Differential impact of selective GH deficiency and endogenous GH excess on insulin-mediated actions in muscle and liver of male mice

Jose Cordoba-Chacon,1,2* Manuel D. Gahele,1,2,3* Owen P. McGuinness,4 and Rhonda D. Kineman1,2

1Research and Development Division, Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois; 2Section of Endocrinology, Diabetes, and Metabolism, Department of Medicine, University of Illinois at Chicago, Chicago, Illinois; 3Department of Cell Biology, Physiology, and Immunology, University of Córdoba, Instituto Maimónides de Investigación Biomédica de Córdoba/Hospital Universitario Reina Sofia, and CIBER de la Fisiopatología de la Obesidad y Nutrición, Córdoba, Spain; and 4Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee

Submitted 9 September 2014; accepted in final form 26 September 2014

Cordoba-Chacon J, Gahele MD, McGuinness OP, Kineman RD. Differential impact of selective GH deficiency and endogenous GH excess on insulin-mediated actions in muscle and liver of male mice. Am J Physiol Endocrinol Metab 307: E928–E934, 2014. First published September 30, 2014; doi:10.1152/ajpendo.00420.2014.—A reciprocal relationship between insulin sensitivity and glucose tolerance has been reported in some mouse models and humans with isolated changes in growth hormone (GH) production and signaling. To determine if this could be explained in part by tissue-specific changes in insulin sensitivity, hyperinsulinemic-euglycemic clamps were performed in mice with adult-onset, isolated GH deficiency and in mice with elevated endogenous GH levels due to somatotrope-specific loss of IGF-I and insulin receptors. Our results demonstrate that circulating GH levels are negatively correlated with insulin-mediated glucose uptake in muscle but positively correlated with insulin-mediated suppression of hepatic glucose production. A positive relationship was also observed between GH levels and endpoints of hepatic lipid metabolism known to be regulated by insulin. These results suggest hepatic insulin resistance could represent an early metabolic defect in GH deficiency.

growth hormone; hepatic glucose production; triglycerides; glucose disposal; hyperinsulinemic-euglycemic clamps

HUMANS WITH LONG-TERM adult-onset growth hormone deficiency (AOGHD) have been reported to have impaired glucose tolerance (5, 27, 32), systemic insulin resistance, and increased hepatic glucose production [HGP; (12, 16, 24)], with a higher prevalence of diabetes (1). However, the direct impact of growth hormone deficiency (GHD) on insulin-mediated glucose homeostasis is not clear cut. AOGHD patients can show reduced (5), normal (12), or elevated (8) insulin levels. A wide spectrum of insulin sensitivity in AOGHD patients may be in part due to the length, severity, multiplicity, and etiology of pituitary deficiencies, leading to variable effects on body composition (1, 9, 14, 30). In fact, insulin sensitivity was shown to be improved in AOGHD subjects compared with age-, sex-, and body mass index (BMI)-matched controls (28). Also, subjects with congenital isolated GH deficiency resulting from an activating mutation in the growth hormone-releasing hormone (GHRH) receptor are more insulin sensitive compared with their unaffected relatives despite increased visceral adiposity and glucose intolerance (27, 32). Glucose intolerance, with improved systemic insulin sensitivity [as measured by insulin tolerance tests (ITT)], is also observed in mouse models with GHD (7) and growth hormone (GH) resistance (11, 13). Notably, the opposite metabolic phenotype (improved glucose tolerance with normal/reduced insulin sensitivity) is observed in mice with elevated heterologous (6) or endogenous (10) GH levels.

The disconnect between systemic insulin sensitivity and glucose tolerance observed in mouse model systems and humans with isolated changes in GH production and signaling (6, 7, 10, 13, 15, 22, 27, 32) suggests GH might differentially alter tissue-specific insulin sensitivity and/or change β-cell function/mass. To differentiate between these possibilities, we have performed studies using mice with adult-onset, isolated GHD [AOGHD (22)] and mice with elevated endogenous GH levels due to somatotrope-specific loss of IGF-I and insulin receptors [HiGH (10)]. In both AOGHD and HiGH mice the modification in GH levels is selective with concomitant changes in IGF-I, without appreciable alterations in expression/secretion/action of other pituitary hormones (10, 22). In addition, AOGHD mice are not dwarves but show a modest decrease in lean mass (7), whereas HiGH mice are not giants but show a modest increase in lean mass (10). Therefore, these models represent a unique opportunity to examine the impact of selectively lowering and raising endogenous GH levels while minimizing the impact of GH/IGF-I on body mass. We have reported that alterations in glucose tolerance in these mouse models (7, 10) could not be attributed to changes in glucose-stimulated insulin secretion from islet cultures or changes in β-cell mass (Ref. 7 and Cordoba-Chacon J, Majumdar N, Pokala NK, Gahele MD, Kineman RD, unpublished observations). The current study was designed to determine if changes in circulating GH levels alter tissue-specific insulin actions. Specifically, hyperinsulinemic-euglycemic clamps with double-isotope labeling (2, 3) were performed in male AOGHD and HiGH mice and their respective controls. Results demonstrate circulating GH levels are negatively correlated with insulin-mediated glucose uptake in muscle but positively correlated with insulin-mediated suppression of HGP.

MATERIALS AND METHODS

Animals and experimental endpoints. All experiments were approved by the University of Illinois at Chicago and Jesse Brown Veterans Affairs Medical Center Institutional Animal Care and Use Committee (IACUC), with the additional approval of the Vanderbilt...
University IACUC for the hyperinsulinemic-euglycemic clamp studies. Mice were housed under a 12:12-h light-dark cycle at 22–24°C and provided standard rodent chow diet [Teklad LM-485 Mouse/Rat Sterilizable irradiated diet (3.51 kcal; fat: 17% kcal; carbohydrate: 58% kcal; protein: 25% kcal) Teklad diets, Madison, WI] or a low-fat (LF) diet [catalog no. 12450B (3.84 kcal/g; fat: 10% kcal; carbohydrate: 70% kcal; protein: 20% kcal) Research Diets, New Brunswick, NJ] as indicated. AOiGHD was induced at 12 wk by treating mice heterozygous for both the rat GH promoter-driven Cre recombinase transgene and the inducible diphertheria toxin (DT) receptor transgene (rGHp-Cre/<−/−;DT<−/−>) with DT to selectively destroy somatotropes, leading to a >60% reduction in circulating GH levels, as previously described (22). DT-treated, rGHp-Cre/<−/−;DT<−/−> littermates (C57Bl/6J background) served as GH-replete controls (22). Hyperinsulinemic-euglycemic clamp was performed 32–34 wk after mice were generated by crossbreeding rGHp-Cre/<−/−;DT<−/−> inductions. Male HiGH mice and their littermate controls (C57Bl/6J background) were generated by crossbreeding rGHp-Cre/<−/−;DT<−/−> HiGH induction. Male HiGH mice and their littermate controls (C57Bl/6J background) were generated by crossbreeding rGHp-Cre/<−/−;DT<−/−> littermates (C57Bl/6J background) as GH-replete controls (22).

**RESULTS**

AOiGHD mice show improved muscle insulin sensitivity, whereas insulin-induced suppression of HGP is impaired. Body weights did not differ between AOiGHD mice and their littermate controls (31.2 ± 0.8 vs. 29.6 ± 0.7 g). Although body composition was not measured in this cohort of mice, we have previously reported AOiGHD mice have reduced lean mass, but no significant change in fat mass, at 40–46 wk of age (28–34 wk GHD) (7). Basal and clamp insulin levels did not differ between AOiGHD (30–32 wk GHD) and control (Fig. 1A) mice. To maintain euglycemia during 50–90 min of the hyperinsulinemic clamp (Fig. 1B), a higher glucose infusion rate

**Table 1. List of primers for qRT-PCR used in this study**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Position</th>
<th>Product Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>NM_007393.3</td>
<td>313</td>
<td>205</td>
</tr>
<tr>
<td>GAPDH</td>
<td>NM_008804.2</td>
<td>735</td>
<td>104</td>
</tr>
<tr>
<td>HPRT</td>
<td>NM_013556</td>
<td>838</td>
<td>183</td>
</tr>
<tr>
<td>Cyclophilin A</td>
<td>NM_008907.1</td>
<td>421</td>
<td>109</td>
</tr>
<tr>
<td>Pck1</td>
<td>NM_011044.2</td>
<td>1496</td>
<td>128</td>
</tr>
<tr>
<td>SREBP1c</td>
<td>NM_006532716.1</td>
<td>1623</td>
<td>70</td>
</tr>
<tr>
<td>AAC</td>
<td>NM_133360.2</td>
<td>6022</td>
<td>158</td>
</tr>
<tr>
<td>FASN</td>
<td>NM_007988.3</td>
<td>7162</td>
<td>200</td>
</tr>
<tr>
<td>SCD-1</td>
<td>NM_009127.4</td>
<td>1106</td>
<td>137</td>
</tr>
</tbody>
</table>

qRT-PCR, quantitative real-time RT-PCR; HPRT, hypoxanthine guanine phosphoribosyl transferase; Pck1, phosphoenolpyruvate carboxykinase 1; SREBP1c, sterol regulatory element binding transcription factor 1; AAC, acetyl-CoA carboxylase; FASN, fatty acyl synthase; SCD-1, stearoyl-coenzyme A desaturase 1.
The differences between control and AOiGHD mice (C, and F) 120 min, controls: 34.90 GIR did not differ between groups (mean GIR of 100, 110, and pared with GH-replete controls (Fig. 1). At steady state, the GIR did not differ between controls and AOiGHD mice (C, D, E, and F).

(GIR mg·kg⁻¹·min⁻¹) was required for AOiGHD mice compared with GH-replete controls (Fig. 1C). At steady state, the GIR did not differ between groups (mean GIR of 100, 110, and 120 min, controls: 34.90 ± 3.99 vs. AOiGHD: 37.58 ± 2.76 mg·kg⁻¹·min⁻¹). However, the vastus lateralis of AOiGHD mice showed improved insulin-mediated glucose uptake (P < 0.006) compared with controls (Fig. 1D), whereas glucose uptake was not significantly altered in the gastrocnemius, soleus, heart, or fat. Basal HGP [=endogenous glucose production (ERa)] did not differ between AOiGHD and control mice (Fig. 1E). At steady state, insulin infusion suppressed HGP in both groups. However, HGP of AOiGHD remained significantly elevated above controls after the clamp (Fig. 1E). Consistent with this observation, phosphoenolpyruvate carboxykinase 1 (Pck1) expression was elevated in AOiGHD livers compared with controls (Fig. 2E). Basal hepatic glucose production (HGP = ERa) did not differ between HiGH and control mice (Fig. 2E). At steady state, insulin infusion suppressed HGP in both groups. However, insulin suppression of HGP during the clamp was significantly amplified in HiGH mice compared with controls (Fig. 2E). Consistent with this observation, Pck1 expression (Fig. 2F) is reduced in HiGH livers, suggesting enhanced insulin actions in the HiGH livers.

**Additional endpoints supporting a positive relationship between circulating GH levels and hepatic insulin sensitivity.** AOiGHD mice displayed reduced hepatic TG content (Fig. 3A), which was associated with a downregulation of SCD-1 gene expression, without significant changes in other lipogenic genes in LF-fed mice (Fig. 3B) and chow-fed mice (data not shown). Reduced hepatic TG content in AOiGHD mice may be due, in part, to an increase in VLDL-TG production based on the fact that circulating TG concentrations were elevated following tyloxapol injection (Fig. 3C).

HiGH mice displayed an increase in hepatic TG content (Fig. 3D), and this was associated with an increase in the

---

**Fig. 1.** Assessment of tissue-specific insulin actions by hyperinsulinemic-euglycemic clamps in adult-onset, isolated growth hormone deficiency (AOiGHD) and control mice, maintained on a low-fat (LF) diet. A: plasma insulin levels before and after hyperinsulinemic-euglycemic clamp. B: blood glucose (B) and glucose infusion ratio (GIR, C) during hyperinsulinemic-euglycemic clamp. D: tissue-specific glucose metabolic index (Rg) of gastrocnemious (GAS), vastus lateralis (VAS L), urogenital fat (UG-fat), soleus (SOL), and heart (HRT). E: endogenous glucose production (ERa) before and after hyperinsulinemic-euglycemic clamp [n = 8–9 mice/group, 30–32 wk of growth hormone deficiency (GHD), LF feeding started at 4 wk of age]. F: hepatic phosphoenolpyruvate carboxykinase 1 (Pck1) expression of AOiGHD and control mice (28 wk of GHD, chow fed, n = 5–14 mice/group). *Significant difference between clamped and basal state within genotype (E). **P < 0.05 and ***P < 0.01, significant difference between control and AOiGHD mice (C, D, E, and F).
expression of lipogenic genes in LF-fed mice (Fig. 3E) and chow-fed mice (data not shown). The increase in hepatic lipid accumulation in HiGH mice may be due, in part, to a reduction in hepatic TG secretion, as assessed after tyloxapol injection (Fig. 3F).

**DISCUSSION**

Using a genetic approach to either modestly decrease (AOiGHD) or increase (HiGH) GH tone, we examined hepatic and peripheral insulin action using a hyperinsulinemic-euglycemic clamp. The regulation of hepatic and peripheral insulin action was differentially regulated by GH. Consistent with prior work (18–20, 23, 25, 29), relative GH excess resulted in mild peripheral insulin resistance, whereas relatively low GH levels modestly improved insulin action. The striking observation was the impact of GH on liver insulin action. A relative excess of GH enhanced hepatic insulin action while relative deficiency of GH impaired hepatic insulin action. Thus, inappropriate GH secretion can have markedly different effects on hepatic and peripheral insulin action.

Hyperinsulinemic-euglycemic clamps revealed male AOiGHD had improved insulin-dependent muscle glucose disposal but impaired insulin-dependent suppression of HGP. We optimized the study design to detect alterations in both hepatic and peripheral insulin action by using a relatively low dose of insulin in the clamps (2 mU insulin·kg⁻¹·min⁻¹). This allowed us to detect an impairment in hepatic insulin action in animals on a LF diet. These results may initially seem at odds with hyperinsulinemic-euglycemic clamp studies performed in AOGHD patients, showing systemic insulin resistance, in addition to increased HGP (12, 16, 24). However, evidence has accumulated suggesting that changes of systemic insulin sensitivity may be secondary to alterations in body composition (increase in visceral adiposity and decrease in lean mass) and not due to GHD per se (14, 30). This is supported by studies showing AOGHD patients can have reduced (5) or normal (12) insulin levels and can even show improved insulin sensitivity compared with BMI-matched controls (28). In the current study, the pituitary defect was induced after sexual maturation and was selective for GH (10, 22). In addition, weight gain was moderated by LF feeding. Therefore, this model system provides an opportunity to more directly explore the impact of adult-onset GHD on tissue-specific insulin actions. Based on our current results, we might speculate that impaired hepatic glucose metabolism observed in AOiGHD mice represents an “early” metabolic defect in GHD, a defect that can be offset by improvement in peripheral insulin sensitivity if caloric intake is in moderation. In fact, we have previously reported that AOiGHD mice fed a LF-diet show normal glucose tolerance [by ip glucose tolerance test (GTT)], but improved insulin tolerance (by ip ITT), compared with GH-replete controls (22). However, in the context of HF feeding, improved insulin tolerance is diminished in AOiGHD mice, and marked glucose intolerance develops after an inperitoneal bolus of glucose
Because we have previously reported that H9252-cell mass and in vitro glucose-stimulated insulin release is not altered in HF-fed AOiGHD mice (7), the current findings suggest AOiGHD-related impairment of insulin-dependent suppression of HGP may contribute to impaired glucose tolerance observed when caloric intake is in excess (22). Glucose intolerance with improved insulin sensitivity is also observed in 6- to 12-mo-old GH receptor antagonist transgenic mice (13), GHRH knockout mice (11), and in patients with isolated GHD due to inactivating mutations in the GHRH receptor gene (27, 32). Interestingly, the opposite phenotype (improved glucose tolerance with normal/impaired insulin sensitivity) is observed in HiGH mice (10) and in mice expressing the bovine GH transgene (6). The current clamp results suggest that the improved glucose tolerance observed in HiGH mice is in part due to improved insulin-mediated suppression of HGP, which could serve to offset impaired insulin-stimulated glucose uptake in muscle, thereby maintaining glucose homeostasis.

Of note, we observed a differential impact of GH alterations in muscle subtype, insulin-mediated, glucose uptake within and between AOiGHD and HiGH. Multiple factors could contribute to these differences, which include: low dose of insulin during the clamp; age at onset of GH alteration in AOiGHD vs. HiGH mice, which may alter muscle subtype development and maintenance; and type of muscle [oxidative (soleus), glycolytic (22)]. Because we have previously reported that β-cell mass and in vitro glucose-stimulated insulin release is not altered in HF-fed AOiGHD mice (7), the current findings suggest AOiGHD-related impairment of insulin-dependent suppression of HGP may contribute to impaired glucose tolerance observed when caloric intake is in excess (22). Glucose intolerance with improved insulin sensitivity is also observed in 6- to 12-mo-old GH receptor antagonist transgenic mice (13), GHRH knockout mice (11), and in patients with isolated GHD due to inactivating mutations in the GHRH receptor gene (27, 32). Interestingly, the opposite phenotype (improved glucose tolerance with normal/impaired insulin sensitivity) is observed in HiGH mice (10) and in mice expressing the bovine GH transgene (6). The current clamp results suggest that the improved glucose tolerance observed in HiGH mice is in part due to improved insulin-mediated suppression of HGP, which could serve to offset impaired insulin-stimulated glucose uptake in muscle, thereby maintaining glucose homeostasis.

To our knowledge, the current study represents the first report examining the impact of selective reduction or increase in circulating GH/IGF-I levels on tissue-specific insulin actions as measured by hyperinsulinemic-euglycemic clamps. However, clamps have been performed in other mouse models where GH output or signaling is altered. Specifically, a very recent paper performed clamps in Ames mice (34). In that study, insulin actions were impaired in muscle, fat, and liver. However, unlike HiGH mice (10), LID mice show impaired response to ITT and dyslipidemia. Finally, Vijayakumar et al. (33) performed clamps in muscle-specific GH receptor knockout (mGHRKO) mice made obese by HF feeding. In that study, mGHRKO mice exhibited normal GH and IGF-I levels with improved muscle and liver insulin actions. In all of these models (including the AOiGHD and HiGH mice in the current report), GH is negatively correlated with insulin-mediated muscle glucose uptake. This is consistent with reports showing GH treatment suppresses insulin-induced glucose uptake in muscle in normal human subjects (20). Although the current study was not designed to investigate the cellular mechanism of GH-mediated inhibition of muscle glucose uptake, our current results suggest skeletal muscles are more sensitive than adipose tissue to changes in endogenous GH levels, with respect to insulin-mediated glucose uptake, as suggested by data generated by others (21, 33).

Of note, we observed a differential impact of GH alterations in muscle subtype, insulin-mediated, glucose uptake within and between AOiGHD and HiGH. Multiple factors could contribute to these differences, which include: low dose of insulin during the clamp; age at onset of GH alteration in AOiGHD vs. HiGH mice, which may alter muscle subtype development and maintenance; and type of muscle [oxidative (soleus), glycolytic (22)].
GH LEVELS AND HEPATIC/MUSCLE INSULIN ACTIONS IN MALE MICE

ACKNOWLEDGMENTS

We thank Neena Majumdar for technical assistance.

GRANTS

This work was supported by the Fundación Alfonso Martin Escudero (to J. Cordoba-Chacon), the “Sara Borrell” program (Grant No. CD11/00276) (to M. D. Gahete), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grants DK-059637 and DK-020893 (to O. P. McGuinness), Department of Veterans Affairs, Office of Research and Development Merit Award BX001114, and NIDDK Grant R01-DK-088133 (to R. D. Kineman).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: J.C.-C., M.D.G., O.P.M., and R.D.K. performed experiments; J.C.-C., M.D.G., and O.P.M. analyzed data; J.C.-C. and R.D.K. interpreted results of experiments; T.C.-C. prepared figures; J.C.-C. and R.D.K. drafted manuscript; J.C.-C., M.D.G., O.P.M., and R.D.K. edited and revised manuscript; J.C.-C., M.D.G., O.P.M., and R.D.K. approved final version of manuscript; R.D.K. conception and design of research.

REFERENCES


