed from log base 10 to log base 2 and then scale normalized. Noncontrol probes that were detected on at least two arrays in either the red or green channel were retained for further analysis. Microarray data are presented on a log2 scale where each unit represents a 2-fold change in expression level.

After correcting for the dye swap, the following linear model was fitted to the data (11) from each probe independently: $y_i =$

$\alpha_i + \beta_i + \varepsilon_i$, where y_i are the normalized expression ratios for probe *i*, α_i is the effect of GH treatment relative to baseline, β_I is the batch effect (*i.e.* which date the array was hybridized on), and ε_i is the residual error.

To account for the additional correlation that is expected from dye-swapped technical replicates, the duplicate correlation among samples was estimated, and the sample ID was used as a blocking variable in the linear model fit. To assess the extent of differential expression, an empirical Bayes, moderated *t* statistic (11) was performed. *P* values were adjusted for multiple testing using the positive false discovery rate (FDR) procedure (12). For genes with multiple probes, the probe with the largest absolute moderated *t* statistic was retained, with all data reported at the gene level. All analyses were performed using R version 2.8.0, and the *limma* package version 2.16 (11), from Bioconductor version 2.3. Differentially expressed genes were defined as those with FDR below 5%.

All raw and normalized microarray data are available via the ARRAYEXPRESS web site (accession number E-MTAB-276).

Functional classification of differentially expressed genes

Functional classification of genes that were differentially expressed after GH treatment were analyzed for identification of pathways. This was performed using gene set enrichment analysis (GSEA version 2.0) (13), using 1000 permutations to assess the FDR, and the c2_all and c5_BP collection of gene sets from the Molecular Signatures Database (MSigDB version 2.5). An enrichment score (ES) was calculated for each of these gene sets, and it reflects the degree to which a gene set is overrepresented at the extremes of the entire gene list. ES was normalized for each gene set to account for differences in gene set size, yielding a normalized ES. The FDR corresponding to each normalized ES was then calculated. Significantly altered pathways were defined as those with FDR below 25% (13). Analyzing the effect of changes in gene expression at the level of gene sets (*e.g.* pathways) improves the robustness of findings because these methods measure the cumulative evidence for coordinated change in expression, over an entire set of functionally related genes. This is true when the magnitude of change is modest.

Gene lists were further analyzed based on their potential relevant functions in adipose tissues. We studied well-known GH-regulated genes, such as *IGF1*, those involved in GH receptor (*GHR*) signaling and cellular response genes; those involved in lipid metabolism, including lipolysis and TG synthesis; those governing adipogenesis; and genes encoding components of extracellular matrix (ECM). Gene abbreviations are used in the text with their full descriptions presented in Table 1.

Quantitative RT-PCR (qRT-PCR)

The Applied Biosystems (Foster City, CA) 7900HT Real-Time PCR System was used to measure the abundance of selected mRNAs in adipose tissue to validate the microarray findings. Total RNA of each individual sample was reverse transcribed using the high-capacity RNA-to-cDNA kit (Applied Biosystems). Five predesigned TaqMan gene expression assays (Applied Biosystems) were used, with the assay ID as follows: Hs01547657 (*IGF1*), Hs02330328 (*SOCS3*),

TABLE 1. Selected GH-responsive genes in adipose tissue with enriched functions

Functional groups	Genes	Description	Direction	Absolute FC
GH action	*IGF1*	IGF-I (somatomedin C)	Up	2.7
	SOCS3	Suppressor of cytokine signaling 3	Up	6.1
	CISH	Cytokine-inducible SH2-containing protein	Up	3.7
Early response genes	*FOS*	V-fos FBJ murine osteosarcoma viral oncogene homolog	Up	11.9
	FOSB	FBJ murine osteosarcoma viral oncogene homolog B	Up	24.4
	EGR1	Early growth response 1	Up	5.8
	JUN	Jun oncogene	Up	2.8
Lipolysis	*CIDEA*	Cell death-inducing DFFA-like effector A	Down	−2.8
	PNPLA3	Patatin-like phospholipase domain containing 3	Up	3.2
TG biosynthesis	*ACSS2*	Acyl-CoA synthetase short-chain family member 2	Up	2.6
	LPIN1	Lipin 1, phosphatidic acid phosphatase-1 (PAP1)	Up	2.7
	DGKG	Diacylglycerol kinase, γ 90 kDa	Down	−6.2
	PLCB2	Phospholipase C, β 2	Up	8.6
Phospholipid signaling	*RASGRP3*	RAS guanyl releasing protein 3 (calcium and DAG-regulated)	Up	2.5
Adipogenesis	*HSD11B1*	Hydroxysteroid (11-β) dehydrogenase 1	Down	−3.1
ECM	*ITGA8*	Integrin, α 8	Down	−6.8
	ITGBL1	Integrin, β-like 1 (with EGF-like repeat domains)	Down	−4.5
	TNC	Tenascin C (hexabrachion)	Down	−7.9
	NPNT	Nephronectin	Down	−6.2
	THBS1	Thrombospondin 1	Down	−4.8
	COL4A6	Collagen, type IV, α 6	Down	−6.3
	LAMA3	Laminin, α 3	Up	2.8
TGF-β signaling pathway	*INHBA*	Inhibin, β A (activin A, activin AB α-polypeptide)	Down	−6.9
	LTBP2	Latent TGFβ binding protein 2	Down	−3.8
	LTBP1	Latent TGFβ binding protein 1	Down	−4.0
	PPP2R2B	Protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), β isoform	Down	−4.8

Fold change (FC) is the expression ratio of GH-treated samples *vs.* baseline. Direction indicates up- or down-regulation of genes. Absolute FC is used to denote changes in expression level between GH-treated samples and baseline (negative number denotes down-regulation).

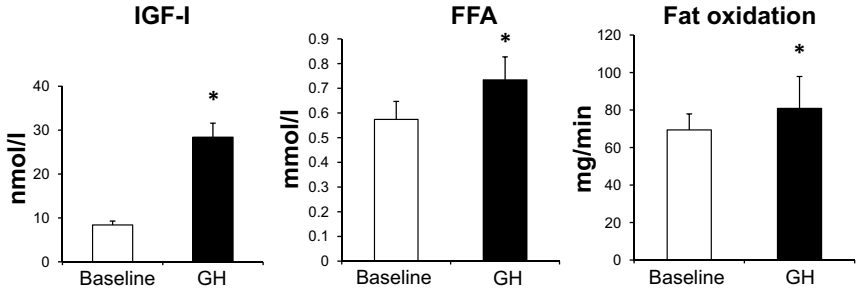

FIG. 1. Mean IGF-I (*left*), FFA (*middle*), and rates of fat oxidation (*right*) in eight hypopituitary men before and after 4 wk treatment with GH (0.5 mg/d). Results are presented as mean ± SE. *, $P < 0.05$ *vs.* baseline, by Student's paired *t* test.

Hs00194153 (*HSD11β1*), Hs00228747 [patatin-like phospholipase domain containing 3 (*PNPLA3*)], and Hs00943410 (*HSL*, referred to as *LIPE* in Agilent array gene symbols). Standard TaqMan cycling conditions were used, and all samples were run in triplicates. The mRNA abundance of each gene was calculated using the standard curve method and adjusted for the expression of β-actin as an internal control.

Results

Biochemical and metabolic changes

The mean (±SE) baseline IGF-I (8.4 ± 0.9 nmol/l) and testosterone (2.6 ± 0.6 mmol/liter) were subnormal, indicating compliance with withdrawal from replacement treatment. GH administration resulted in a significant increase ($P < 0.05$) in circulating IGF-I level (Fig. 1, *left*). At the end of 4 wk GH treatment, plasma testosterone still remained subnormal at 1.8 ± 0.7 mmol/liter. GH treatment significantly increased plasma FFA from 0.57 ± 0.07 to 0.73 ± 0.09 mmol/liter ($P < 0.05$) (Fig 1, *middle*). GH stimulated whole-body fat oxidation ($P < 0.05$) (Fig. 1, *right*).

Identification of differentially expressed genes after GH treatment

Of the 44,000 probes (Agilent G4112F arrays) analyzed, 41,201 probes representing 28,791 genes were detected on at least two arrays. The distribution of unadjusted *P* values (Fig. 2A) was markedly shifted toward low *P* values, with some 3918 genes with $P < 0.05$, far exceeding 2060 (the expected number according to random chance), indicating that GH had a significant effect. A total of 246 genes were differentially expressed in adipose tissue after GH treatment (FDR < 0.05), of which 135 were up-regulated and 111 down-regulated. These genes are represented as *open circles* in Fig. 2B, which compares the FDR and log-base-2 fold change of each gene, indicating that all differentially expressed genes have fold change larger than 1.9-fold, with an average of 9.3-fold. Among the up-regulated genes, the fold change ranged from 218-fold for the gene Zic family member 1 (*ZIC1*) to 1.8-fold for the gene splicing factor proline/glutamine-rich (*SFPQ*). Among the down-regulated genes, there was a maximal 80-fold reduction for the gene chordin-like 2 (*CHRDL2*) and minimal 1.7-fold reduction for the gene serine/threonine kinase 24 (*STK24*). A complete list of GH-responsive genes is attached separately as supplemental data (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Functional profiling of GH-responsive genes

No significant cluster of enriched functions, in connection with current knowledge of GH biological process, was identified by gene set enrichment analysis (data not shown). Further analysis of GH-regulated individual genes, based on their potential function, revealed groups of genes with enriched functions. GH-responsive genes with significant biological function annotation are listed in Table 1.

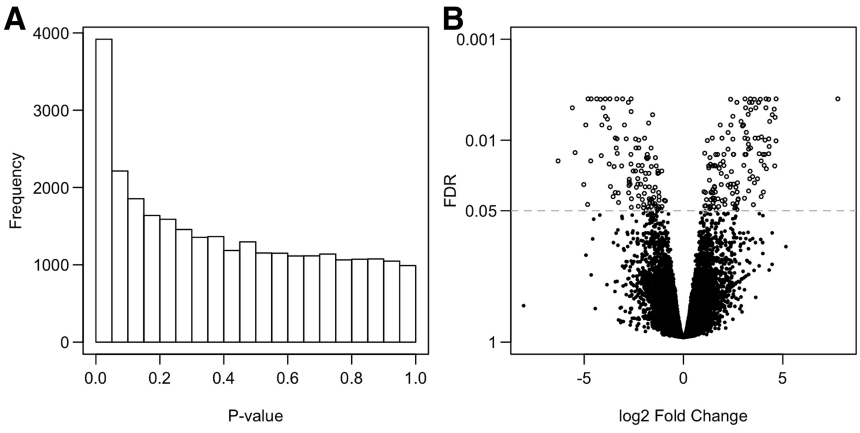

FIG. 2. Gene expression profiles of adipose tissue were measured using microarray before and after GH treatment. Differentially expressed genes were identified using a moderated *t* test producing *P* values that which were then corrected for multiple testing using the FDR (see *Subjects and Methods*). A, Histogram of *P* values (unadjusted for multiple correction); B, volcano plot comparing the log-base-2 fold change of each gene with its FDR (−log scale); ○, genes with FDR below 0.05. Positive values for log fold change indicate genes up-regulated due to GH treatment, and vice versa.

Cellular action

GH increased the expression of *SOCS3* and *CISH* by 6.1- and 3.7-fold, respectively. The expression level of other suppressor of cytokine signaling (SOCS) genes did not change significantly. No significant change in expression of any of the signal transducer and activator of transcription (STAT) genes, a family of transcriptional fac-

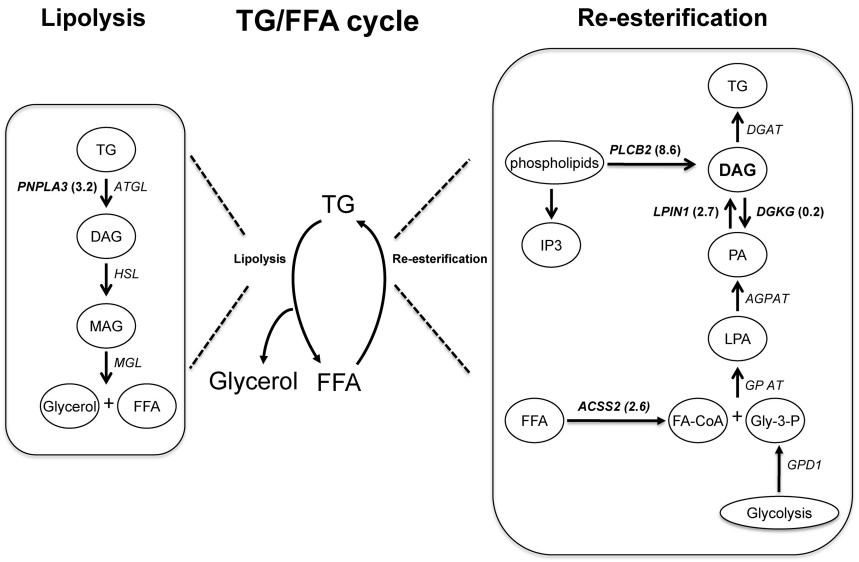

FIG. 3. Schematic representation of the TG/FFA cycle. Within adipocytes, TG is hydrolyzed to glycerol and FFA, which is released into plasma. Nearly 40% is recycled by esterification to TG. The biochemical processes of lipolysis and reesterification are expanded in detail in *flanking boxes*. Genes in *bold* are differentially expressed with *numbers in parentheses* denoting fold change. AGPAT, 1-Acyl-*sn*-Gly-3-P acyltransferase; DGAT, DAG acyltransferase; GPAT, Gly-3-P acyltransferase; IP3, inositol 1,4,5-trisphosphate; LPA, lysophosphatidic acid; MAG, monoacylglycerol; PA, phosphatidic acid.

tors that plays a central role in GH signaling, was detected (data not shown). Early response genes are activated rapidly in response to a wide variety of cellular stimuli. Among this family of genes, GH enhanced the expression of *FOS*, *FOSB*, *EGR1*, and *JUN* with a substantial transcriptional stimulation of *FOS* and *FOSB* in excess of 10-fold (Table 1). GH induced the expression of *IGF1* by 2.7-fold. No significant differential expression was detected for genes encoding any member of the IGF-binding protein family (data not shown).

Lipid metabolism

Lipolysis

GH treatment did not significantly change the expression of genes encoding key enzymes governing lipolysis, including adipose TG lipase (*ATGL*), *HSL*, and monoglyceride lipase (*MTGL*) (Fig. 3). GH enhanced expression of the gene *PNPLA3* by 3.2-fold. PNPLA proteins, designated as patatin-like phospholipase domain containing proteins, are a mammalian family of lipid hydrolases that play critical role in lipid hydrolysis (see *Discussion*). GH suppressed the expression of the gene cell death-inducing DFFA-like effector A (*CIDEA*) by 2.8-fold. *CIDEA* is a recently discovered gene playing a significant role in the regulation of apoptosis, lipolysis, and lipid droplet formation (see *Discussion*). GH treatment did not change the expression of the gene *LPL* (lipoprotein lipase).

Triglyceride biosynthesis

Triglyceride biosynthesis in adipose tissue involves esterification of FFA to a glycerol backbone (Fig. 3). FFA is derived from lipolysis or from hydrolysis of circulating lipoproteins by lipoprotein lipase (LPL). It is converted to fatty acid coenzyme A (FA-CoA) before esterification to glycerol-3-phosphate (Gly-3-P). GH did not change significantly the expression of *LPL* or genes involved in FA transport, such as *CD36*, and fatty acid-binding protein 4 (*FABP4*). It did not induce any change in expression of Gly-3-P dehydrogenase 1, the enzyme involved in the production of Gly-3-P from glycolysis (Fig. 3).

GH significantly induced differential expression of several enzymes involved in esterification of Gly-3-P (Fig. 3). GH increased the expression of *ACSS2* by 2.6-fold, which catalyzes the conversion of FFA to FA-CoA. As shown in Fig. 3, GH increased the expression of *LPIN1* and *PLCB2* and repressed that of *DGKG*. *LPIN1* encodes for phosphatidic acid phosphatase 1, which catalyze the conversion of phosphatidic acid to diacylglycerol (DAG). *PLCB2* catalyzes the hydrolysis of phospholipids to generate DAG and inositol 1,4,5-trisphosphate. *DGKG* phosphorylates DAG to produce phosphatidic acid. Overall, GH regulated genes enhance the pool of DAG in adipose tissue.

Adipogenesis

Transcription factors peroxisome proliferator activated receptor γ (PPARγ), CCAAT/enhancer binding protein, α (CEBPα), and sterol regulatory element-binding protein (ADD1/SREBP) are established transcriptional regulators of adipogenesis. None of these transcripts changed significantly with GH treatment. However, GH reduced by 3.1-fold the expression of hydroxysteroid 11-β dehydrogenase 1 (HSD11β1), an enzyme that converts inactive cortisone to active cortisol.

Others

Extracellular matrix

GH induced differential expression of several genes encoding components of the ECM (Table 1). Most of these were suppressed except for laminin, α 3 (*LAMA3*), which increased by 2.8-fold. These ECM components include members of glycoproteins, integrins and thrombospon-

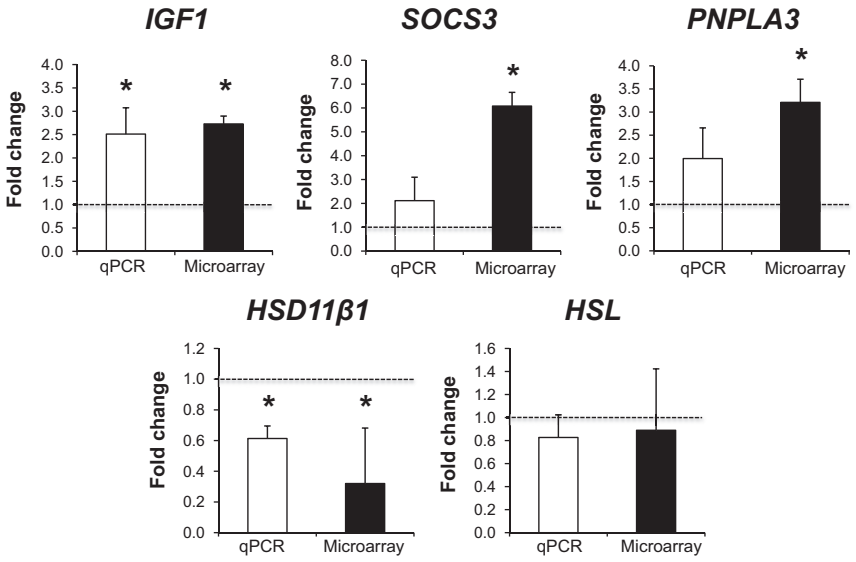

FIG. 4. Microarray results and qRT-PCR verification of selected genes. Results are displayed as fold change (mean ± SE) denoting the expression ratio of GH-treated samples *vs.* baseline. *Dotted lines* at the level of fold change = 1 indicate no expression changes between GH-treated samples and baseline. *, $P < 0.05$ *vs.* baseline.

din, adhesion proteins, and tenascin-C as well as members of the collagen family, *COL4A6*. ECM complex serves important roles in cell migration, adhesion, proliferation, differentiation, and inflammation.

TGF-β signaling pathway

GH significantly suppressed the expression of several genes involved in the TGF-β signaling pathway, including *INHBA*, *LTBP1*, *LTBP2*, and *PPP2R2B*. The protein encoded by *INHBA* is a subunit of both activin and inhibin. Inhibin is a growth/differentiation factor, whereas *LTBP1* and *LTBP2* belong to the family of latent TGF-β binding proteins, which target latent complexes of TGF-β to the ECM, where the latent cytokine is subsequently activated. *PPP2R2B* belongs to the phosphatase 2 regulatory subunit B family, implicated in the negative control of cell growth and division.

Verification by qRT-PCR

The transcript changes of five genes were quantified by qRT-PCR (Fig. 4). Sufficient material was available for qRT-PCR from five of the eight subjects only. The transcript changes measured by qRT-PCR and those obtained from microarray analysis for the same gene demonstrated high similarity. As shown in Fig. 4, there was concordance between the two methods with *IGFI, SOCS3*, and *PNPLA3*, which were up-regulated, and *HSD11β1*, which was down-regulated, although the changes by qRT-PCR did not reach statistical significance for *SOCS3* and *PNPLA3*. The gene *HSL* did not show differential expression in qRT-PCR in concordance with the microarray data.

Discussion

The aims of this study were to identify GH-responsive genes regulating lipid metabolism in adipose tissue, in particular genes regulating lipolysis. We used whole-genome microarray technique to study gene expression profiles of adipose tissue in a group of well-characterized hypopituitary men before and after GH treatment for 1 month. In this study, GH increases expression of *SOCS3* gene and induced concordant changes in circulating IGF-I and IGF-I expression in adipose tissue. GH increased circulating FFA, and in adipose tissue obtained concomitantly, it increased expression of PNPLA3, a novel TG hydrolase, but not of classical TG hydrolases including HSL. GH repressed CIDEA, a novel lipid droplets-associated storage protein. GH differentially regulated genes promoting diacylglycerol syntheses. GH suppressed HSD11β1, which activates local cortisol production and genes encoding components of ECM and TGF-β signaling pathway.

The cellular action of GH is mediated principally by the Janus kinase (JAK)-STAT pathway, the activity of which is terminated by SOCS proteins. In our study, GH significantly increased expression of two members of the SOCS family, namely SOCS3 and cytokine-inducible SH2-containing protein (CISH), in adipose tissue, providing evidence of a positive direct effect of treatments. Our data showed that GH enhanced the expression of IGF-I in adipose tissue. Animal and cell culture studies have reported *GHR* signaling and IGF-I expression in adipose tissue in response to GH (14). In agreement with our study, a recent study on GH signaling in humans also reported increased SOCS3 and IGF-I mRNA in adipose tissue from normal subjects after GH infusion using qRT-PCR (15). It provides evidence that GH elicits an effect on adipose tissue. This may be indirect as well as direct.

Lipolysis, which results in an increase in plasma FFA, is a robust metabolic action of GH. Our microarray data, however, revealed no significant effect on any of the key genes involved in the classical lipolytic pathway, *e.g. ATGL*, *HSL*, or *MTGL* (Fig. 3). Due to the small amount of tissue sample, we were unable to study whether a change in the protein level and/or phosphorylation state of these genes could explain the stimulation of lipolysis. However, we found GH increased the expression of PNPLA3, a novel adipocyte protein. PNPLA3 is predomi-

nantly expressed in human liver and adipose tissue (16). It encodes 481 amino acids and, among the classical TG hydrolases, has the greatest homology with ATGL, suggesting that these two enzymes share similar function in TG hydrolysis (17, 18). Indeed, PNPLA3 displays potent lipolytic activity *in vitro* (19, 20). Taken together, we speculate that GH stimulates lipolysis in part by enhancing expression of PNPLA3 (Fig. 3).

We observed that GH significantly repressed the expression of *CIDEA*. Initially characterized as a mitochondrial activator of apoptosis, it has emerged as an important negative regulator of lipolysis and energy balance. CIDEA is found in high abundance in human white adipose tissue (21). In obesity, CIDEA levels are low and associated with increased basal lipolytic activity, both of which normalize after weight reduction (21, 22). Depletion of CIDEA by RNA interference markedly elevates lipolysis in human adipocytes (23). CIDEA colocalizes around lipid droplets with perilipin and enhances TG deposition in adipocytes (24). It greatly increases lipid droplet size when ectopically expressed (24). Deletion of CIDEA in mice causes leanness and resistance to diet-induced obesity and diabetes (25). The collective evidence indicates that CIDEA inhibits lipolysis and increases TG storage. Therefore, suppression of *CIDEA* may explain in part the facilitation of lipolysis by GH. Based on the changes in expression profiles, we hypothesize a mechanistic pathway for the stimulation of lipolysis by GH (Fig. 5). We propose that GH reduction of CIDEA expression leads to suppression of TG deposition in the cell and facilitates the availability of TG for hydrolysis by PNPLA3.

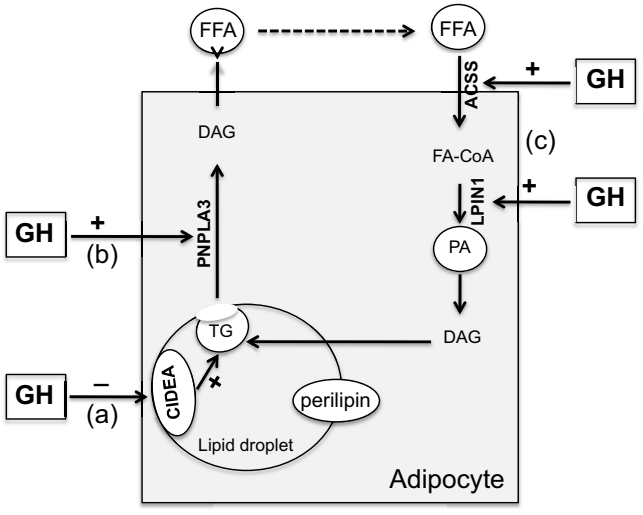

FIG. 5. Schematic representation of GH regulation of the TG/FFA cycle. GH reduction of CIDEA expression leads to destabilization of TG deposition in the cell (a), facilitating the availability of TG for hydrolysis by PNPLA3 (b). Concurrently, GH stimulation of ACSS2 and LPIN1 enhance TG biosynthesis (c) for the coordinated stimulation of the TG/FFA cycle. ATGL, Adipose TG lipase; PA, phosphatidic acid.

Our findings of an up-regulation of *ACSS2*, *LPIN1*, and *PLCB2* and down-regulation of *DGKG* (Fig. 3) suggest that GH may also enhance TG biosynthesis by increasing the pool of DAG. These findings are supported by the report that GH stimulated the formation of DAG in rat hepatocytes (26) and in mouse preadipocytes (27). The stimulation of DAG formation by GH was accompanied by an increase in levels of phosphocholine, suggesting mediation by phospholipase C, which hydrolyzes phosphatidylcholine (phospholipid) to phosphocholine (27). In a clinical study of fatty acid metabolism, withdrawal of GH from GH-deficient adults resulted in a fall in glycerol and FFA, a change consistent with a reduction in lipolytic activity (28). The fall occurred concurrently with a proportionate reduction in FFA uptake, suggesting that GH-induced dynamics of FFA release and reesterification are linked. Thus, the stimulatory effect of GH on genes involved in lipolysis and in TG synthesis suggests coordinated regulation of the TG/FFA cycle (Fig. 5).

We observed a potent suppression of *HSD11β1* mRNA by GH in adipose tissue. This finding confirms the data in a previous randomized placebo-controlled double-blind study in GH-deficient patients that HSD11β1 mRNA is reduced by 66% in adipose tissue 4 months after GH treatment (29). Two isoenzymes of 11β-hydroxysteroid dehydrogenases (HSD11β1 and HSD11β2) catalyze the interconversion of inactive cortisone and active cortisol. Reduction of *HSD11β1* mRNA transcripts, if translated to a reduction in enzyme expression, will reduce adipocyte generation of active cortisol from cortisone. Studies in adipose stromal cells demonstrate that local metabolism of glucocorticoid via HSD11β1 activity regulates adipocyte differentiation and visceral fat mass (30, 31). In animal studies, transgenic mice that overexpressed *HSD11β1* selectively in adipose tissue develop visceral obesity (32). Conversely, adipose-specific *HSD11β1* knockout mice exhibit reduced visceral fat (33). The observation from clinical studies supports a positive association between fat mass with adipose tissue HSD11β1 mRNA expression and activity (34, 35). Taken together, the evidence that GH suppresses HSD11β1 is strong, and this effect is likely to inhibit adipocyte differentiation and growth.

There is increasing evidence that the ECM plays a significant role in regulating a range of tissue functions including cell signaling, differentiation, and migration. A recent study in obese individuals has reported that up-regulation of ECM genes predispose to an inflammatory response, and the overexpression of these genes was reversed upon weight reduction (36). This observation suggests that down-regulation of the ECM transcriptions, such as that after GH, may reduce inflammation in adipose tissue. There is some evidence linking GH to a dampening

of inflammatory status. Circulating markers of inflammation are reduced after GH therapy in GH-deficient adults (37) and in obese postmenopausal women (38). A possible related observation in our study is the significant downregulation of genes involved in the TGF-β signaling pathway given the role of TGF-β in inflammation (39). The implications of ECM transcriptional repression in response to GH action are unknown; however, available evidence suggests a favorable influence on adipose tissue inflammation.

One limitation of this study is that it was not placebo controlled. The small sample size is also a weakness. Another limitation is the focus on gene transcript changes to provide mechanistic insights into the action of GH on adipose tissue. Changes in mRNA levels do not necessarily reflect a parallel alteration in proteins or biological function. For example, we found no significant change in LPL expression in contrast to Richelsen *et al.* (40), who observed an increase in LPL activity after GH treatment in obese women. Due to the limited amount of biopsy samples, we were not able to investigate parallel changes in protein expression or enzyme activity. Another limitation of this study design is that it does not distinguish acute (*e.g.* hours) from longer-term (*e.g.* weeks) transcript changes. However, the fat samples were obtained at the same time as all blood samples and metabolic measurements.

In summary, GH regulates the expression of novel genes that enhance TG hydrolysis, reduce TG storage, and promote DAG synthesis. Within adipose tissue, GH represses the expression of *HSD11β1*, an effect expected to reduce local cortisol synthesis, and represses genes encoding components of ECM and TGF-β signaling pathway. We conclude that GH stimulates the TG/FFA cycle in adipose tissue where it also represses adipocyte growth, differentiation and inflammation.

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