Tissue-Specific Increase Of Insulin-Like Growth Factor I Gene Expression In Human Adipose Tissue After Abdominal Surgery

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Citation

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Abstract

The growth hormone (GH)/insulin-like growth factor I (IGF-I) axis in catabolic conditions is believed to be severely altered, based on measurements of biochemical markers in plasma and clinical observations. To test the hypothesis that GH sensitivity may be differentially regulated in different tissues, we analysed GH receptor (GHR) and IGF-I gene expression in adipose tissue and skeletal muscle, before and after major surgery, and compared it with the corresponding gene products in plasma, IGF-I and GH-binding protein (GHBP).

A significant increase ($p<0.05$) in IGF-I mRNA levels was found in adipose tissue after surgery whereas there were no changes in IGF-I mRNA levels in skeletal muscle or IGF-I concentrations in plasma. Furthermore, GHR mRNA levels did not change after surgery whereas GHBP in plasma decreased postoperatively ($p<0.01$).
This study shows that catabolism induced by surgical trauma is associated with differential regulation of IGF-I gene expression in adipose tissue and skeletal muscle and that plasma measurements of IGF-I and GHBP may not accurately reflect changes in gene expression in different tissues. These observations may have implications in the planning and conduction of clinical studies in catabolic states.

Ruth Wickelgren and Gunnel Hellgren contributed equally to this manuscript and should both be considered first authors.

INTRODUCTION

It is generally believed that a wide range of catabolic conditions, including surgical stress, sepsis and burns, are associated with acquired growth hormone (GH) resistance and a reduced anabolic response to exogenous GH ([1]). In humans, these assumptions have mainly been based on clinical observations and changes in biochemical markers in plasma, for example decreased levels of insulin-like growth factor I (IGF-I) and GH-binding protein (GHBP) in conditions associated with increased or normal GH secretion ([1]). Little is known, about alterations in GH sensitivity in specific tissues in catabolic conditions, mainly due to the difficulty of obtaining tissue samples from patients. However, we have previously shown decreased GH receptor (GHR) mRNA levels ([2]) and decreased gene expression of IGF-I ([3]) in skeletal muscle in response to surgical stress, indicating a decreased sensitivity for GH in this tissue after abdominal surgery. Furthermore, studies in rats show decreased GHR levels in liver and decreased serum concentrations of IGF-I after induction of sepsis, indicating GH resistance mediated by a reduced amount of hepatic GHR ([4,5]).

In an attempt to improve protein synthesis and preserve lean body mass, high doses of GH have been used in clinical trials ([6]). However, two large multicentre placebo-controlled studies in patients in intensive care with acute critical illness showed that administration of high doses of GH increases mortality and morbidity ([7]), indicating a more complex picture of acquired GH resistance than previously assumed. For example, high doses of GH may be required to obtain beneficial effects in some tissues, such as skeletal muscle, where GH sensitivity seems to be blunted in catabolic patients, whereas an increase in GH responsiveness in other

tissues could be deleterious. This is difficult to demonstrate, as the origin of biochemical markers, such as plasma IGF-I and GHBP, cannot be determined in ordinary plasma measurements.

We therefore measured GHR and IGF-I gene expression in adipose tissue and skeletal muscle to study changes in GH sensitivity in these two important target tissues. The changes were compared with changes in corresponding plasma protein concentrations, i.e. GHBP and IGF-I, in addition to changes in IGF-binding protein 3 (IGFBP-3), the major binding protein for IGF-I in plasma.

METHODS

SUBJECTS AND SAMPLES

Six women and four men undergoing elective abdominal surgery at St Göran Hospital, Stockholm, Sweden were included in the study. None of the patients had systemic illness or was taking medication that could effect metabolism, were included in the study. The study was approved by the Ethics Committee of Karolinska Institute, Stockholm, Sweden. The procedure for the study, the possible discomfort and the risks involved were explained to the patients, and their informed consent was obtained before participation in the study. Clinical characteristics of the patients are given in Table 1.

Figure 1

Table 1: Clinical characteristics of the patients.

Patient No.	Sex	Age (yrs)	BMI	Type of surgery	Blood loss (mL)
1	Female	70	20.0	Right-sided hemicolectomy	100
2	Female	71	25.4	Ileocaecal resection	150
3	Female	78	20.9	Sigmoid resection	150
4	Female	71	25.0	Sigmoid resection	150
5	Male	52	23.3	Sigmoid resection	200
6	Male	75	25.1	Sigmoid resection	200
7	Male	74	23.5	Left-sided hemicolectomy	1100
8	Female	45	21.6	Anterior resection of rectum	1400
9	Male	73	27.9	Left-sided hemicolectomy	300
10	Female	74	19.0	Sigmoid resection	100

All sampling was performed after an overnight fast. Preoperatively, after induction of anaesthesia (day 0), a biopsy was taken from subcutaneous abdominal adipose tissue and a muscle biopsy was taken from the lateral portion of the quadriceps femoris using the percutaneous needle biopsy technique. Three days after surgery (day 3), a second adipose tissue biopsy was taken under local anaesthesia confined to the skin, and a second muscle biopsy was taken under local anaesthesia confined to the skin and fascia only. The local anaesthesia did not contain adrenaline. At the time of the biopsies, fasting blood samples were drawn from the antecubital vein for determination of GHBP, IGF-I and IGFBP-3. Tissue samples were frozen in liquid nitrogen and stored at –80 °C, and plasma was stored at –20 °C until analysis. The patients were given standardized postoperative parenteral nutrition as a continuous infusion, containing 0.15 g nitrogen/kg/day and including a balanced amino acid solution not containing glutamine. Energy was provided as glucose and fat (10% glucose and 20% Intralipid; Pharmacia & Upjohn, Stockholm, Sweden), given at 1.2 times the calculated need according to the Harris-Benedict formula. During the day of surgery, 75% of this nutritional regimen was given.

MEASUREMENTS OF GHR, IGF-I AND CYCLOPHILIN MRNA

Total RNA was isolated from the biopsies using guanidium thiocyanate-phenol-chloroform extraction essentially as described by Chomczynski and Sacchi ([8]). GHR, IGF-I and cyclophilin mRNA levels were measured by competitive quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR) based assays as described previously ([2,3]). The intra-assay coefficients of variation of the Q-RT-PCR assays were 10%, 12% and 16% for GHR, IGF-I and cyclophilin, respectively. For each patient, samples from day 0 and day 3 were measured in the same Q-RT-PCR assay and, for each sample, aliquots of the same cDNA synthesis were used as templates in PCR amplification of GHR, IGF-I and cyclophilin genes. However, only seven and eight samples were available for IGF-I mRNA measurements in skeletal muscle and adipose tissue, respectively.

5'-RAPID AMPLIFICATION OF CDNA ENDS (5'-RACE)

5'-untranslated regions (5'-UTRs) of the GHR transcripts were reverse transcribed and amplified from adipose tissue and skeletal muscle RNA obtained from patient numbers 3, 5 and 9 on days 0 and 3 using a SMART™ RACE cDNA amplification kit (Clontech, Palo Alto, CA, USA) according to the protocol supplied by the manufacturer. To select for

transcripts containing sequences upstream of exon 2, the PCR sample was diluted 1:1000 and 2.5 µl of the diluted sample were used as template in a half-nested PCR. The half-nested PCR was performed as described above, using the universal primer mix and a nested GHR-specific primer (5'-GCAGCTGCCAGAGATCCATACCTGT-3') which included six bases upstream of the ATG translation initiating site. Amplified cDNA fragments were subcloned into pCR 2.1 (Invitrogen, San Diego, CA, USA) and sequenced using the ABI Prism big dye terminator cycle sequencing ready reaction kit (Applied Biosystems Division, Perkin Elmer, Foster City, CA, USA).

CROSS-SPECIES ANALYSIS

Regions that are evolutionary conserved are thought to be important for gene regulation and function. In order to address this issue regarding the 5-UTRs of the GHR we performed a cross-species analysis between human and mouse. We compared both the human (Homo sapiens chromosome 5 clone RP11-116B13 and contig GA_x54KREALWUN) and mouse (contig 15.3000001-4000000 and contig GA_x6K02T2N9RL) sequences from the public database (Ensembl) and Celera using the MultiPipMaker tool (http://bio.cse.psu.edu/cgi-bin/multipipmaker). For this analysis, human repetitive sequences were first masked with the RepeatMasker program (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker/) ([9]).

IMMUNOASSAYS

Plasma levels of GHBP were measured by a ligand-mediated immunofunctional assay as described previously ([10]). All samples were measured in the same assay, and the intra-assay coefficient of variation was 7%. The reagents were kindly provided by Genentech Inc, San Francisco, CA, USA.

Plasma levels of IGF-I and IGFBP-3 were measured by radioimmunonoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). IGF-I was separated from binding proteins using an acid-ethanol and alkaline precipitation step. The intra,- and interassay coefficients of variation were less than 10% in the concentration range measured in the study.

STATISTICAL ANALYSIS

For evaluation of changes from day 0 to day 3 after surgery, Wilcoxon's signed rank test was used. Correlations between changes of GHR and changes of IGF-I gene expression in adipose tissue and skeletal muscle were sought by calculating Spearman's rank correlation coefficient (r_s) using the difference (day 0-day 3) in gene expression before and after surgery. Changes were considered significant if $p<0.05$. Data are expressed as mean(SD).

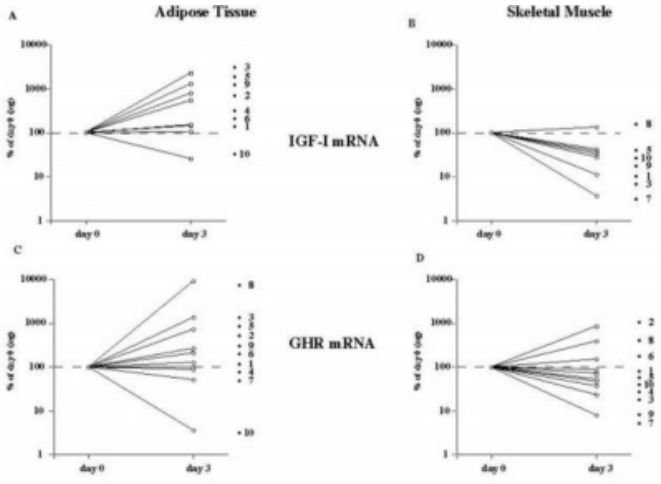
RESULTS

EFFECT OF SURGICAL TRAUMA ON IGF-I AND GHR GENE EXPRESSION

To investigate the effect of surgical trauma on IGF-I and GHR gene expression in different GH target tissues, gene expression was measured in adipose tissue and skeletal muscle. While IGF-I mRNA levels increased significantly on day 3 compared with day 0 in adipose tissue (Fig. 1a, $p<0.05$), there was no significant change in skeletal muscle (Fig. 1b, $p=0.09$), although the IGF-I mRNA levels were lower in six out of seven patients after surgery in this tissue. GHR gene expression did not change significantly after surgery either in adipose tissue (Fig 1c, $p=0.44$) or in skeletal muscle (Fig. 1d, $p=0.72$).

Figure 2

Figure 1: IGF-I (A, B) and GHR (C, D) gene expression in adipose tissue (A, C) and skeletal muscle (B, D) from patients undergoing abdominal surgery. Samples were obtained on day 0 and day 3 after surgery. Data shown are IGF-I or GHR transcripts per cyclophilin transcripts. The mRNA levels on day 0 are given as 100% and the values on day 3 are expressed as a percentage of the values on day 0. Numbers 1-10 refer to the individual patients (Table 1).

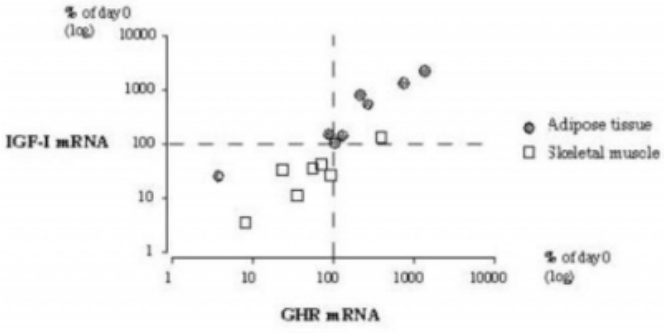


In order to examine whether changes in GHR could contribute, at least partly, to the changes of IGF-I gene expression, changes in GHR mRNA levels were plotted against the changes in IGF-I mRNA levels (Fig. 2). In both

adipose tissue and skeletal muscle, the postoperative changes in GHR mRNA levels correlated with the postoperative changes in IGF-I mRNA levels (r_s=0.88, p<0.05 and r_s=0.86, p<0.05, respectively).

Figure 3

Figure 2: Changes in GHR mRNA levels plotted against changes in IGF-I mRNA levels in adipose tissue and skeletal muscle from patients undergoing abdominal surgery. Samples were obtained on day 0 and day 3 after surgery. Data shown are IGF-I or GHR transcripts per cyclophilin transcripts on day 3, expressed as a percentage of the values on day 0 for each patient.

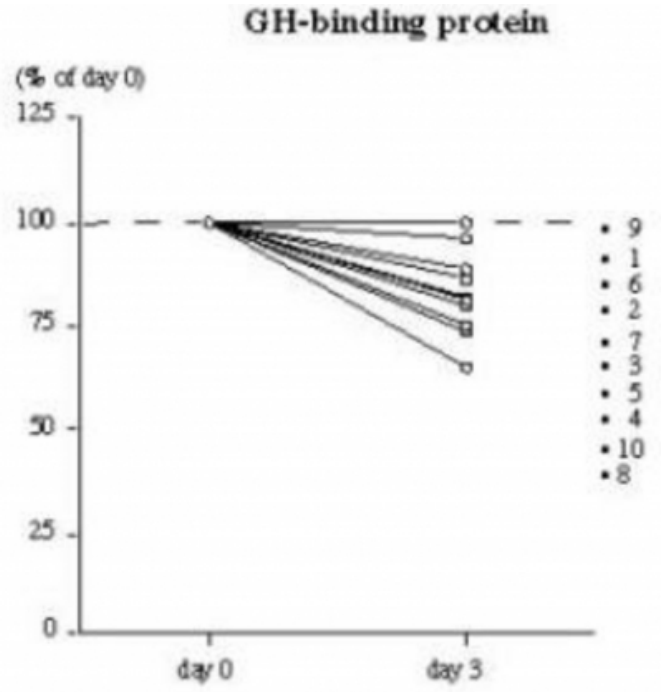


PLASMA LEVELS OF GHBP, IGF-I AND IGFBP-3

To compare the changes of IGF-I and GHR gene expression in the investigated tissues with the corresponding gene products in plasma, plasma concentrations of GHBP and IGF-I were measured in blood samples taken on day 0 and day 3. Plasma concentrations of IGFBP-3 were also measured. In contrast to the GHR gene expression in adipose tissue and skeletal muscle, where no significant changes occurred, the plasma concentration of GHBP was significantly reduced on day 3 compared with day 0 [116.0(47.3) pmol/l vs 142.5(58.5) pmol/l, p<0.01;Fig. 3]. However, plasma levels of IGF-I and IGFBP-3 did not change significantly from day 0 to day 3 [123.4(76.0) µg/l vs 160.5(111.2) µg/l, p=0.09] and [2.1(0.75) mg/l vs 1.9(0.76) mg/l, p=0.11, respectively].

Figure 4

Figure 3: Plasma concentrations of GHBP in patients undergoing abdominal surgery. Concentrations of GH-binding protein were measured on day 0 and day 3 after surgery. The GHBP concentration on day 0 is given as 100% and the values on day 3 are expressed as a percentage of the values on day 0. Numbers 1-10 refer to the individual patients (Table 1).



IDENTIFICATION OF GHR 5'-UTRS CROSS-SPECIES ANALYSIS OF THE GHR GENE

The correlation between the changes in IGF-I gene expression and the changes in GHR gene expression suggests that the GHR expression could have influenced IGF-I gene expression. Previous studies have shown that the GHR contains multiple alternative exon 1 ($_{11,12,13}$) (GeneBank AF230800; GeneBank AF230801) and most of these are likely to be driven by unique promoters. In order to obtain some information about the GHR promoter regions of importance for adipose tissue and skeletal muscle we first identified the alternative exon 1:s by 5´-RACE and then analyzed the promoter region for putative binding-sites for transcription factors. The sequences we found consisted of both previously described (V2, V3 and V9) and a novel GHR 5'-UTR, here named V10 (Genebank AY216680). The predominant GHR 5'-UTR in both adipose tissue and skeletal muscle was V2. No obvious difference was found in the expression of different GHR 5'-UTRs before and after surgery or between tissues (data not shown).

By comparing the genomic 5' region of the GHR gene retrieved from Ensembl with the corresponding region from Celera we show that the region containing V2, V3 and V9 is located at least 247 kb upstream of the start codon of the GHR gene. The novel exon, V10, is located at least 187 kb upstream the translation start site between the regions (V8, V4, V1 and V7) and (V2, V3 and V9) (Fig. 4A).

Figure 5

Figure 4a: Genomic organisation and location of the V2, V9 and V3 UTRs and exon 2 in the GHR gene.

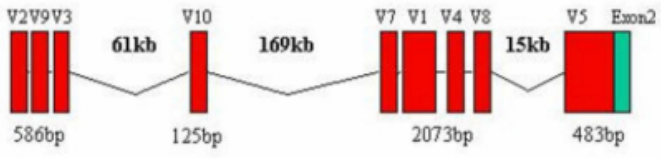


Figure 6

Figure 4b: Cross species comparison using percentage identity plot (pip) between the human and mouse sequences in the region containing V2, V9 and V3 from both the public and Celera database. A scale representing the degree of sequence identity, indicated as percentage, is located to the right of each row. The human sequence from the public database was used as template for the comparison. The abbreviations h and m, indicate human and mouse, respectively.

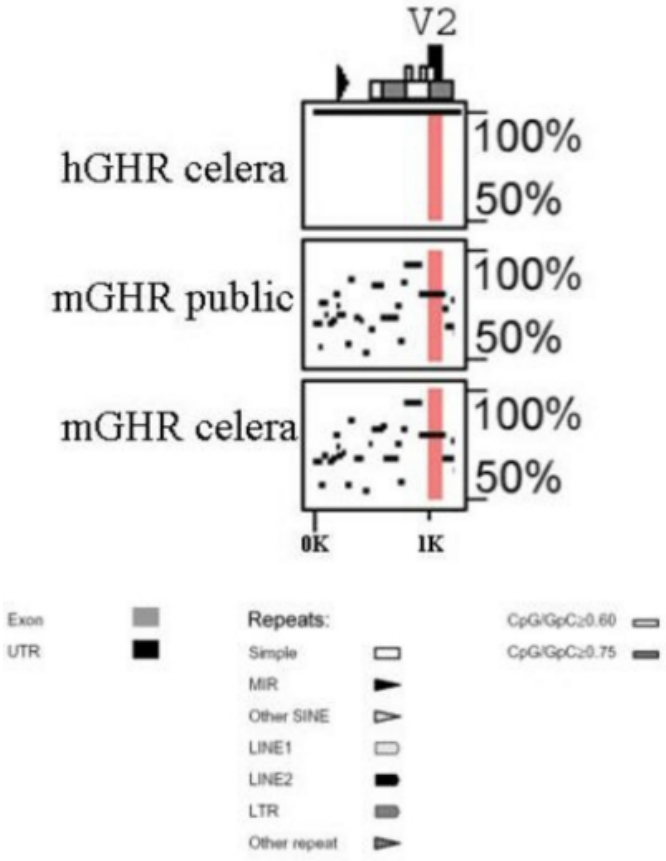
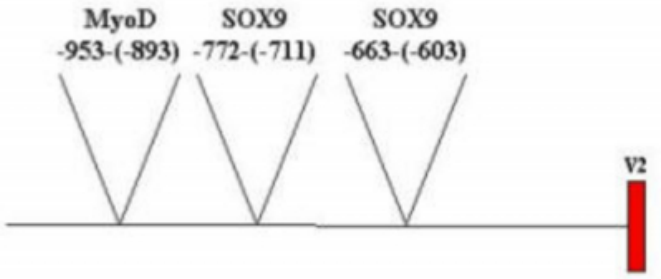


Figure 7

Figure 4c: Identified transcription factor binding sites upstream of the V2 5'-UTR of the GHR gene.



Comparison of the human and mouse GHR genes showed that all the different 5'-UTRs, except V8, are highly homologous between species (Fig. 4B). Since the level of

homology in the regions flanking the UTR regions are high one might speculate that several other 5'-UTRs exist and/or that the promoters are highly conserved. A motif search on the sequence upstream of the most abundant 5'-UTR, V2, revealed transcription binding sites MyoD and SOX9 (Fig 4C).

DISCUSSION

The results of this study indicate that GH sensitivity in man may vary between tissues and suggest that catabolism, induced by surgical trauma, is not associated with generalized GH insensitivity. We have also demonstrated that changes in plasma levels of a biochemical marker may not accurately reflect the changes in local expression in target tissues. Plasma IGF-I levels did not change significantly, which could be interpreted as the GH/IGF-I axis being not affected by the surgical trauma. However, IGF-I gene expression increased in adipose tissue, while it was unchanged in skeletal muscle. Furthermore, plasma GHBP levels decreased significantly, whereas GHR mRNA levels were unchanged in adipose tissue and skeletal muscle. GHBP levels in serum have been proposed to roughly parallel hepatic GHR expression ([14,15,16]). Thus, the results of this study indicate a regulation of hepatic GHR rather than general changes in GHR expression throughout the body.

Most studies of tissue-specific expression in catabolic conditions have been performed in animals ([5, 17,18,19,20,21,22]). Human studies have been hampered by difficulties in obtaining human tissues. Thus, the conclusions have been drawn from animal experiments. However, conclusions from animal studies may not always be extrapolated to humans, as many systems, including the GH/IGF-I system under certain conditions, differ between humans and rodents. For example, starvation blunts GH secretion in rats but leads to increased GH secretion in man ([23,24]). Therefore, data on the GH/IGF-I system generated from animal studies may not always reflect the biological situation in man.

In the present study, we have shown a difference between the local gene expression of IGF-I and GHR in human tissues and plasma levels of corresponding gene products. Plasma levels of IGF-I, IGFBP-3 and GHBP are often used as markers for GH responsiveness. For example, in the investigation of short stature, a variety of IGF-I and IGFBP-3 generation tests exists where IGF-I and IGFBP-3 are measured in plasma before and after GH treatment to evaluate GH responsiveness. Some of these may be informative, although they sometimes have poor reproducibility and low specificity that may limit their usefulness ([25]). Our results suggest that a greater knowledge of the detailed mechanisms of local expression of genes in the GH/IGF-I axis is of importance when interpreting the mechanisms behind changes in the GH/IGF-I axis in catabolic conditions.

Our previous data show that GHR mRNA ([2]) and IGF-I mRNA ([3]) levels were decreased in skeletal muscle in response to surgery and that GH administration prevented a decrease in IGF-I mRNA in skeletal muscle ([3]). These results indicate that GH sensitivity is decreased in skeletal muscle after major surgery, which may explain the observation that high doses of GH are required to obtain anabolic effects on protein metabolism in skeletal muscle in catabolic patients ([26,27,28,29,30]). In the present study, there was an increase in IGF-I gene expression in adipose tissue after abdominal surgery, in contrast to skeletal muscle where no changes were observed. The reason for the increase in IGF-I expression in adipose tissue is not clear. However, there was a correlation between changes in IGF-I gene expression and changes in GHR gene expression, indicating the GHR abundance may affect IGF-1 expression. The importance of the level of GHR expression is supported by the minimal, but discernible, gene dose effect on growth rate shown in heterozygous GHR knock-out mice ([31]) and the observation that mutations in one allel of the GHR gene result in partial GH insensitivity in children ([32]).

For many genes, alternative promoter usage has been observed to be involved in the regulation of gene expression in different tissues and developmental stages ([33,34]). Examination GHR 5'-UTRs in this study indicated that both tissues predominantly used the same variant of exon 1 (V2). A motif search on the sequence upstream of V2 revealed binding sites for two transcription factors, MyoD and SOX9. MyoD is specifically expressed in muscle cells and plays an important role in muscle cell differentiation. Previous studies have shown that the GH/IGF-I system is involved in the regulation of myoD ([35,36]), however, novel studies are needed to understand the possible role of MyoD in regulation of GHR expression in skeletal muscle. Defects in the SOX9 gene result in campomelic dysplasia, a severe dwarfism syndrome, which affects cartilage-derived structures and partial or complete sex reversal ([37,38,39]). This suggests a role for SOX9 in male sexual differentiation, bone formation and longitudinal bone growth. Obviously, lack of functional GHRs results in Laron type dwarfism ([40]). The

identification of a SOX9 cis-element upstream of V2 in the growth hormone receptor gene is interesting and may suggest that Sox9, in fact, is an important regulator of GHR gene expression.

Other ways of influencing the tissue-specific expression of IGF-I may be possible, such as regulation of genes and/or gene products downstream of the GHR. Interestingly, the tissue-specific regulation of IGF-I in this study is in agreement with another study in which rats were infused with tumour necrosis factor-a, a catabolic cytokine ($_{17}$). IGF-I gene expression decreased in skeletal muscle, heart and liver, while it increased in the kidney, indicating a tissue-specific regulation of IGF-I in a catabolic condition. Although the exact mechanism for the increase of IGF-I in adipose tissue in our study is unknown, the results indicate that the IGF-I gene expression is regulated differently in adipose tissue than in skeletal muscle in man after abdominal surgery.

It is possible that GHR mRNA is also affected by the amount of nutrition available, as it decreased postoperatively when hypocaloric nutrition was given ($_{2}$). In the present study, in which an adequate amount of parenteral nutrition was given, this change was not so pronounced postoperatively. This can be explained by a possible influence of nutrition on the degree of decreased GHR gene expression in skeletal muscle seen in catabolic conditions. The importance of well-controlled glucose levels in catabolic conditions has been shown in a recently published study in which a group of patients in intensive care were treated with individual doses of insulin in order to keep glucose levels within the normal range ($_{41}$). This regimen was shown to decrease the morbidity and mortality of these patients.

In summary, we found a tissue-specific regulation of IGF-I gene expression after major surgery, with increased gene expression in adipose tissue and no significant change in skeletal muscle. This may indicate that IGF-I gene expression is differentially regulated in skeletal muscle and adipose tissue in catabolic conditions. Furthermore, there was a discrepancy between changes in GHR and IGF-I gene expression in GH target tissues and changes in plasma levels of their corresponding gene products, GHBP and IGF-I. This illustrates that plasma levels of IGF-I and GHBP may be inadequate as markers of GH sensitivity.

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