Decreased hexosamine biosynthesis in GH-deficient dwarf rat muscle. Reversal with GH, but not IGF-I, therapy

KATHERINE A. ROBINSON,1 STEVEN M. WILLI,2 SARAH BINGEL,3 AND MARIA G. BUSE1,4

1Division of Endocrinology, Diabetes, and Medical Genetics, Department of Medicine, and Departments of 2Pediatrics, 3Laboratory Animal Resources, and 4Biochemistry/Molecular Biology, Medical University of South Carolina, Charleston, South Carolina 29425-2222

Robinson, Katherine A., Steven M. Willi, Sarah Bingle, and Maria G. Buse. Decreased hexosamine biosynthesis in GH-deficient dwarf rat muscle. Reversal with GH, but not IGF-I, therapy. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E435–E442, 1999.—Enhanced glucose flux via the hexosamine synthesis pathway (HNSP) has been implicated in insulin resistance. We measured L-glutamine:D-fructose-6-phosphate amidotransferase (GFAT, a rate-limiting enzyme) and concentrations of UDP-N-acetyl hexosamines (UDP-HexNAc, major products of HNSP) in muscle and liver of growth hormone (GH)-deficient male dwarf (dw) rats. All parameters measured, except body weight, were similar in 5-wk-old control and dw rats. Muscle GFAT activity declined progressively with age in controls and dw rats but was consistently 30–60% lower in 8- to 14-wk-old dw rats vs. age-matched controls; UDP-HexNAc concentrations in muscle were concomitantly 30% lower in dw rats vs. controls (P < 0.01). Concentrations of UDP-hexoses, GDP-mannose, and UDP in muscle were similar in control and dw rats. Muscle HNSP activity was similarly diminished in fed and fasted dw rats. In liver, only a small difference in GFAT activity was evident between controls and dw rats, and no differences in UDP-HexNAc concentrations were observed. Treatment with recombinant human GH (rhGH) for 5 days restored UDP-HexNAc to control levels in dw muscles (P < 0.01) and partially restored GFAT activity. Insulin-like growth factor I treatment was ineffective. We conclude that GH participates in HNSP regulation in muscle.

L-glutaminolfructose-6-phosphate amidotransferase activity in muscle and liver; UDP-N-acetyl hexosamine concentration in muscle and liver; regulation of hexosamine biosynthesis by growth hormone in muscle; growth hormone-induced insulin resistance

INSULIN RESISTANCE is a hallmark of non-insulin-dependent diabetes mellitus and is also associated with uncontrolled insulinopenic diabetes in humans and rodents. In the former condition, insulin resistance is thought to be genetically determined, whereas in the latter, it is reversed by normalizing circulating glucose concentrations and attributed to “glucose toxicity” (10). On the basis of studies in adipocytes in primary culture, Marshall et al. (19) proposed in 1991 that increased flux of glucose via the hexosamine synthesis pathway (HNSP) may contribute to glucose-induced insulin resistance of glucose transport. The rate-limiting enzyme for glucose flux via HNSP is L-glutaminolfructose-6-phosphate amidotransferase (GFAT), which catalyzes the conversion of fructose 6-phosphate and glutamine to glucosamine 6-phosphate (GlcN-6-P) and glutamate. The pathway generates obligatory substrates for the synthesis of glycoproteins and glycolipids; major HNSP products that accumulate in cells are UDP-N-acetyl hexosamines (UDP-HexNAc), i.e., UDP-N-acetyl glucosamine (UDP-GlcNAc) and UDP-N-acetyl galactosamine (UDP-GalNAc) in an ~3:1 ratio (reviewed in Refs. 19, 25). Glucosamine (GlcN) enters cells via the same transporter as glucose and is rapidly phosphorylated to GlcN-6-P, bypassing GFAT. GlcN has been extensively used to demonstrate that accumulation of HNSP products can induce insulin resistance of glucose transport and glycosgen synthesis in skeletal muscle and fat, both in vitro and in vivo (4, 19, 25, 27).

The major site of glucose-induced insulin resistance is skeletal muscle (10). Prolonged hyperglycemia results in the accumulation of UDP-HexNAc in muscle (26), and insulin clamp studies in rats under various experimental conditions suggest a relationship between the development of insulin resistance and increased UDP-HexNAc in muscle (13, 14). Transgenic mice, overexpressing GFAT in muscle and fat, develop insulin resistance with age (15). In two other models of insulin resistance, the ob/ob mouse and transgenic mice overexpressing the glucose transporter isofrom GLUT-1 in muscle, GFAT activity and UDP-HexNAc concentrations in muscle are also increased (6, 7).

The growth hormone (GH)-deficient dwarf (dw) rat arose as an autosomal recessive mutation in the Lewis (Lw) rat. Although the gene defect has not been identified, the phenotype has been well characterized (8). GH synthesis and storage are reduced by ~90% in dw rats, whereas the production of other pituitary hormones is normal (8). These animals manifest decreased growth rate and body weight, which is reversible with GH treatment (8, 28, 32). GH-releasing factor mRNA is increased in the hypothalamus of dw rats, and somatotroph cell number is markedly reduced (reviewed in Ref. 5). Somatotrophs isolated from dw rats show decreased ability to increase cAMP production in response to various stimuli. The impairment in maximal cAMP production, although not affecting acute GH release, may underlie the defect in somatotroph cell number and GH content in the dw pituitary (5). Because chronic GH excess causes insulin resistance (reviewed in Ref. 29), and GH deficiency may enhance insulin sensitivity (21, 30), we examined whether HNSP activity was affected in muscles of this GH-deficient animal model.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
RESEARCH DESIGN AND METHODS

Animals. Homozygous dw rat breeding pairs (dw-4-ola-hsd) were obtained from Harlan Olac (Bicester, UK) and bred (32). Because of significant sexual dimorphism of GH production in rats, only male offspring were used for experiments. Because the original mutation was observed in Lw rats, age-matched male Lw rats were obtained from Harlan Sprague Dawley (Indianapolis, IN) and used as controls (32). Rats were housed in controlled animal facilities (23°C, 12:12-h light-dark cycle); Lw rats were acclimated to the laboratory for ≥1 wk before use. Animals were fed standard rat chow ad libitum, unless otherwise indicated, and had free access to water at all times. In some experiments food was withdrawn for 18 h before animals were killed (i.e., fasted). In other experiments, controls were pair fed with dw rats; i.e., animals were housed in individual cages, and the amount of food consumed by dw rats per gram of body weight on 1 day was offered to control rats (adjusted for body weight) on the following day. Food was withdrawn from all rats 2 h before they were killed.

In some experiments, rats were treated with recombinant human growth hormone (rhGH, Nutropin, Genentech, South San Francisco, CA; sp ac 3 IU/mg) or with rh insulin-like growth factor I (rhIGF-I, Genentech). Each hormone was administered subcutaneously, 200 μg/day, in two daily doses for 2 or 5 days. The last injection was given 2 h before rats were killed. Nontreated rats received subcutaneous injections of saline on the same schedule.

Rats were killed between 9 and 10 AM under pentobarbital sodium anesthesia (50 mg/kg ip). Blood was withdrawn by cardiac puncture into heparinized syringes and centrifuged at 4°C; the serum was stored at −70°C for later analyses of serum glucose and insulin. Hindlimb muscles (gastrocnemius and soleus) were rapidly dissected, freed of connective tissue, frozen in liquid N2, and powdered in a mortar cooled in liquid N2. Muscles from one leg were used for analysis of nucleotide sugar concentrations and muscles from the other were used for assay of GFAT activity. Weighed aliquots of frozen tissue powder (∼0.5 g) were processed immediately for these assays, as will be described. In some experiments, samples of liver were also removed, frozen in liquid N2, and processed.

Analysis of nucleotide-linked hexoses and hexosamines. The method used and its validation have been described previously (26). Briefly, frozen tissue powder was homogenized at 4°C in 0.3 M perchloric acid (PCA), precipitates were pelleted by centrifugation, and PCA was extracted from the supernatant with 1:4 triethylamine, 1:2-trichloro-trifluoro-ethane. The aqueous phase was stored at −70°C until analysis by high-performance liquid chromatography (HPLC) within 1 wk. HPLC was performed on a Whatman Partisil anion exchange column (4.6 × 250 mm) eluted with a concave gradient of ammonium phosphate from 15 mM, pH 3.8, to 1 M, pH 4.5, over 50 min at a flow rate of 1 ml/min. UDP-HexNAC, UDP-hexoses (UDP-Hex), GDP-mannose, and UDP were quantified by ultraviolet absorption (A254) and comparison with standards. With this method, UDP-GlcNAc coelutes with UDP-GaINAc, and UDP-glucose coelutes with UDP-galactose. The ratio of glucose to galactose containing sugar nucleotides is typically 3:1, and their absorption coefficients are very similar (26). Therefore, UDP-GlcNAc and UDP-glucose were used as standards for UDP-HexNAC and UDP-Hex, respectively.

GFAT enzyme activity. GFAT activity was assayed as previously described (6, 26). Frozen tissue powder was homogenized in 4–5 vol of extraction buffer (25 mM HEPES, pH 7.5, 4°C, 5 mM EDTA, 100 mM KCl, 5 mM glucose-6-phosphate, and protease inhibitors (6)). Extracts were centrifuged at 4°C (60,000 g for 15 min, and the supernatant was centrifuged at 100,000 g for 60 min). The supernatant was spin-filtered over Sephadex G-25 columns equilibrated with assay buffer (25 mM K2PO4, pH 7.5, 1 mM EDTA, 50 mM KCl) to reduce the concentration of small molecules that may modify GFAT activity. Aliquots of gel-filtered cytosolic extracts were incubated for 60 min at 37°C with 6 mM fructose-6-phosphate and 12 mM glutamine. Reactions were stopped with PCA, samples were centrifuged, and PCA was extracted from the supernatants as we have described. The aqueous phase was stored at −70°C until analysis within 1 wk. GlcN-6-P, the product of the GFAT-catalyzed reaction, was measured fluorometrically after separation by HPLC, as described in Ref. 26. Blank readings were <10% of measured activity and were subtracted. GFAT activity was normalized to the protein concentration in the gel-filtered extract, which was measured spectrophotometrically using Coomassie protein assay reagent (Pierce, Rockford, IL) and bovine serum albumin standards. GFAT activity was expressed as picomoles of GlcN-6-P generated per milligram of protein per minute.

Measurement of plasma glucose and insulin. Plasma glucose was determined by the glucose oxidase method using a Beckman Glucose Analyser II. Plasma insulin was measured by a radioimmunoassay kit for rat insulin (Linco Research, St. Charles, MO).

Statistical analyses. Means ± SE are shown. Where error bars are not shown, they are too small for graphic representation (i.e., <3% of the mean). The significance of differences between means was analyzed by two-tailed Student’s t-test. When multiple means were compared, analysis of variance with post hoc analysis by Tukey’s test to accommodate unequal sample sizes was used. Regression analysis with indicator variables was used to assess the relationship between age and GFAT, UDP-HexNAC, and UDP-Hex. P < 0.05 was considered statistically significant.

Materials. Reagents used were of the highest purity available and were purchased from Sigma or as indicated in the text.

RESULTS

Animal characteristics. Male dw rats and controls were studied between 5 and 14 wk of age. Dw rats weighed 26% less than controls at 5 wk of age (P < 0.01), 34% less than controls between 8 and 10 wk (P < 0.001), and 40% less than controls between 12 and 14 wk (P < 0.001, Fig. 1). Dw rats consumed less food than the controls at each age studied, but food intake was not significantly different between groups when normalized to body weight (−8 g·100 g body wt−1·day−1, Fig. 1). Concentrations of plasma glucose and insulin were measured in 9- to 10-wk-old control and dw rats in the fed and fasted states. Plasma insulin concentrations were higher in fed than in fasted rats but were not significantly different between control and dw rats in either condition. Plasma glucose tended to be slightly lower in dw rats than in controls, but the difference (11%) was significant only in the fasted state (n = 9, P < 0.02, Fig. 2).

Developmental changes. GFAT activity and nucleotide sugar concentrations in muscle were measured in male control and dw rats at various ages between 5 and 14 wk (Table 1). GFAT activity in muscle was greatest in 5-wk-old prepubertal rats and decreased with maturation. Regression analysis revealed that muscle GFAT activity was expressed as picomoles of GlcN-6-P generated per milligram of protein per minute.
activity decreased with age in dw and in control rats, 
($r = -0.8285$ and $-0.673$ for dw and control rats, 
respectively; $P < 0.0001$ for both, $n = 28$ and $31$, 
respectively). There was no significant difference in 
muscle GFAT activity between 5-wk-old control and dw 
rats. Muscle GFAT activity was 30–40% lower in dw 
rats than in controls at 8–10 wk of age ($P < 0.01$) and 
60% lower than in controls at 12–14 wk ($P < 0.01$). Dw 
rats and age-matched controls were always studied in 
the same experiment and analyzed in parallel.

UDP-HexNAc concentrations in muscle were 10% 
lower in 5-wk-old dw rats than in controls, but this 
difference was not statistically significant ($P > 0.1$, 
Table 1). They were 16% lower in dw rats than in 
controls at 8 wk of age ($P < 0.01$) and were consistently 
30% lower between 9 and 14 wk of age ($P < 0.01$). 
Regression analysis suggested an age-dependent de- 
crease in UDP-HexNAc concentrations in muscles of 
dw rats ($r = 0.4736$, $P = 0.026$, $n = 25$) but not in 
control rats ($r = 0.1156$, $P = 0.582$, $n = 25$).

UDP-Hex concentrations in muscle were lower in 
8- to 14-wk-old rats than in 5-wk-old rats. They tended 
to be slightly (~10%) lower in 8- to 14-wk-old dw rats 
than in controls, but the differences were not statisti- 
cally significant (Table 1). Regression analysis sug- 
gested an age-related decrease in UDP-Hex concentra- 
tions in muscles of dw rats ($r = 0.4606$, $P = 0.021$, $n = 25$). There 
were no significant differences in the concentrations of 
GDP-mannose or UDP in muscle between fed control 
and dw rats in any age group studied (data not shown).

Comparison of fed and fasted rats. Because GFAT 
activity in muscle may be regulated by the ambient 
concentrations of glucose and insulin (9), we examined 
whether the differences between dw and control rats
were dependent on the nutritional state (Fig. 2). Nine to ten-week-old dw and control rats were either fasted overnight or fed ad libitum, with feed withdrawn 2 h before experiments. GFAT activity in muscle was 40% lower in dw rats than in controls, both in the fed and the fasted states (P < 0.01 in both conditions, Fig. 2). Similarly, UDP-HexNAc concentrations in muscle were 30% lower in dw rats than in controls under both fed (P < 0.01) and fasted (P < 0.05) conditions (Fig. 2). The concentrations of UDP-Hex were similar in muscles from dw and control rats in the fed and fasted states (Fig. 2). The ratio of UDP-HexNAc to UDP-Hex was 1.82 ± 0.2 and 1.87 ± 0.1 in fasted and fed controls, respectively, and 1.41 ± 0.1 in both fasted and fed dw rats (P < 0.01 dw vs. control). UDP concentrations in muscle were similar in control and dw rats in the fed and fasted states (data not shown). GDP-mannose concentrations in muscle were significantly lower in dw rats vs. controls, but only in the fasted state (6.2 ± 0.61 vs. 9.1 ± 0.79 nmol/g muscle, P < 0.01).

Liver. To test whether the decreased HNSP activity in muscles of dw rats was tissue specific, GFAT activity and the concentrations of nucleotide sugars were measured in livers from 5-, 8-, and 10-wk-old rats. As previously reported (26), GFAT activity and the concentrations of nucleotide sugars were much higher in liver than in muscle (compare Fig. 3 with Fig. 2). In contrast to muscle, no marked age-associated changes in GFAT activity were observed in liver. In 5-wk-old rats, hepatic GFAT activity was 810 ± 50 pmol·mg protein·min⁻¹ in controls vs. 780 ± 69 pmol·mg protein·min⁻¹ in dw rats (n = 3/group). When data from 8- to 10-wk-old rats were pooled for analysis (n = 7/group, Fig. 3), hepatic GFAT activity was slightly lower in the dw animals (14.5%, P < 0.05) than in the controls. No differences in the level of UDP-HexNAc or UDP-Hex were observed between livers of control and dw rats in any age group.

Treatment with rhGH or rhLGF-1. To assess whether the decreased HNSP activity observed in muscles of dw rats was reversible with GH treatment, ad libitum-fed, 10- to 14-wk-old dw rats were injected subcutaneously with 100 µg rhGH twice daily, at 9 AM and 8 PM, for 2 or 5 days. In each experiment, treated and untreated control and dw rats were of the same age and were analyzed in parallel. In some experiments, an additional set of untreated control rats was pair fed to the untreated dw rats on a weight-adjusted basis. Control rats were treated with GH for only 2 days. To facilitate data presentation, measurements in the different groups were expressed as a percentage of the mean value observed in untreated control rats from the same experiment (Fig. 4). Pair feeding did not affect GFAT activity or nucleotide sugar concentrations in muscle (Fig. 4). There were no significant differences in food intake per gram body weight between any of the groups (data not shown).

Two days of treatment with GH increased GFAT activity in muscles of control and dw rats by 23%, but the increases were not statistically significant; the concentrations of nucleotide sugars in muscle were not significantly changed by this treatment (Fig. 4). In these experiments, the mean GFAT activity in muscles of dw rats was 51% of the activity of control rats (P = 0.00016), whereas UDP-HexNAc was 72% of controls (P = 0.00014). Treatment with rhGH for 2 and 5 days increased GFAT activity to 64 and 75% of that in controls, respectively. Although neither increase was

---

Table 1. Age-related differences in GFAT activity and nucleotide sugar concentrations in muscles of ad libitum-fed male control and dwarf rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, wk</th>
<th>n</th>
<th>GFAT Activity, pmol·mg⁻¹·min⁻¹</th>
<th>UDP-HexNAc, nmol/g muscle</th>
<th>UDP-Hex, nmol/g muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>3</td>
<td>74.2 ± 5.30</td>
<td>26.5 ± 0.74</td>
<td>20.3 ± 0.44</td>
</tr>
<tr>
<td>Dwarf</td>
<td>5</td>
<td>3</td>
<td>70.4 ± 6.93</td>
<td>23.8 ± 0.60</td>
<td>20.6 ± 1.00</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>7 (4)</td>
<td>38.6 ± 4.07*</td>
<td>22.6 ± 0.80</td>
<td>15.5 ± 0.91</td>
</tr>
<tr>
<td>Dwarf</td>
<td>8</td>
<td>7 (4)</td>
<td>26.2 ± 3.52†</td>
<td>18.9 ± 0.63†</td>
<td>13.4 ± 0.50</td>
</tr>
<tr>
<td>Control</td>
<td>9–10</td>
<td>16 (13)</td>
<td>23.6 ± 2.29</td>
<td>23.5 ± 1.85</td>
<td>12.5 ± 0.59</td>
</tr>
<tr>
<td>Dwarf</td>
<td>9–10</td>
<td>14 (11)</td>
<td>14.7 ± 1.94†</td>
<td>16.2 ± 1.27†</td>
<td>11.6 ± 0.90</td>
</tr>
<tr>
<td>Control</td>
<td>12–14</td>
<td>6 (6)</td>
<td>19.6 ± 1.28</td>
<td>21.2 ± 0.65</td>
<td>13.5 ± 0.74</td>
</tr>
<tr>
<td>Dwarf</td>
<td>12–14</td>
<td>4 (4)</td>
<td>7.5 ± 1.99†</td>
<td>15.4 ± 0.27†</td>
<td>11.9 ± 1.44</td>
</tr>
</tbody>
</table>

Control and dw rats were age matched and studied in parallel in the same experiments. Muscles were frozen and analyzed as described in RESEARCH DESIGN AND METHODS. Muscle L-glutamine:D-fructose-6-phosphate amidotransferase (GFAT) activity was measured in all rats. UDP-HexNAc, UDP-N-acetyl hexosamines; UDP-Hex, UDP-hexoses. Values are means ± SE; n, no. of animals studied; nos. in parentheses, nos. of rats with nucleotide sugar analyses. Significant differences between dwarf and age-matched control rats: *P < 0.05; †P < 0.01.

---

**Fig. 3.** GFAT activity and nucleotide sugar concentrations in livers of control and dw rats. Values are means ± SE; n = 7/group. *Significant difference between control and dw rats, P < 0.05.
statistically significant, GFAT activity in muscles of dw rats was no longer significantly different from that in controls after 5 days of treatment with GH. UDP-HexNAc concentrations in muscles from dw rats increased significantly after 5 days of treatment with rhGH ($P < 0.0012$) and were restored to levels observed in control rats ($P < 0.021; \text{Fig. 4}$). UDP-Hex concentrations also increased in dw muscles after GH treatment for 5 days ($P < 0.00075$) to levels exceeding those in untreated controls ($P < 0.021; \text{Fig. 4}$). Therefore, the UDP-HexNAc-to-UDP-Hex ratio continued to be significantly lower in muscles from GH-treated dwarf rats than in controls.

We also assessed whether the effects of GH treatment on HNSP in muscles of dw rats could be mimicked by IGF-I treatment. Ad libitum-fed, 10-wk-old dw rats were injected with 100 $\mu$g rhIGF-I or with saline twice daily for 5 days, as described above for GH treatment. This dose was chosen because it has been previously demonstrated to achieve weight gain and IGF-I levels in dw animals that are greater than or equal to those induced by 200 $\mu$g of GH daily (28, 32). In contrast to GH treatment, 5 days of IGF-I treatment had no effect on any of the parameters measured (Fig. 5). Treatment with IGF-I for 2 days, using the same protocol, was also ineffective ($n = 3$, data not shown).
**DISCUSSION**

The inherited defect in homozygous dw rats is a paucity of pituitary somatotrophs, which results in a marked (~90%) reduction in pituitary GH content and secretion. The responsiveness of somatotrophs to GH-releasing hormone (GHRH) and somatostatin and the sex-specific pulsatility of GH secretion are maintained, although the amplitude of individual pulses is markedly reduced in dw rats (5, 17). Circulating IGF-I is 25–30% of that observed in controls (28, 32). The metabolic abnormalities observed in this model are direct or indirect consequences of GH deficiency. Therefore, our data suggest that GH participates in the regulation of HNSP in skeletal muscle in young, postpubertal male rats. This conclusion is supported by the consistent reduction in GFAT activity in muscles of 8- to 14-wk-old dw rats, as well as the reduced concentration of the major products of HNSP, UDP-HexNAc. Furthermore, treatment with rhGH for 5 days restored UDP-HexNAc concentrations in muscles of dw rats to the level observed in controls and appeared to partially restore GFAT activity. Of interest is the apparent tissue specificity of HNSP regulation by GH, i.e., dw rats showed a greater percent reduction in GFAT activity in muscle than in liver, and UDP-HexNAc concentrations were reduced only in muscle. From these data, we conclude that GH deficiency reduces HNSP activity in skeletal muscle more than in liver. Similar tissue specificity was previously observed in ob/ob mice, where GFAT activity and UDP-HexNAc concentrations in muscle were significantly greater than in lean controls, without concomitant hepatic changes (6).

During the first 3 wk of life, there are no significant differences in growth rate between control and dw rats, but growth differences are clearly manifest between 3 and 5 wk of age (Ref. 8 and Fig. 1). In a preliminary study (n = 3/group), we did not detect significant differences in muscle HNSP activity between 5-wk-old control and dw rats. The emergence of differences between 5 and 8 wk of age (Table 1) coincides with the onset of puberty in rats, which is accompanied by a fall in somatostatin mRNA, a rise in GHRH mRNA expression in the hypothalamus, and the appearance of high-amplitude GH secretory pulses in male rats (1, 17).

Developmental regulation of GFAT activity has been observed in many rodent tissues (24). In general, GFAT activity in fetal or neoplastic tissues is higher than that in adults. It is 10-fold lower in muscles of adult rats than in fetal muscle tissue (24). In cultured myocytes derived from human skeletal muscle, GFAT activity is an order of magnitude higher than in human muscle biopsies (9). A gradual decline in muscle GFAT activity and UDP-HexNAc concentrations in muscles between 8 and 14 wk of age has been observed in mice (6). In the present study, the major decline of GFAT activity in rat muscle occurred between 5 and 9 wk, when circulating GH tends to be high (1, 17). Therefore, although our data indicate that GH acts as a positive modulator of GFAT activity and hexosamine synthesis in muscle, other unidentified factors may act as negative regulators during development. To our knowledge there is no information concerning possible sex-related differences in HNSP regulation in muscle.

The age-dependent decline in muscle UDP-HexNAc concentrations was smaller than that of GFAT activity (see Table 1), supporting the concept that glucose flux via HNSP reflects several metabolic parameters in addition to GFAT activity. These include the rate of glucose transport into the cell (7, 19) and the activity of competing pathways of glucose metabolism, such as glycolysis and glycogen synthesis (14, 26). Furthermore, UDP-GlcNAc is a potent inhibitor of GFAT activity (see reviews in Refs. 19, 26), which would dampen product accumulation in vivo. It is also possible that the downstream metabolism of UDP-HexNAc is developmentally regulated.

Our results with GH and IGF-I therapy suggest that GH rather than IGF-I regulates HNSP activity in muscle. In previous studies, which compared the effects of rhGH and rhIGF-I in dw rats, GH was much more effective than IGF-I in stimulating bone growth, although changes in body weight were similar, and serum IGFI was higher in IGF-I-treated than in GH-treated dw rats (8). However, GH and IGF-I exert differential effects on the expression of IGF-I binding proteins and on IGF-I expression in muscle of dw rats (18). GH infusion enhances IGF-I expression in muscles of dw rats primarily by inducing IGF-I production in fibroblasts residing between muscle fibers (31). GFAT activity in muscle mainly reflects GFAT expression by muscle cells (23). Thus, although IGF-I may participate via autocrine or paracrine mechanisms in the regulation of HNSP by GH in muscle, a direct effect of circulating IGF-I seems unlikely.

GH exerts dual effects on glucose metabolism in muscle. A rapid, transient, insulin-like effect appears to be mediated by tyrosine phosphorylation of insulin receptor substrate (IRS) 1 by janus kinase 2 (J AK2), which associates with and is activated by the dimerized, occupied GH receptor (2, 11). Prolonged GH treatment results in insulin resistance, as manifested by increased circulating insulin concentrations, decreased glucose utilization by muscle, increased lipolysis, and eventually hyperglycemia (reviewed in Ref. 29). The mechanism of this insulin resistance is poorly understood. Insulin receptor number is increased in muscle, GLUT-4 expression and subcellular distribution are unchanged, but GLUT-1 expression is diminished (22, 29) in the presence of prolonged GH exposure. An impairment in insulin-stimulated phosphorylation on tyrosine residues of the insulin receptor β-subunit and of IRS-1 has been proposed as a cause of GH-induced insulin resistance (29), and impaired activation of muscle glycogen synthase by insulin has been reported (3). In our studies, plasma glucose concentrations were similar in nonfasted dw rats with or without GH treatment for 5 days. Mean plasma insulin was higher in the GH-treated group, but the difference was not significant (data not shown). UDP-glucose is the obligatory substrate of glycogen synthase, and the concentration of UDP-Hex in muscle reflects the rate of
glucose transport and phosphorylation, on one hand, and the rate of UDP-glucose utilization, (i.e., glycogen synthesis) on the other (reviewed in Ref. 26). The fact that UDP-Hex increased significantly in muscles of dw rats after 5 days of GH treatment suggests that glycogen synthase activity may have been reduced. Increased fatty acid (FFA) oxidation promotes glucose flux via HNSP, possibly by inhibiting glycolytic flux at the level of phosphofructokinase (14). FFA oxidation in muscle is increased by GH (3, 21) and may thereby contribute to increased UDP-HexNAc concentrations in muscles of GH-treated dw rats. Whether GH deficiency increases insulin sensitivity is controversial (21, 30) and has not been investigated in dw rats. Although in this study fasting glycemia was significantly lower in dw rats than in controls, neither the serum insulin concentrations nor the insulin-to-glucose ratios were altered significantly (Fig. 2 and data not shown, respectively).

HNSP has been implicated in mediating the induction of certain growth factors in response to hyperglycemia in vascular smooth muscle cells and in renal tubular and mesangial cells (reviewed in Refs. 16, 20). Of particular interest is a recent report indicating that increased flux via HNSP may mediate the hyperglycemia-induced overexpression of transforming growth factor-β, in mesangial cells, which in turn leads to increased matrix production (16). Dw rats are in great part protected from developing the renal complications of diabetes, i.e., renal and glomerular hypertrophy and microalbuminuria (12). Immunocytochemical studies suggest that GFAT protein may be induced in glomerular epithelial and mesangial cells by uncontrolled diabetes (23). Whether GH participates in the regulation of HNSP in the kidney needs to be established.

In summary, the data presented indicate that GH acts as a positive regulator of HNSP in skeletal muscle. Whether GH’s effect is direct or is mediated by muscle-derived IGF-I or is secondary to other more direct effects of GH on intermediary metabolism is not known. Assuming that our findings in a GH-deficient model may apply to conditions with chronic GH excess, one may speculate that increased glucose flux via HNSP may contribute to GH-induced insulin resistance.

We gratefully acknowledge the gift of rhGH-β by Genentech to S. M. Willi. We thank Drs. Nancy Wright and Lyndon Key for providing the dwarf rats used in initial experiments and breeding. Dr. Key for the generous gift of rhGH, and J effrey S. Koning for excellent technical assistance and Barbara Wojciechowsi for expert statistical advice and analysis. This work was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-02001.

Address for reprint requests: M. G. Buse, Dept. of Medicine, Division of Endocrinology, Medical Univ. of South Carolina, 171 Ashley Ave., Charleston, SC 29425-2222.

Received 29 April 1998; accepted in final form 6 November 1998.

REFERENCES


18. Lemmy, A. B., J. Glassford, H. C. Flicksmith, J. M. P. Holly, and J. M. Pell. Differential regulation of tissue insulin-like growth factor-binding protein (IGFBP) -3, IGF-1 and IGF type 1 receptor mRNA levels, and serum IGF-1 and IGFBP concentra-