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Stimulation of Lipolysis but Not of Leptin Release by Growth Hormone Is Abolished in Adipose Tissue from Stat5a and b Knockout Mice

John N. Fain,*[,1] James H. Ihle,*[,‡] and Suleiman W. Bahouth†

Department of Biochemistry, †Department of Pharmacology, College of Medicine, University of Tennessee, Memphis, Tennessee 38163; and ‡Howard Hughes Medical Institute, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38105

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The present studies examined the effects of growth hormone (GH) on lipolysis and leptin release by adipose tissue from mice incubated for 24 h in primary culture. In adipose tissue from control mice GH enhanced lipolysis without affecting leptin release. The lipolytic action of GH was unaffected in adipose tissue from Stat5b−/− male mice but leptin release was enhanced by GH in fat from Stat5b−/− mice. In adipose tissue from Stat5ab−/− female mice no significant lipolytic action of GH was seen but leptin release was enhanced by GH. An insulin-like effect of GH on glucose conversion to lactate was also seen in mice deficient in Stat5ab−/−. These results suggest that the lipolytic action of GH involves the Stat5 proteins while the insulin-like effects of GH on glucose metabolism and leptin release involve different mechanisms.

Key Words: **lipolysis; dexamethasone; adipose tissue; insulin; growth hormone; Stat5ab.**

The disruption of the genes for both Stat5a and 5b results in mice with severe growth retardation, a 50% reduction in the serum IGF-1 content, and dramatic reductions in the level of the major urinary proteins [1]. These proteins are a family of α2-microglobulin-related liver secretary proteins that are excreted in male mouse urine at levels at least 3-fold higher than in females and growth hormone is required for their expression [2].

The present studies were designed to determine whether the effects of GH on mouse adipose tissue are affected by simultaneous disruption of both Stat5a and 5b proteins. There is a well established lipolytic effect of GH on rat adipose tissue that is delayed in onset and is seen in the presence of glucocorticoids [3–4]. It is now thought that this anti-insulin effect of GH involves decreases in the level of the $G_{i\alpha 2}$ protein [5, 6]. There is another effect of GH that is insulin-like, immediate in onset and is best seen in adipose tissue from hypophysectomized rats [3–4].

A delayed lipolytic effect of GH has been reported in mouse adipose tissue [7]. Furthermore an insulin-like effect of GH has also been seen after pre-incubation of mouse adipose tissue [8]. The present studies indicate that the lipolytic action of GH is abolished in adipose tissue from mice deficient in both Stat5a and 5b proteins (Stat5ab−/−) while the insulin-like effects of GH are enhanced in adipose tissue.

MATERIALS AND METHODS

Epididymal adipose tissue for each experiment except those involving Stat5ab−/− mice was obtained by pooling the tissue from 4–6 fed male mice. The pooled adipose tissue was cut into small pieces and incubated in 5 ml of a modified DMEM:Ham's F12 medium for 24 h [9, 10].

Stat5b−/− mice and Stat5ab−/− mice (C57/B6 × 129SV strain) were prepared as described by Teglund *et al.* [1]. The Stat5b−/− male mice were compared to littermate controls (+/+) and both groups were fed laboratory chow. However, Stat5ab−/− mice have less than 20% of the fat pad content of controls if fed laboratory chow. Stat5ab−/− mice as well as their controls were therefore fed for 4–8 weeks a pelleted high fat diet containing 27% casein, 20% Crisco, 46% sucrose, 2% RP vitamin mix and 5% RP mineral mix #10 that was supplied by Purina Mills of Richmond, IN. The control group was composed of two-thirds heterozygotes (+/−) for Stat5ab and one-third Stat5ab (+/+) mice. While feeding mice this diet enhanced the fat content of both groups the weight of the fat pads averaged 300 and 450 mg respectively in male and female Stat5ab−/− mice and 500 and 840 mg in control male and female mice. Some dwarf Stat5ab−/− mice [about 10–20%] weighed less than 11 g and were not used since these mice had insufficient fat for the studies. Similarly controls that weighed over 28 g were excluded since they had much larger amounts of adipose tissue. The weight of Stat5ab−/− mice averaged 17 g in male and 16 g in female mice while male controls averaged 26 g and 23 g for female controls in agreement

Abbreviations used: GH, growth hormone; STAT, signal transducers and activators of transcription.

[1] To whom correspondence should be addressed. Fax: 901-448-7360. E-mail: jfain@utmem.edu.

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TABLE 1

The Effects of GH on Lipolysis Were Unaffected in Stat5b Knockout Mice

Parameter	Stat5b+/+ [21]	Stat5b−/− knockouts [12]
Basal lipolysis in μmol/g	27.8 ± 2.7	25.0 ± 2.7
% increase due to 10 nM GH	+38 ± 6%*	+31 ± 11%*
Basal leptin release in ng/g	168 ± 18	182 ± 18
% change due to 10 nM GH	−3 ± 5%	+21 ± 8%*
Weight of mice (g)	27.5 ± 1.1	29.7 ± 2.4
Weight of epididymal adipose tissue/mouse (mg)	470 ± 40	590 ± 90
Serum leptin (ng/ml)	4.6 ± 1.2	4.8 ± 1.0

Note. Cut pieces of epididymal adipose tissue from male mice fed laboratory chow (80–260 mg/tube) were incubated in 5 ml of medium for 24 h in the presence of 25 nM dexamethasone. The number of determinations for each group are shown in brackets. The increases in lipolysis due to GH were statistically significant in both groups and of leptin release in the Stat5b knockouts ($p < 0.025$ indicated by an asterisk).

with prior studies [1]. Each experiment using adipose tissue from Stat5ab−/− mice utilized tissue from a single animal divided between 4–8 tubes and the same was true for controls.

Aliquots (20 to 50 μl) of the medium were used to measure the leptin content using radioimmunoassay kits with antibody raised against rat or mouse leptin and with rat or mouse leptin standards from Linco Research, Inc. (St. Charles, MO). Lipolysis was measured by analysis of glycerol release into the medium (10 to 50 μl aliquots) by the procedure of Boobis and Maughan [11]. Lactate was measured by the same procedure using lactate dehydrogenase [11]. The GH preparation was human recombinant GH (lot PS9033AX) produced by Genentech Inc. The insulin was bovine insulin obtained from Sigma as were the other hormones and reagents.

The effects of added agents were calculated as the percentage change from the incubation control in each experiment since this resulted in a more normal distribution of the data. Statistical comparisons were made using Student's *t* test on the paired differences.

RESULTS

Effect of deletion of the Stat5b gene on GH action in mouse adipose tissue. GH increases the phosphorylation of Stat5b which is a substrate for the JAK2 kinase activated by the GH receptor [12]. The lipolytic action of GH was unaffected in Stat5b−/− mice (Table 1). There was no effect of the Stat5b deletion on body weight, serum leptin and only a small, but not statistically significant, increase in the amount of epididymal fat (Table 1). The data on body weight are in agreement with those previously reported by Teglund *et al.* [1].

We did not see any increase in leptin release due to GH in the adipose tissue from control mice in the presence of 25 nM dexamethasone. However, a statistically significant increase (+21%) in leptin release due to GH was seen in Stat5b−/− mice (Table 1).

The stimulation of lipolysis by GH is not seen in adipose tissue from Stat5ab−/− mice. There is another Stat5 gene [Stat5a] that encodes a protein which is 95% identical in its amino acid sequence to Stat5b and these proteins may be functionally redundant [1]. We therefore turned to studies using Stat5a and 5b double knockout mice. While Stat5b−/− mice have the same or a slightly higher weight and fat content that is not the case for Stat5ab−/− mice which have 20 to 40% decrease in body weight and an 80% reduction in fat pad weight [1]. In our initial studies using Stat5ab−/− mice there was so little body fat in animals fed laboratory chow that it was not possible to obtain enough adipose tissue for *in vitro* studies. We therefore fed the control and Stat5ab−/− mice for 4–8 weeks after weaning on a synthetic diet containing 20% fat.

Basal lactate formation was 63% higher in adipose tissue from Stat5ab−/− mice (Table 2). There was a stimulation of lactate formation due to the combination of insulin plus T_3 in adipose tissue from control which was also 63% greater in fat from Stat5ab−/− mice. However, while GH increased lactate formation by 34% in adipose tissue from Stat5ab−/− mice in the presence of insulin plus T_3 it had no effect in tissue from controls (Table 2). Furthermore there was no effect of GH in the absence of insulin plus T_3 in Stat5ab−/− mice. These data indicate that there is a higher rate of glucose uptake and conversion to lactate in tissue from Stat5ab−/− mice and that GH, in the presence of T_3 and insulin, has an insulin-like effect in adipose tissue of these animals.

We found that serum leptin levels were elevated by 52% in female mice but decreased by 28% in male Stat5ab−/− mice (Table 3). The amount of fat in the Stat5ab−/− mice was 40% less in the male mice and 49% less in the female mice (Table 2). It is unclear why the serum leptin was elevated in the Stat5ab−/− female mice.

TABLE 2

Comparison of GH Effects on Lipolysis and Lactate Formation in Stat5ab Double Knockout Mice

Parameter	Controls n = 17	Stat5ab−/− n = 18
Basal lactate formation in μmol/g	115 ± 11	188 ± 19
% change due to GH	−9 ± 6%	−6 ± 4%
% change due to insulin + T_3	+24 ± 4%**	+39 ± 8%**
% change due to GH in presence of insulin + T_3	+1 ± 7%	+34 ± 11%**
Basal lipolysis in μmol/g	38.5 ± 4.0	43.3 ± 2.8
% change due to GH	+19.0 ± 6.2**%	+7.3 ± 4.9%

Note. Pieces of epididymal or parametrial adipose tissue (approximately 80 mg/tube from control and 42 mg/tube from Stat5ab−/− fed the high fat diet were incubated for 24 h in 5 ml of medium containing 25 nM dexamethasone without, with 10 nM GH, or with 10 nM insulin plus 10 nM T_3. The values are from 17 control mice (6 females and 11 males) and 18 Stat5ab−/− mice (9 females and 9 males). Statistically significant effects of added hormones are as follows based on the paired differences: *$p < 0.05$ and **$p < 0.01$.

TABLE 3

The Lipolytic Effect of GH Is Abolished in Adipose Tissue from Stat5ab−/− Mice

Parameter	Control	Stat5ab−/−	% difference
Mouse weight in grams			
♂	26.4 ± 0.7	17.0 ± 0.8*	−36%
♀	23.2 ± 0.2	16.1 ± 0.4*	−30%
Fat weight in milligrams			
♂	500 ± 20	300 ± 40*	−40%
♀	800 ± 60	410 ± 35*	−49%
Serum leptin			
♂	7.6 ± 0.7	5.5 ± 0.8	
♀	7.3 ± 1.0	11.1 ± 1.1*	+52%
Lipolysis at 24 h in μmol/g			
♂	35 ± 4	46 ± 4	
♀	36 ± 4	40 ± 3	
% change due to GH			
♂	+1 ± 4%	+14 ± 8%	
♀	+23.4 ± 5.1%	+4 ± 4.8%*	−83%

Note. Ten male and 18 female controls were compared to 10 male and 20 female Stat5ab−/− mice. All mice were fed the high fat diet. Basal leptin release and lipolysis were measured over 24 h in the presence of 25 nM dexamethasone. The amount of adipose tissue incubated per tube averaged 65 mg for male and 96 mg of female controls while from the Stat5ab−/− mice it averaged 40 mg for males and 50 mg of females. The values are shown as the mean ± SEM and significant differences between the two groups are indicated by an asterisk ($p < 0.05$). The effect of 10 nM GH on lipolysis is shown as the % change based on paired analysis. Only the increase in lipolysis due to growth hormone in female controls was significant ($p < 0.001$).

The studies on the Stat5ab−/− mice utilized mice fed the high fat diet (Tables 2–3). The data from Stat5ab−/− mice of both sexes were combined in the studies shown in Table 2. There was a 19% increase in lipolysis due to GH in adipose tissue from control mice that was statistically significant (Table 2). In contrast there was no significant increase in lipolysis using adipose tissue from Stat5ab−/− mice. Examination of the data indicated that the increase in lipolysis due to GH was primarily in the female controls so we expanded the series to 58 mice and the data are shown in Table 3 for each sex. For reasons that are unclear, lipolysis was only increased by GH in the adipose tissue from the female controls of this strain fed the high fat diet. There was a 23% increase in lipolysis due to GH in fat from female mice fed the high fat diet. Basal lipolysis was unaffected in adipose tissue from female Stat5ab−/− mice but there was no significant effect of GH on lipolysis in the fat from these mice (Table 3).

We could not demonstrate any effect of GH on leptin release in control female mice fed the high fat diet in either the absence or presence of dexamethasone (Fig. 1). However the release of leptin by adipose tissue from Stat5ab−/− mice was reduced by half in the absence of dexamethasone (Fig. 1). Furthermore this defect was reversed in the presence of GH which stimulated leptin release by +107 ± 24% ($p < 0.05$) in the 7 paired experiments using adipose tissue from female Stat5a/b−/− mice (Fig. 1). There was also an increase in leptin release due to GH in tissue from Stat5a/b−/− mice if incubated in the presence of 200 nM dexamethasone but the effect was one-third that seen in the absence of dexamethasone and statistically insignificant (+33 ± 30%) in the experiments shown in Fig. 1. The data in Fig. 1 also demonstrate that the ability of dexamethasone to stimulate leptin release was unaffected by Stat5a/b deletion.

DISCUSSION

The lipolytic action of GH on rat adipose tissue first reported by Fain *et al.* [13] requires the presence of glucocorticoids and is now thought to be secondary to a reduction in the levels of $G_{i\alpha 2}$ in adipocyte membranes [5, 6]. While a reduction in the level of $G_{i\alpha 2}$ in adipocytes might explain the lipolytic action of GH, the insulin-like effects of GH on leptin release and lactate formation may not be linked to a reduction in $G_{i\alpha 2}$. It appears that GH has multiple effects on rodent adipose tissue and that leptin release is stimulated when lipolysis is unaffected and the reverse is seen with respect to lipolysis.

It is well-established that the addition of GH to adipose tissue from hypophysectomized rats results in an inhibition of lipolysis and stimulation of glucose metabolism [3, 4]. We saw a stimulation by GH of glucose conversion to lactate in adipose tissue from Stat5ab−/− mice in the presence of insulin plus T_3 (Table 2). This is an insulin-like effect of GH. Furthermore the basal formation of lactate was 63% higher in fat from the Stat5ab−/− mice. One complication in the interpretation of the data from the Stat5ab−/− mice was the reduced adipose tissue mass in both male (40%) and female (46%) Stat5ab−/− mice even on the high fat diet (Table 3). Therefore it is difficult to determine which effects of deletion of the Stat5a and Stat5b gene products are due to direct effects versus indirect effects due to a reduction in fat content.

GH signaling involves the JAK2 tyrosine kinase which has been reported to initiate a wide variety of pathways including the Ras, mitogen-activated protein kinase, Stat5, insulin receptor substrate, phosphatidylinositol 3-kinase, Elk-1, and p125 focal adhesion kinase pathways [14–17]. Since GH can apparently activate most if not all of the pathways activated by insulin it is unclear why GH and insulin have such different effects on metabolism and why GH is a physiological antagonist of insulin [12].

There is evidence that the glucocorticoid receptor is a transcriptional co-activator for Stat5. The formation of casein in mammary epithelial cells is synergistically induced by either GH or prolactin in the presence of glucocorticoid [18]. Stocklin *et al.* [19] found that Stat5 forms a complex with the glucocorticoid receptor which

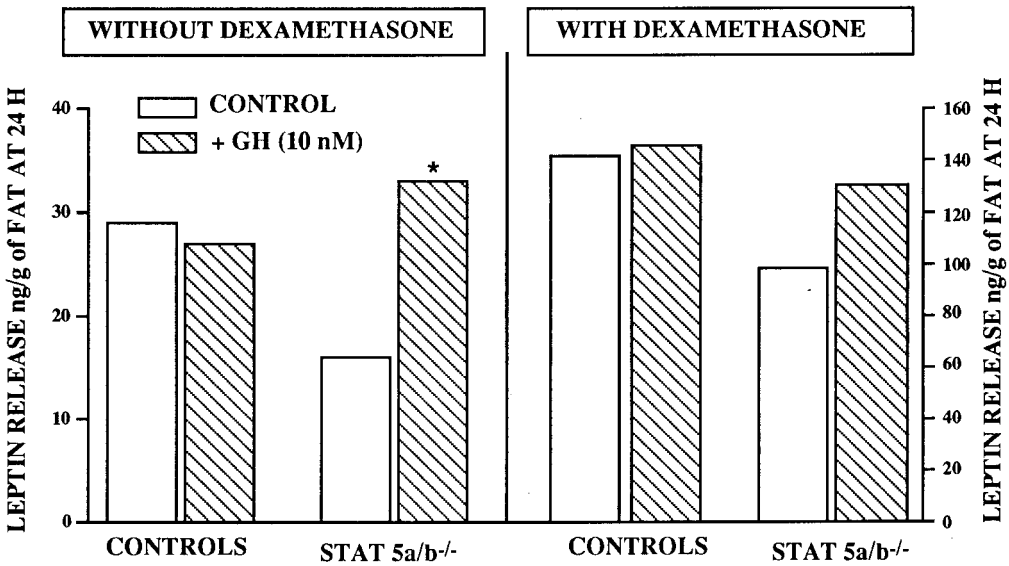

FIG. 1. GH stimulates leptin release in adipose tissue from mice deficient in both Stat5a and 5b in the absence of dexamethasone. Seven female controls were compared to 7 Stat5ab−/− female mice. All mice were fed the high fat diet. Leptin release was measured over 24 h in the absence or presence of 200 nM dexamethasone either without or with 10 nM GH. The amount of adipose tissue incubated per tube averaged 130 mg for controls while from the Stat5ab−/− mice it averaged 75 mg. The values are shown as the means of 7 paired experiments for each group. Statistically significant effects of GH are indicated by an asterisk based on paired differences ($p < 0.05$).

binds to DNA independently of the glucocorticoid response element and upregulates Stat5-response element-containing promoters. In contrast the complex of Stat5 with the glucocorticoid receptor diminishes the effects of glucocorticoids mediated through a promoter containing the glucocorticoid response element [18]. Our data suggest that the stimulation of lipolysis due to GH in the presence of glucocorticoids, at least in female mice, may involve Stat5 proteins in a manner similar to that for the synergistic stimulation of casein mRNA formation by GH and glucocorticoids in mammary cells [19].

However there was a stimulation of leptin release by GH alone in adipose tissue from Stat5b−/− mice (Table 1) or Stat5ab−/− mice (Fig. 1) and an insulin-like effect of GH on lactate accumulation under appropriate conditions in Stat5ab−/− mice (Table 2). Yamauchi *et al.* [14] have shown that GH can stimulate the tyrosine phosphorylation of the insulin receptor substrate proteins secondary to JAK2 kinase activation and our results suggest that this effect is enhanced in fat from Stat5ab−/− mice.

An indication of some of the complexities in the regulation of lipid metabolism by GH is the recent report that in a transgenic strains of rats carrying a low copy numbers of a transgene for GH had low GH levels in plasma but a normal nose to tail length [20]. However, there was massive obesity in these animals which resulted in a doubling in body weight by 25 weeks. It is unclear what was responsible for the obesity but the administration of GH for a week resulted in a decrease in fat content [20].

In Stat5b−/− mice there was no impairment of the lipolytic action of GH and Teglund *et al.* [1] found no growth retardation of these mice. However, Udy *et al.* [2] reported that in male but not in female mice growth during the first 6 weeks of life was markedly retarded. They also reported that the mice had significantly less adipose tissue during the first 9 weeks of life but that subsequently some of the Stat5b knockout mice developed obesity [2]. We found only a small increase in adipose tissue in Stat5b deficient mice. Furthermore both we and Teglund *et al.* [1] found a marked retardation of growth as well as fat accumulation in Stat5ab−/− mice which could not be corrected even on a high fat diet. In conclusion we suggest that the activation by GH of lipolysis involves Stat5ab while the mechanism for stimulation of leptin release and glucose conversion to lactate appears to involve other pathways.

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