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# Growth hormone-mediated alteration of fuel metabolism in the aged rat as determined from transcript profiles

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**Tollet-Egnell, Petra, Paolo Parini, Nina Ståhlberg, Ingrid Lönnstedt, Norman Lee, Mats Rudling, Amilcar Flores-Morales, and Gunnar Norstedt.** Growth hormone-mediated alteration of fuel metabolism in the aged rat as determined from transcript profiles. *Physiol Genomics* 16: 261–267, 2004. First published November 11, 2003; 10.1152/physiolgenomics.00093.2002.—Age-related changes in body composition and serum lipids resemble symptoms of adult-onset growth hormone (GH) deficiency. GH treatment has been shown to normalize these changes in both GH-deficient adult patients and elderly subjects. The aim of this study was to identify GH-responsive genes that might mediate positive effects of GH treatment on fuel metabolism and body composition. cDNA microarrays were used to analyze age- and GH-induced changes in gene expression patterns in male rats. Tissues analyzed were liver, adipose tissue, and skeletal muscle from animals on or off GH treatment. A value of 1.5 was chosen to denote differences (increased or decreased expression) in the level of mRNA expression. In the liver, 7.3% of the expressed genes were affected by age and 6.5% by GH. Similar values for the other tissues were 8.3% and 5.3% (fat), and 7.9% and 9.6% (muscle), respectively. Among the differentially expressed genes, we identified several that encode proteins involved in fuel metabolism. Old rats were shown to have induced expression of genes involved in hepatic glucose oxidation and lipid synthesis, whereas these pathways were reduced in adipose tissue. GH treatment induced the expression of genes for lipid oxidation in liver and for glucose oxidation in skeletal muscle. In adipose tissue, GH reduced the expression of genes involved in lipogenesis even further. Changes in transcript levels were reflected in serum in terms of altered lipid profiles. Serum levels of triglycerides, high-density lipoprotein (HDL) cholesterol, and total cholesterol were higher in the old animals than in the young and normalized by GH treatment.

cDNA microarray; aging; growth hormone; body composition

GROWTH HORMONE (GH) is a pleiotropic hormone of increasing clinical importance, with uses ranging from the treatment of short stature in children to potential slowing of the aging process in the elderly (27). GH is the major regulator of postnatal somatic growth (13, 28), evidenced by the short stature of GH-deficient children, but GH is also affecting various metabolic pathways (2, 8, 15). The symptoms of GH-deficiency in adults thus include the results of altered intermediary metabolism, leading to reduced muscle mass and increased body fat (7, 40). The fact that the secretion of GH progressively decline during aging (7, 16) led Rudman (37) to postulate that senescent changes in body composition and organ function might be causally linked to a decline in GH levels with age. Strong support for this hypothesis was provided by results obtained from studies on elderly subjects treated with GH (38). A number of trials of GH replacement have since then shown that such treatment results in a loss of body fat and an increase in lean body mass (14, 48), reflecting a combination of the lipolytic and anabolic effects of GH. It may also result in a feeling of well being, which is thought to reflect in part increased availability of fuels for physical work, i.e., non-esterified fatty acids and glucose in serum. Furthermore, GH has been implicated to play a protective role in cardiovascular disease, due to its suppressive effect on serum cholesterol levels (29, 36, 46).

The various physiological actions muscle, GH enhances amino acid uptake and protein synthesis (4, 17). Following GH exposure, the stimulation of protein synthesis, in combination with a reduced level of transamination and amino acid oxidation in the liver, attenuates urea excretion and promotes nitrogen retention. GH also has an effect on hepatic glucose production (4), probably involving stimulation of gluconeogenesis. Most of the actions of GH on adipose tissue are to prevent lipid accumulation and to enhance lipid mobilization (33, 34).

Since GH exposure has both anabolic and lipolytic actions, it is not surprising that GH therapy has beneficial effects on body composition. However, the sites of action at the molecular level are still not precisely located, and the available data are sometimes conflicting. It appears to be of great importance to elucidate the molecular mechanisms behind physiological changes exerted by clinically used compounds such as GH. An increased understanding of how GH therapy affects distinct tissues should provide leads for better diagnosis and design of GH analogs with fewer side effects. The major aim of this study was to identify GH-induced changes in gene expression patterns that may explain the overall anabolic and lipolytic actions of GH in old animals. We (44) and others (19–21, 50) have previously shown that the aging process can be monitored at the level of mRNA expression through the application of microarray analysis. In the present study, we have collected transcript profiles from three metabolically important tissues, to enable a simultaneous comparison of tissue-specific transcriptional changes. The effects of aging and daily injections of GH were studied in male rat liver, adipose tissue and skeletal muscle, using cDNA microarrays representing 3,200 genes.

## MATERIALS AND METHODS

*Animals.* Ten old (18 mo of age) and five younger (2 mo of age) male Sprague-Dawley rats, obtained from B&K Universal (Stock-

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holm, Sweden), were maintained under standardized conditions of light and temperature, with free access to animal chow and water. All animals ($n$ = 5 rats/group) were kept in the same environment during the experiment. Five old rats were treated with vehicle only (placebo) and five with recombinant human GH (hGH) by two daily injections (at 8 AM and 5 PM). Young rats were treated with vehicle only. Recombinant hGH, a kind gift from Pharmacia and Upjohn (Stockholm, Sweden), was administered to animals at a daily dose of 1 μg/g body wt. After 1 wk of treatment, the rats were killed between 10 and 11 AM (2 h after the last injection) without prior food withdrawal. Liver, visceral adipose tissue, and soleus muscle were removed and immediately frozen in liquid nitrogen for further analysis. The animal experiments were approved by the institutional animal care and use committee.

*cDNA microarray analysis.* Generation of cDNA microarrays has been described previously (12, 44). The arrays represented ~3,200 cDNA clones selected from the TIGR Rat Gene Index (**http://www.tigr.org**) and our own collection of rat cDNA libraries (42).

Total RNA was isolated by homogenization of tissue specimen using a Polytron PT-2000 (Kinematica, Switzerland) and TRIzol Reagent (Life Technologies), according to the protocol supplied by the manufacturer. The RNA concentration was carefully determined spectrophotometrically, and the quality of the RNA samples was examined on a denaturing agarose gel. The aim of this study was not to investigate individual variations but rather the common changes representative of the whole group. Therefore, equal amounts of total RNA from five animals in the same experiment group were pooled before cDNA labeling. Although pooling of samples might lead to the signals being confounded by mixed individuals, it also minimizes the biological noise. The protocol employed for probe labeling and purification was essentially as described previously (12, 44). Twenty micrograms total RNA was used from each group of animals for each experiment. Labeled cDNA was produced by an oligo-dT primed reverse transcription reaction using SuperScript II (Life Technologies). Oligo-dT primers and Cy-labeled nucleotides were obtained from Amersham Pharmacia Biotech (Sweden). In the first set of experiments, each hybridization compared Cy3-labeled cDNA reverse transcribed from total RNA isolated from young male rats (2 mo of age), with Cy5-labeled cDNA isolated from old males (18 mo of age). In another set of experiments, Cy3-labeled cDNA derived from tissues of nontreated old animals was compared with Cy5-labeled cDNA from old animals that had been treated with hGH for 1 wk. Each experiment was analyzed in triplicate. In addition, an extra round of hepatic RNA isolation and labeling was performed including dye swaps. This particular analysis utilized a 6,200-cDNA array, including the 3,200 gene products on the smaller array. Results obtained using hepatic RNA were therefore derived from seven independent hybridizations. The labeled and purified cDNA was added to the array and put in a sealed hybridization chamber (Corning, NY), as described before (12, 44). After the hybridization, which took place at 65°C for 15–18 h, the array was washed and dried.

The array was scanned using a GMS 418 scanner (Affymetrix). Image analysis was performed using the GenePix Pro software (Axon Instruments). Automatic flagging was used to localize absent or very weak spots (less than 1.4 times above background), which were excluded from analysis. Fluorescence ratios were normalized independently for each array by applying a single scaling factor to all fluorescent ratios from each array, as described (1). This scaling factor was computed so that the median fluorescence ratio of well-measured spots (>1.4 times the background) on each array was 1.0. A gene was considered expressed if its signal was >1.4 times the background in two of three replicated measurements. The total number of expressed genes for each tissue was calculated as the mean number of expressed genes in the two different experimental situations (aging and GH treatment of old rats). The significance of the expression ratios of both age and GH replacement studies were estimated using the significance analysis of microarray (SAM) technique (45). A $q$ value was assigned for each of the detectable (expressed) genes in the array. This value is similar to the familiar $P$ value, measuring the lowest false discovery rate (FDR) at which the differential expression (the ratio between control and experimental cDNA) of a gene is called significant. In this study, genes with a $q$ value of less than 10% were considered significantly differentially expressed. To this statistically based criterion, a further requirement was added for differential expression based on the absolute changes in gene expression ratios. A value of 1.5 was chosen to denote differences (increased or decreased expression) in the level of hybridization between control and experimental cDNA. This cutoff was empirically chosen based on previous validation studies using different independent techniques. We and others have shown that Cy5/Cy3 ratios around 1.5 correspond to higher ratios when calculated using results obtained from RNase protection or Northern blot analysis (11, 12, 30, 44). In terms of fold regulation, DNA array analysis thus tended to underestimate differences compared with RNase protection assay. Genes listed as differentially expressed in Tables 1, 2, and 4 thus meet both the criteria of having a $q$ value less than 10% and having a Cy5/Cy3 (upregulated) or a Cy3/Cy5 (downregulated) ratio larger than 1.5. The whole set of data is available at **http://www.cmm.ki.se/endoged**.

*Determination of serum lipid concentrations.* The rats ($n$ = 5 rats/group) used in the microarray analysis described above, were also analyzed for serum lipids. Blood was drawn by cardiac puncture under light methoxyflurane anesthesia. Blood was transferred into EDTA-containing tubes, and plasma was collected by centrifugation. Triglyceride, total cholesterol, and high-density lipoprotein (HDL) cholesterol were determined individually in fresh plasma using a Monarch automated analyzer (ILS Laboratories Scandinavia, Sollentuna, Sweden). Data are presented as means ± SE. The significance of differences between groups concerning lipid measurements in serum was tested by one-way ANOVA, followed by planned comparisons (Statistica Software; StatSoft, Tulsa, OK).

Table 1. *Degree of differentially expressed genes in different tissues from male rats as an effect of age or GH treatment in old rats*

| | Liver | Fat | Skeletal Muscle |
|---|---|---|---|
| Total number of expressed genes | 1,793 | 2,319 | 1,570 |
| Reduced by old age, % of total | 2.7 | 4.4 | 2.5 |
| Induced by old age, % of total | 4.6 | 3.9 | 5.4 |
| Reduced by GH in old rats, % of total | 4.5 | 1.5 | 2.0 |
| Induced by GH in old rats, % of total | 2.0 | 3.8 | 7.6 |
| Reversed by GH replacement, % of total | 1.0 | 0.6 | 0.9 |
| Super-induced by GH replacement, % of total | 1.7 | 0.1 | 0.3 |
| Super-reduced by GH replacement, % of total | 3.6 | 0.4 | |

Table 1 was generated from a comparison of changes in gene expression in old versus young rats and growth hormone (GH)-treated vs. untreated old rats ($n$ = 5 rats/group) determined by cDNA microarray analysis. The arrays represented approximately 3,200 cDNA clones. A gene was considered expressed if it could be detected (1.4 times above background) in 2 of 3 replicated hybridizations with cDNA derived from fat or skeletal muscle, and in 4 of 7 replicated hybridizations with hepatic cDNA. The total number of expressed genes is given as the mean number of expressed genes in the 2 experimental situations (effect of age and effect of GH). A $q$ value less than 10% and a ratio value of more than 1.5 was chosen as criteria to denote differences in the level of hybridization between control and experimental cDNA. The number of differentially expressed genes was calculated for the different tissues in relation to the total number of expressed genes. Genes were called “super-induced” or “super-reduced” if the effect of GH treatment in old rats was greater than the effect of age.



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## RESULTS AND DISCUSSION

*Age-induced changes in gene expression patterns of different tissues in male rats.* cDNA microarrays were used to examine molecular events behind aging and GH replacement therapy in different tissues from old rats. Initially, transcriptional responses were determined by comparing liver, adipose tissue, or skeletal muscle from young (8 wk of age) and old (18 mo of age) male rats. Of 3,200 genes printed on the microarrays, ~1,800 were detected in the liver. Among these, 82 transcripts (4.6%) displayed a statistically significant increase (at least 1.5-fold) in expression levels during aging, whereas 49 (2.7%) were reduced. These results are summarized in Table 1, together with similar values from the other tissues investigated. To compare the effects of aging on specific cellular pathways between these different tissues, the regulated gene products were next grouped into functional categories. The whole data set can be found at the Endocrinology Gene Expression Database (EndoGED; **http://www.cmm.ki.se/endoged**), and the differentially expressed gene products categorized as being important in cellular fuel metabolism are listed in Table 2. The aged liver was found to have an increased expression of genes involved in glycolysis and lipogenesis, confirming our previous study on hepatic gene expression during aging (42, 44). An age-associated increase in hepatic de novo lipid synthesis is in line with increased levels of plasma triglycerides in old animals, previously reported by us (31) and others (47). An increase in hepatic lipogenesis and lipid output might lead to a general switch in fuel utilization with age, from glucose to fatty acids in peripheral tissues. This is in agreement with the increased expression of fatty acid translocase/CD36 in skeletal muscle. Since myocytes that oxidize fatty acids will have a reduced uptake and metabolism of glucose due to the glucose-fatty acid cycle, it has been discussed whether the resulting elevation in serum glucose levels could be one of the mechanisms behind insulin resistance (6, 10, 35).

In adipose tissue, a big group of age-regulated genes encoded proteins involved in glucose oxidation (Table 2). In contrast to the situation in liver, gene products from the glycolysis, tricarboxylic acid cycle, and lipid synthesis pathways were reduced in fat obtained from the old animals. The coregulation of genes such as pyruvate kinase, fatty acid synthase, and stearoyl-CoA desaturase has been shown before (25) and reflects the cellular coordination of carbohydrate oxidation and de novo lipid synthesis. Similarly, a coordinated reduction of genes involved in glucose oxidation and mitochondrial function was recently demonstrated in humans with insulin resistance and diabetes (23, 32). In healthy individuals, an increase in serum glucose and insulin levels after a meal will lead to an increased activity of these pathways, mediated by increased activities of GLUT4, pyruvate dehydrogenase, acetyl-CoA carboxylase, and fatty acid esterification. The age-induced attenuation of these pathways, observed in this study, might therefore contribute to the effect (or cause) of reduced insulin sensitivity in adipose tissue from aged rats (3, 9).

*GH-induced changes in gene expression patterns of different tissues from GH-treated old rats.* To identify GH-responsive genes that might mediate positive effects of GH treatment during aging, we next performed similar experiments to compare patterns of mRNA expression in tissues from old untreated rats with that of rats treated for 1 wk by two daily injections of GH. As determined by the number of differentially expressed genes, GH treatment of the old rats changed the expression levels in 6.5% of the total number of genes expressed in liver, 5.3% in adipose tissue, and 9.6% in skeletal muscle (Table 1). In the liver, GH was found to mainly regulate genes involved in lipid metabolism (Table 2). GH reduced the hepatic expression of genes for lipid synthesis, such as fatty acid synthase and stearyl-CoA desaturase, in parallel with an increase of genes for fatty acid oxidation, including carnitine palmitoyl transferase 1. These results suggest that the GH-treated old rat might have a lower degree of hepatic lipid production, and therefore lipid output, compared with untreated old rats. This is in agreement with previously published data (31). An elevated rate of hepatic β-oxidation in GH-treated rats may increase the ability of the liver to produce glucose by gluconeogenesis. A final picture of a GH-induced change in the type of fuel leaving the liver could therefore be envisioned. This is again in agreement with the observed changes in skeletal muscle. As shown in Table 2, four gene products from the glycolysis pathway were increased and two transcripts encoding proteins involved in the uptake of fatty acids were reduced in skeletal muscle upon GH treatment. These GH-induced changes in gene expression might lead to a switch from fatty acid to glucose oxidation in GH-treated old rats, which in turn could reverse a potential risk of elevated blood glucose levels with age. In adipose tissue, as in liver, GH treatment lead to a reduced expression of gene products involved in lipogenesis, such as fatty acid synthase and stearyl-CoA desaturase. These changes at the level of gene expression might therefore, if translated into altered protein levels, lead to a further suppressed rate of adipose lipid synthesis from the already reduced state due to old age. In summary, a GH-mediated increase in hepatic fatty acid oxidation, together with a reduced lipogenesis in liver and adipose tissue, might add up to a final picture of decreased body fat upon GH treatment. A summary of the transcriptional changes induced by age and GH treatment in old rats is illustrated in Fig. 1.

*Age- and GH-induced changes in serum lipid levels.* We next wanted to determine whether the alterations in transcript levels also could be reflected in terms of blood lipids. Serum levels of triglycerides, HDL cholesterol, and total cholesterol were therefore determined in the different groups of animals. As shown in Table 3, the old animals had a higher level of blood lipids, with significantly increased levels of HDL cholesterol and total cholesterol. This age-induced increase was reversed by GH treatment, which is in agreement with the findings stated above. Both the age- and GH-induced changes in blood lipids may at least partly be explained by an altered hepatic production and secretion of triglycerides and cholesterol, which might be a consequence of the transcriptional changes demonstrated in this study. Although other important mechanisms behind these effects on serum lipids were missed in the present study of transcript profiles, it may be concluded that the expression data presented here fits with expected changes in physiological parameters. We (31) and others (5) have previously shown that serum levels of total cholesterol and triglycerides increase in old male rats compared with young and that GH treatment of old rats can reverse this hyperlipidemia.



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Table 2. *Effects of old age and GH therapy on the expression of genes involved in fuel metabolism*

| GenBank Accession No. | Gene Product | Liver | | Fat | | Skeletal muscle | |
|---|---|---|---|---|---|---|---|
| | | Δ Age | Δ GH | Δ Age | Δ GH | Δ Age | Δ GH |
| Glucose oxidation | | | | | | | |
| AW140600 | Glucose-6-phosphate isomerase* | | 0.66 | | | | **1.71** |
| U09256 | Transketolase | **1.57** | 0.53 | 0.39 | | | |
| M10149 | Fructose-bisphosphate aldolase B | **1.89** | | | | | |
| M25558 | Glycerol-3-phosphate dehydrogenase 1 | | | | | | **2.23** |
| BM986368 | Glyceraldehyde-3-phosphate dehydrogenase | **2.28** | | 0.53 | | | |
| X02610 | Enolase 1, alpha | | | 0.65 | | | **1.72** |
| X15800 | Pyruvate kinase, muscle, Pkm2 | | | 0.52 | | | **1.64** |
| NM_012624 | L-type pyruvate kinase | | 0.47 | | | | |
| BM986359 | Pyruvate dehydrogenase alpha chain 1 | **1.72** | | 0.52 | | | |
| AW142079 | Pyruvate dehydrogenase kinase 1 | | | 0.46 | | | |
| AF034577 | Pyruvate dehydrogenase kinase 4 | **1.80** | **2.94** | | | **2.71** | |
| NM_017005 | Fumarate hydratase | **2.18** | | | | | |
| AW140553 | Malate dehydrogenase 1 | | | 0.45 | | | |
| Glucose synthesis | | | | | | | |
| BC028342 | Phosphoenolpyruvate carboxykinase 1 | | 0.42 | **1.70** | | | |
| NM_017025 | Lactate dehydrogenase A | 0.62 | | | | | |
| Pentose phosphate pathway | | | | | | | |
| X07467 | Glucose-6-phosphate dehydrogenase | **1.95** | 0.46 | 0.44 | | | |
| Lipid mobilization | | | | | | | |
| V01222 | Albumin | | 0.48 | | **3.18** | | |
| NM_012824 | Apolipoprotein C-1 | | 0.45 | | | | |
| BU671024 | Apolipoprotein E | | 0.55 | **2.37** | | | **2.10** |
| AW140541 | Scavenger receptor class B, member 1 | | | | | | **2.23** |
| M14201 | Acyl-CoA BP/diazepam binding inhibitor | | 0.49 | | | | |
| L19658 | Fatty Acid translocase/CD36 | **4.07** | 0.19 | | | **1.74** | 0.54 |
| NM_012598 | Lipoprotein lipase | | | | | | 0.54 |
| Lipid synthesis | | | | | | | |
| K01934 | Thyroid hormone responsive SPOT14 | | 0.43 | 0.56 | | 0.39 | |
| D90109 | Fatty acid CoA ligase, long chain 2 | **3.40** | 0.47 | | | | |
| AB012933 | Fatty acid CoA ligase, long chain 5 | | 0.43 | | | | |
| BF281429 | Fatty acid synthase | **2.62** | 0.41 | 0.14 | 0.51 | | |
| M95591 | Farnesyl diphosphate farnesyl transferase 1 | | | | 0.62 | | |
| M34477 | Farnesyl diphosphate synthase | | | 0.57 | | | |
| J02585 | Stearoyl-coenzyme A desaturase 1 | **2.40** | 0.27 | 0.12 | 0.58 | 0.14 | |
| AB032243 | Stearoyl-coenzyme A desaturase 2 | | | 0.28 | | | |
| Lipid oxidation | | | | | | | |
| L07736 | Carnitine palmitoyltransferase 1 | | **1.84** | | | | |
| U08976 | Enoyl coenzyme A hydratase 1 | | **1.61** | **1.66** | | | |
| AW141050 | Cytosolic acyl-CoA thioesterase 1 | 0.30 | **5.25** | | | | |
| AW142131 | Dodecenoyl-CoA delta isomerase | | **3.55** | | | | |
| M33648 | 3-Hydroxy-3-methylglutaryl-CoA synthase 2 | | | **2.16** | | | |
| Amino acid turnover | | | | | | | |
| M29599 | Glutamine synthetase 1 | | | **1.52** | | **3.56** | |
| X14044 | Glutamate dehydrogenase 1 | | **2.34** | | | | |
| M11842 | Ornithine aminotransferase | | 0.55 | **1.55** | | **1.80** | |
| D10354 | Glutamic-pyruvate transaminase | **1.94** | | | | | |
| AW141014 | Tissue-type transglutaminase | **2.11** | | | **1.85** | | |

Table 2 was generated from a comparison of changes in gene expression in old vs. young rats (Δ age) and GH-treated vs. untreated old rats (ΔGH) ($n = 5$ rats/group), using arrays representing approximately 3,200 cDNA clones. Data are presented as ratios between the levels of test to reference cDNA that are hybridized to spotted DNA. The significance of the expression ratios of both age and GH replacement were estimated using the "significance analysis of microarray" technique. The magnitude of the changes was calculated as the mean of 3 (fat and muscle) or 7 (liver) independent experiments. Values highlighted in bold are those representing mRNAs that were increased at least 1.5 times and values in plain text are those that were decreased at least 1.5 times. *98% similar to human and mouse glucose-6-phosphate isomerase.

*Age- and GH-induced changes in the expression of previously characterized GH-regulated gene products.* It is obvious that the age-induced changes in gene expression reported in this study are not merely a consequence of decreased GH secretion. As demonstrated in Table 2, only a fraction of genes involved in fuel metabolism were "normalized" after GH replacement in old rats. The total number of genes found to be reversed by GH treatment was only about 1% in all three tissues investigated (Table 1), questioning the importance of GH in age-related alterations. The number of genes found to be either super-induced (induced by GH without being reduced by age) or super-reduced (reduced by GH without being induced by age) after GH replacement is also presented in Table 1. Most notable is the result obtained for hepatic genes, with a high degree of genes altered in the "wrong" direction after GH treatment. Although additional age- and GH-regulated genes might be discovered with higher density gene arrays, these results suggest that GH replacement through daily injections in old male rats is more likely to result in a final pattern of altered gene expression than reconstituting the situation in young rats.



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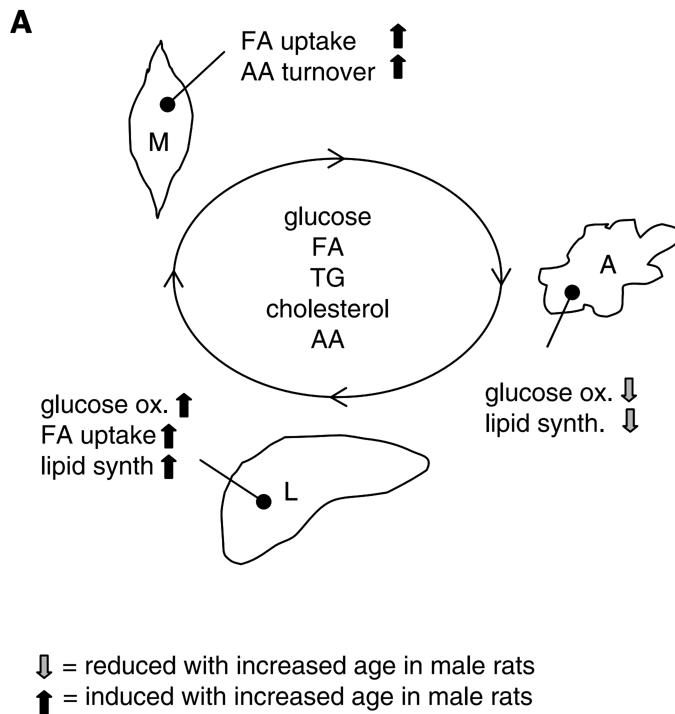


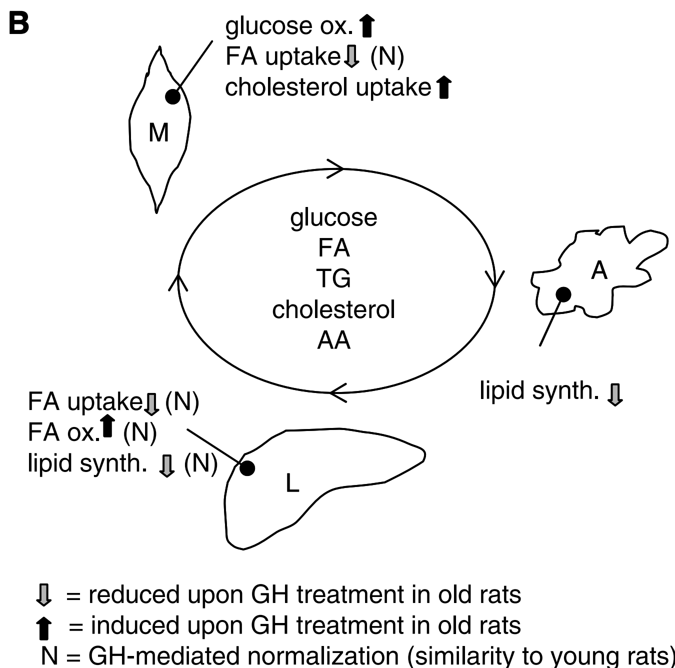


Fig. 1. Schematic overview of transcriptional changes described in this study, including effects of aging and growth hormone (GH) treatment of old male rats, generated from data presented in Table 2, comparing changes in gene expression in old vs. young rats (*A*) and GH-treated vs. untreated old rats (*B*), as determined by cDNA microarray analysis. M, skeletal muscle; A, adipose tissue; L, liver; ox, oxidation; synth, synthesis; AA, amino acid; TG, triglycerides; FA, fatty acid.

Table 3. *Effects of aging and GH treatment of old male rats on serum lipid levels*

| | Young | Old | Old + GH |
|---|---|---|---|
| Triglyceride, mmol/l | 1.91±0.33 | 2.52±0.14 | 1.88±0.32 |
| HDL cholesterol, mmol/l | 1.06±0.08 | 1.67±0.28* | 1.19±0.19 |
| Total cholesterol, mmol/l | 1.89±0.19 | 2.84±0.43* | 1.90±0.29† |

Values are mean ± SE. There were five animals in each group. *$P < 0.05$, vs. young and †$P < 0.05$, vs. old; one-way ANOVA followed by planned comparisons.

Since hGH was used in the present study, and hGH can bind to both lactogenic and somatogenic receptors with high affinity, it might be reasoned that some of the GH-induced effects could be mediated through the prolactin (PRL) receptor. However, Le Stunff et al. (18) have shown that all hepatic effects of hGH parallel the responses to rat GH, indicating that most of the actions of hGH in the liver are mediated by the GH receptor rather than by the PRL receptor. It was suggested that the diminished response to PRL might be secondary to the high density of short PRL receptor isoforms in the liver, which do not participate effectively in ligand-induced signal transmission. However, further studies are needed to distinguish between GH receptor- and PRL receptor-mediated effects in hGH-treated animals. This could include microarray studies comparing the effects of continuous infusion of hGH, bovine GH (which only acts through the GH receptor), and ovine PRL (which only acts through the PRL receptor) on hepatic gene expression.

It may also be argued that the treatment regimen was insufficient to fully restore normal GH levels in serum. Since male rats were used in this study, the mode of GH administration (daily injections) was chosen to specifically mimic the intermittent secretion of GH in male rats (41). It has previously been demonstrated that 18-mo-old male rats have a reduced secretion of GH, with lower mean GH concentrations in serum compared with young (4 mo old) male rats (39) and that the diminished pulsatile release of GH is associated with reduced pulse amplitude but unchanged trough GH values. Treatment of GH-deficient hypophysectomized rats of either sex by daily injections of GH, which is believed to mimic the male-specific pattern of GH secretion, is well known to induce various

Table 4. *Effects of old age and GH therapy in old rats on the hepatic expression of genes previously characterized as GH regulated*

| GenBank Accession No. | Gene Product | Liver Δ Age | Liver Δ GH |
|---|---|---|---|
| AA963258 | Insulin-like growth factor I (IGF-I) | 0.45 | 1.98 |
| AA819611 | IGF binding protein 3 (IGFBP-3) | 0.52 | 1.48 |
| AA925350 | IGFBP, acid labile subunit (ALS) | 0.62 | 2.12 |
| J00737 | α-2u-globulin | 0.09 | 0.34 |
| U09742 | Testosterone 6-β-hydroxylase (CYP3A2) | 0.19 | 0.13 |
| AF037072 | Carbonic anhydrase 3 | 0.13 | 0.72 |

Table 4 was generated from a comparison of changes in gene expression in old vs. young rats (Δ age) and GH-treated vs. untreated old rats (ΔGH) ($n$ = 5 rats/group). Data are presented as ratios between the levels of test to reference cDNA that are hybridized to spotted DNA. The magnitude of the changes was calculated as the mean of four independent experiments, using microarrays representing 6,200 cDNAs.



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male-specific genes. This collection of male-specific and GH-regulated genes includes α-2u-globulin, carbonic anhydrase III, and CYP3A2 (49). The effects of aging and GH treatment obtained in the present study (using microarrays) for these and other well-characterized GH-regulated genes are summarized in Table 4. Expected effects of GH treatment were observed for insulin-like growth factor I (IGF-I), IGF binding protein 3 (IGFBP-3), and IGFBP complex acid labile subunit (ALS). The hepatic expression of these GH-regulated genes is not sexually differentiated and not sensitive to different modes of GH administration. Surprisingly, none of the male-specific genes was induced by GH in this study. Carbonic anhydrase III was unchanged, whereas α-2u-globulin and CYP3A2 were super-reduced in the treated animals. This indicates that the animals had been able to respond to GH, but only partly, which may explain the low degree of reversed genes in this study. An interesting aspect of these findings is the correlation to the effect of SOCS-2 ("suppressor of cytokine signaling-2") deficiency in mice on the expression of GH-regulated genes. SOCS-2 deficiency was reported to be associated with reduced levels of α-2u-globulin but unchanged levels of IGF-I in giant mice (22). It may therefore be speculated that normal levels of SOCS-2 are needed for mediating the correct effect of GH to certain hepatic genes. Since we have shown that hepatic expression of SOCS-2 is reduced in old rats (22, 43), this might at least partly explain the high degree of super-induced and super-reduced genes in the liver of GH-treated old rats.

We have recently reported the effect of continuous infusion of GH through osmotic minipumps on the expression of genes in livers from old male rats (44). In contrast to daily injections, this treatment is thought to mimic the more continuous pattern of GH secretion found in female rodents and has been shown to induce female-predominant hepatic genes. A comparison could thus be made between these two different modes of GH administration in terms of hepatic changes in transcript levels. Genes involved in glucose oxidation and lipid synthesis were induced during aging in both studies, but the different types of GH treatment induced opposite changes on the same set of genes. Both fatty acid synthase and stearyl-CoA desaturase were induced by continuous treatment but reduced by intermittent. Also, infusion of GH led to the expected induction of female predominant gene products (CYP2C12 and CYP2C7) and suppression of male-specific transcripts (α-2u-globulin, carbonic anhydrase III' and CYP3A2) (44). This implies that the previously reported pattern-dependent effects of GH on hepatic gene expression might include genes involved in lipid metabolism. This finding deserves further investigations.

In summary, the data presented here describe tissue-specific alterations in gene expression patterns as a result of the aging process per se and the effects of GH treatment in old rats. Briefly, old rats were shown to have increased expression of genes involved in hepatic glucose oxidation and lipid synthesis, whereas these pathways were reduced in adipose tissue. GH treatment induced the expression of genes for lipid oxidation in liver and for glucose oxidation in skeletal muscle. In adipose tissue, GH reduced the expression of genes involved in lipogenesis even further. Thus it is tempting to speculate that GH may (at least partly) exert its lipolytic action through increased levels of gene products for hepatic β-oxidation in combination with reduced levels of gene products for lipogenesis in fat. The anabolic effect of GH might be secondary to the increased use of fatty acids in the liver (fueling hepatic gluconeogenesis), increased glucose output, and sparing of protein stores. Although a very small number of age-affected genes were shown to be reversed by GH in this study, the set of genes described above could be important mediators of positive effects of GH treatment during aging. Further studies focused on these specific gene products in relation to age-related biological changes are required to unravel this. It should also be noted that since changes in mRNA levels not always result in a parallel alteration in protein levels or biological function, expression data have to be complemented by further studies. Nevertheless, it seems likely that sets of genes from specific metabolic pathways may be regulated in a coordinated manner and that changes in hormonal status with increased age could have an important impact on the long-term regulation of both mRNA and protein expression levels. By comparing promoter sequences in groups of genes behaving similarly in response to age and/or GH, in addition to investigating levels of potentially interesting transcription factors and signaling molecules in animals of different ages and GH status, one might obtain hints as to what mechanisms could be underlying the observed alterations in transcript profiles.

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