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Glucose homeostasis and insulin sensitivity in growth hormone-transgenic mice: a cross-sectional analysis

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Abstract

In contrast to its stimulatory effects on musculature, bone, and organ development, and its lipolytic effects, growth hormone (GH) opposes insulin effects on glucose metabolism. Chronic GH overexposure is thought to result in insulin insensitivity and decreased blood glucose homeostatic control. Yet, despite the importance of this concept for basic biology, as well as human conditions of GH excess or deficiency, no systematic assessment of the impact of GH overexpression on glucose homeostasis and insulin sensitivity has been conducted. We report that male and female adult GH transgenic mice have enhanced glucose tolerance compared to littermate controls and this effect is not dependent on age or on the particular heterologous GH transgene used. Furthermore, increased glucose-stimulated insulin secretion, augmented insulin sensitivity, and muted gluconeogenesis were also observed in bovine GH overexpressing mice. These results show that markedly increased systemic GH concentration in GH-transgenic mice exerts unexpected beneficial effects on glucose homeostasis, presumably via a compensatory increase in insulin release. The counterintuitive nature of these results challenges previously held presumptions of the physiology of these mice and other states of GH overexpression or suppression. In addition, they pose intriguing queries about the relationships between GH, endocrine control of metabolism, and aging.

Keywords

gluconeogenesis; glucose tolerance; growth hormone transgenic; insulin sensitivity

Introduction

Growth hormone (GH) is a key regulator of growth and metabolism, possessing biological activities that are both catabolic and anabolic. GH is known to strongly influence carbohydrate and lipid metabolism by exerting both insulin-like and anti-insulin effects in various tissues (Davidson, 1987). The acute, transient insulin-like effects of GH have been

documented only in tissues with limited GH exposure, and there is ambiguity about their physiological significance (Davidson, 1987). The anti-insulin or diabetogenic activities of GH are delayed and require relatively long-term exposure *in vitro* or *in vivo* and are generally accepted to be the principal physiological effect of GH on carbohydrate and lipid metabolism (Davidson, 1987). Among the delayed anti-insulin effects of GH are hyperglycemia, hyperinsulinemia, increased lipolysis, decreased glucose metabolism, increased serum levels of non-esterified fatty acids, decreased glucose transport, and insulin resistance (reviewed by Kopchick et al., 1999). In fact, GH-mediated insulin resistance is commonly observed in acromegalics, approximately 50% of whom often go on to develop overt diabetes (Hansen et al., 1986). In addition, GH therapy in GH-deficient children with physiological or pharmacological doses was reported to cause insulin resistance, hypo-high molecular weight-adiponectinemia, hypertriacylglycerolemia, and sometimes diabetes (Lippe et al., 1981; Ibanez et al., 2010).

The long-term effects of GH have been studied in various mouse models with chronic elevation of GH expression and plasma levels under the control of different GH transgene-promoter combinations. The use of different promoters results in distinct developmental timing of gene expression; for instance, the phosphoenolpyruvate carboxykinase (PEPCK) promoter initiates GH expression just after birth (Kopchick et al., 1999). The choice of the heterologous GH gene is crucial as human GH (hGH) has both somatotropic and mammatropic effects, whereas bovine, ovine, porcine or rat GH are strictly somatotropic in rodents (Kopchick et al., 1999). Genetic background and the species of GH used notwithstanding, GH overexpressing mice are characterized by accelerated growth, increased size, organomegaly, and a drastic reduction in lifespan (Pendergrass et al., 1993; Kopchick et al., 1999; Bartke, 2003). Bovine (b) GH transgenic mice exhibit elevated circulating levels of insulin-like growth factor 1 (IGF-1) and hyperinsulinemia despite normal fasting blood glucose levels, which has been construed to be a reflection of an insulin-resistant state (McGrane et al., 1990). Alterations in glucose metabolism have been previously reported in PEPCK-bGH mice, including reduced hepatic expression of genes for glucose transporter 2 and for enzymes involved in the gluconeogenic and glycogenic pathways (Valera et al., 1993). Dominici et al. (1999) reported that female PEPCK-bGH mice exhibited a loss of sensitivity to early events in the insulin signaling pathway in the liver and reduced tyrosine phosphorylation of insulin receptor and insulin receptor substrate-1 in skeletal muscle in response to insulin stimulation.

On the basis of these observations, it is widely speculated that insulin sensitivity in bGH transgenic mice is compromised. However, data on blood glucose homeostasis and insulin sensitivity in GH overexpressing transgenic mouse models is limited. In addition, owing to the drastically reduced lifespan of these mice and the age-related and gender-specific changes in body composition (Palmer et al., 2009), it is of interest to evaluate measures of glucose tolerance and insulin sensitivity across different ages and both genders. In this cross-sectional study, we studied measures of glucose homeostasis and insulin sensitivity in male and female mice of different ages expressing the bovine- or human-GH transgenes.

Results

Determination of glucose homeostasis

To assess whether high circulating insulin levels alter glucose metabolism, we analyzed glucose tolerance in GH-transgenic (GH-Tg) mice. Figure 1 shows the plasma glucose responses to an intraperitoneal (i.p.) glucose challenge. Injection of glucose resulted in a rapid and protracted rise in glucose concentrations in both normal and GH overexpressing mice. For mice of different gender, age, or carrying bovine or human transgene the glucose excursion following glucose challenge was significantly different in the GH-Tg mice relative to the littermate controls (Figure 1). It is of interest to note that PEPCK-bGH Tg male mice displayed superior glucose clearance relative to littermates in response to a glucose challenge [$p=0.015$ for 3-month-old (m.o.) male (Figure 1A); $p=0.016$ for 9 m.o. males (Figure 1D)]. Compared to their respective controls, female PEPCK-bGH mice also demonstrated better glucose disposal after a glucose load in our study ($p=0.032$, see Figure 1C) as did the male GH-Tg mice carrying the human GH transgene ($p=0.001$, Figure 1B).

Hepatic glucose production was assessed by measuring blood glucose levels after an i.p. injection of sodium pyruvate. The glucose response to the pyruvate tolerance test was markedly blunted ($p < 0.003$) in bGH-Tg mice relative to the controls at all time points excluding initial and final (Figure 2). These results suggest that the GH overexpressing Tg mice have a reduced ability to convert pyruvate to glucose compared to wild-type littermates. In addition, gene expression analysis revealed a downregulation in the expression of genes for both gluconeogenic enzymes PEPCK and glucose-6-phosphatase in the livers of GH-Tg mice with regard to controls (unpublished data). The suppressed gluconeogenic capacity of GH-Tg mice is further evidence of their enhanced hepatic insulin sensitivity.

Insulin sensitivity

We evaluated the blood glucose excursion in Tg mice after an insulin injection [0.75 United States Pharmacopeia Units (USPU) per kg body weight] during an insulin tolerance test (ITT). As seen in Figure 3A, female Tg animals displayed similar sensitivity to insulin as controls (not statistically significant, i.e., $p>0.05$ at all time points measured); the reduction of blood glucose levels in response to the insulin injection paralleled that of wild-type animals. When challenged with a stronger insulin bolus (2 USPU/kg body weight), the glucose response of female GH-Tg mice still did not differ significantly from control littermates (Figure 3C; $p>0.05$). Because skeletal muscle and adipose tissue are responsible for insulin-stimulated glucose uptake, these results seem to suggest that peripheral tissues of female Tg mice are not resistant to the physiological effects of insulin. Interestingly, we observed that the glucose-lowering effect of insulin was significantly enhanced in male GH-Tg mice at different ages (Figure 3B and D), indicating that male GH-Tg mice were, in fact, more insulin-sensitive than the wild-type littermates.

We examined glucose-stimulated insulin secretion (GSIS) as a measure of islet/ β -cell function, and observed that insulin levels in the fasted basal state and after glucose injection differed markedly between GH overexpressing mice and littermate controls (Figure 4). It

reflects the previously reported basal hyperinsulinemia of GH overexpressing mice along with robust β -cell response, which would explain the improved glucose disposal in the GH-Tg mice. Differences in basal insulin levels failed to reach statistical significance; however, sample sizes could simply be too low to appropriately power the detection of such an effect size (approx. 70% increase in GH-Tg relative to normal counterparts), given the variation exhibited within each group. Nonetheless, glucose-induced elevated insulin levels were significantly different in the two groups ($p=0.002$ at 5 min and $p=0.007$ at 15 min). Furthermore, as seen in Figure 4, the slope of the curve between 0 and 5 min differed dramatically for the two groups, suggesting that enhanced glucose tolerance in GH-Tg mice is attributable to increased insulin secretion into the bloodstream.

Discussion

Here, we document an increased clearance of an exogenous glucose bolus in GH-Tg mice relative to their littermate controls (Figure 1). This result is consistent across gender, age, and bovine and human GH transgenes, and is thus an inherent characteristic of GH overexpression. Of note is that the peak blood glucose value was similar across groups: 15 min time point in 3 m.o. males (Figure 1A), 15 min time point in 12 m.o. males (Figure 1B), 10 min time point in 6–8 m.o. females (Figure 1C), and 10 min time point in 9 m.o. males (Figure 1D). This suggests that the kinetics of GSIS are similar in all groups and that only the magnitude of the response differs amongst groups and ultimately determines the results. This is supported by the results on GSIS, in which the initial surge of insulin secretion occurred just 5 min after the glucose injection for both GH-Tg mice and their normal controls, yet the magnitude of that secretion was greater in the GH-Tg mice (Figure 4).

Furthermore, when separated by gender, these findings suggest that during the interval examined there is no aging-associated decrease in glucose tolerance (whether magnitude or kinetics) in either group, as the male littermate control suffers increases in blood glucose to approximately 22.22 mM (400 mg/dl), and the male GH-Tg to approximately 16.67 mM (300 mg/dl), regardless of age. Aging-associated glucose intolerance might have been detected if older mice were tested, but the early mortality of the GH-Tg mice made that impracticable. Moreover, the effects of GH transgenesis on glucose tolerance were also seen to extend to mice carrying the human GH transgene, as PEPCK-hGH Tg mice displayed superior glucose clearance post-load at 12 months old (Figure 1B).

In addition to the insulin-mediated ability to assimilate postprandial blood glucose, the ability of an endocrine system to suppress endogenous glucose production is crucial for proper blood glucose homeostatic regulation, arguably more important than the former for humans with metabolic syndrome (Bouche et al., 2004; Wu et al., 2005). Therefore, we also assessed hepatic gluconeogenic potential by virtue of a pyruvate tolerance test in old (9–10 months old) male bGH-Tg mice and their controls. We observed strongly decreased glucose levels in GH-Tg mice relative to their littermates after injection of sodium pyruvate; suggestive of decreased gluconeogenic potential or proclivity. Whether the failure of GH-Tg mice to produce as much glucose as their controls is due to passively decreased capacity for such production or to actively repressed propensity is an unresolved matter for future analyses of the macromolecular regulators of gluconeogenesis in the livers of these

physiologically divergent mice. There is also the likelihood of a contribution of enhanced glucose clearance in the GH-Tg mice to the muted increases in blood glucose levels upon pyruvate administration.

Consistent with previous reports (McGrane et al., 1990; Dominici et al., 1999), we observed markedly elevated plasma insulin levels in GH-Tg mice (as young as 3 months of age) relative to littermate controls (unpublished data). The life-long hyperinsulinemia of GH-Tg mice would be predicted to result in decreased sensitivity of target tissues in these mice to the glucose-clearance stimulatory effects of insulin. Surprisingly, this putative insulin insensitivity of GH-Tg mice was not observed in either gender, and instead a considerable increase in insulin sensitivity was observed in male GH-Tg mice (Figure 3). The hypersensitivity of PEPCK-bGH Tg mice to an insulin bolus has been previously reported along with their normal glucose tolerance to a glucose gavage (McGrane et al., 1990). It seems plausible that the degree of cell-autonomous insulin resistance induced by GH transgenesis is superseded by the combined histological effect of substantially increased percentage of lean body mass in the GH-Tg mice (Palmer et al., 2009), ultimately resulting in a net beneficial effect of GH transgenesis on insulin sensitivity (e.g., a 50% decrease in the ability of each GH-Tg myocyte to respond to insulin would be overcompensated for by a >2-fold increase in the number and/or density of myocytes in Tg mice relative to their counterparts). This increased insulin sensitivity of GH-Tg males is remarkable because mutant mice with GH deficiency or resistance are known to be hypoinsulinemic and show heightened insulin sensitivity (Bartke, 2008). One possible explanation for the insulin sensitivity of male GH-Tg mice could lie in body composition differences. The testosterone-fueled increased growth of males leads to a greater difference in lean body weight between male GH-Tg mice and their counterparts than that seen between female mice of those types (Palmer et al., 2009). Furthermore, the marked surge in blood insulin concentration after injection of glucose (as indicated by a comparison of the slopes of the lines connecting the basal insulin measurement with the 5-min measurement) in Tg mice, relative to their controls, is putatively representative of a massive release of insulin from the enlarged pancreatic β -cells of GH-Tg mice.

How can we reconcile the absence of insulin resistance in PEPCK-bGH Tg mice with the long-held and widely accepted association of elevated plasma GH levels and induction of insulin resistance? We believe that there could be more than one explanation for our findings. First, as GH has considerable potency in the regulation of body composition, growth factor production and intermediary metabolism, the overall endocrinological phenotype of bGH Tg mice could chiefly be the result of their improved body composition (i.e., increased lean muscle weight and proportion), especially because enhanced insulin sensitivity is seen in the male GH-Tg mice but not in female mice. Second, analysis of risk factors promoting the development of glucose intolerance in acromegaly revealed that amongst other factors, higher age and longer duration of the disease predisposed the tendency for developing overt diabetes (Nabarro, 1987). As was mentioned in the introductory section of this article, average lifespan of GH-Tg mice is drastically reduced (Pendergrass et al., 1993), apparently owing to an increased incidence of cancer (Bartke, 2003) and an early onset of kidney damage (Doi et al., 1988). It might be conjectured here that the early mortality of the GH-Tg mice precludes progressive β -cell failure, the

subsequent loss of compensatory hyperinsulinemia, and the onset of glucose intolerance as seen in patients with acromegaly. Third, GH is known to exert effects on the cardiovascular system and, in fact, chronic GH excess is known to induce a specific cardiomyopathy. It has been demonstrated that impaired glucose tolerance is significantly correlated with the severity of acromegalic cardiomyopathy (Colao et al., 2000). Although cardiac hypertrophy is a consistent finding in various animal models of GH overexpression (Bollano et al., 2000; Izzard et al., 2009; Palmer et al., 2009), data on deterioration of cardiac function in these GH-Tg mice models is not as compelling. Recently, Izzard et al. (2009) reported that despite cardiac enlargement and an increase in systolic blood pressure in bGH mice, cardiac vasculature and vascular contractile function was normal. This encourages us to speculate that cardiovascular disease might not be a major factor in the accelerated aging phenotype of these transgenic mice, thereby also preventing any deterioration of glucose homeostasis in these GH-Tg mice. Lastly, IGF-1 is recognized as an insulin sensitizer in humans (Moses et al., 1996) and studies using a GH antagonist plus IGF-1 have shown that the insulin resistance that ensues in acromegaly is primarily attributable to GH hypersecretion (O'Connell and Clemmons, 2002). Moreover, it has been suggested that in acromegaly, the net effect on carbohydrate metabolism is the result of direct insulin-antagonizing actions of GH and the insulin-sensitizing actions of IGF-1 (Clemmons, 2002). It is, therefore, conceivable that in the GH-Tg mice under study that IGF-1 is acting to enhance insulin sensitivity and overcome the GH-induced antagonism of insulin action.

A most-interesting feature of this study is the dichotomy between the results of GH transgenesis on ultimate survivorship and those on blood glucose homeostatic regulation. With increasing age, GH-Tg mice perish at a higher rate than their littermates (reviewed by Bartke et al., 2002), and the decreased sample size of old (9–10 months of age, an age that previous survivorship experiments proves as being decidedly old for GH-Tg mice) Tg males in this study (n=3), relative to the sample size of the littermates (n=12), is indicative of this. Yet, the Tg animals establish superior insulin sensitivity and blood glucose homeostatic management in young adulthood (Figure 1A) and maintain it throughout life (Figures 1 and 3). This dissociation is noteworthy insofar as the hypothesized causality of enhanced insulin sensitivity and/or blood glucose homeostatic control for longevity of GH-deficient and GH-resistant mice (Bartke, 2008). This study suggests that other factors that might affect ultimate survivorship and that arise from heightened proliferative potential [such as increased tumorigenic potential (Wanke et al., 1991) and increased rates of inflammation (Miquet et al., 2008)] might be antagonistic to the beneficial effects of such heightened proliferative potential (such as enhanced blood glucose homeostatic control owing to increased musculoskeletal mass, decreased fat mass, and putatively increased pancreatic islet size). To the best of our knowledge, this could be one of very few and perhaps the only experimental example of antagonistic pleiotropy in a gerontological context (i.e., in animals of demonstrably differing survival) (Leroi et al., 2005).

In conclusion, we herein report markedly superior performance in GH-Tg mice relative to their littermate controls, with regard to insulin sensitivity and glucose tolerance, pyruvate conversion, and GSIS. Whether these apparently beneficial effects of GH excess could be applicable to other domains (such as adipocytokine-mediated insulin sensitivity or GH-mediated bone mineral density, and/or bone marrow adiposity; Menagh et al., 2010) is

subject to future study of GH-Tg mice. Finally, considering the age-independent nature of the results presented here, characterizing the effects of caloric restriction (CR) in GH-Tg mice would be of considerable interest. CR has been documented for nearly a century to be beneficial for delaying, mollifying, or forestalling aging-associated phenotypes (Rous, 1914), and these studies have long-since been extended to the beneficial effects of CR on ultimate survivorship (McCay et al., 1935), including that of GH-Tg mice (unpublished data). CR has been reported to reduce insulin levels and improve insulin sensitivity; thus, determining the effects of CR on the already-remarkably well-regulated glucose homeostasis in GH-Tg mice is a very intriguing outstanding question of relevance to endocrinology, gerontology, and the association between the two.

Materials and methods

Animals

The transgenic (Tg) mice that overexpress the bGH gene under the control of the rat PEPCK promoter employed in this study have been previously described (McGrane et al., 1988). PEPCK-hGH Tg mice were produced by introduction of the entire human (h) GH structural gene fused with PEPCK promoter into male pronuclei as described previously (Milton et al., 1992). Normal siblings of Tg mice were used as controls, thus littermates used as controls in these studies differed from transgenics only by the absence of the transgene and otherwise were identical in every respect, including genetic background (derived from C57Bl/6J and C3H/J inbred strains), maternal effects, and intrauterine environment. The mice were housed three to five per cage in a room with controlled light (12 h light per day) and temperature ($22\pm 2^{\circ}\text{C}$). The animals had free access to food (Lab Diet Formula 5001 containing a minimum of 23% protein, 4.5% fat, and a maximum of 6% crude fiber; Purina Mills Inc., St. Louis, MO, USA) and tap water. Experiments were performed using young (3–4 months old), middle aged (6–8 months old), and old (9–10 months old or 12 months old) Tg and normal mice of both sexes, and were approved by the institutional Laboratory and Animal Care and Use Committee.

Experimental protocols for *in vivo* studies—Blood samples were taken from the nicked tip of the tail of mice at times indicated in Figures 1–4, and blood glucose was analyzed by using a One Touch Ultra 2 Glucometer (Lifescan, Inc., Milpitas, CA, USA).

Intraperitoneal glucose tolerance test (IPGTT)—For blood glucose homeostasis studies, after baseline blood glucose measurements, overnight (14–16 h) fasted mice were injected with 2 g of glucose/kg body weight i.p., and blood glucose was checked at regular intervals as graphically indicated (at either 0, 15, 30, 45, 60, and 120 min intervals or 0, 10, 20, 30, 40, 50, 60, 75, 90, and 120 min intervals).

Insulin tolerance test (ITT)—For assessment of systemic insulin sensitivity, mice were injected i.p. with porcine insulin (Sigma, St. Louis, MO, USA) at 0.75 United States Pharmacopodia Units (USPU)/kg or 2 USPU/kg of body weight as indicated, and blood glucose was measured at different time points as indicated, as for the IPGTT above.

Pyruvate tolerance test (PTT)—For assessment of gluconeogenic execution, overnight fasted mice were injected i.p. with pyruvate (sodium pyruvate, Sigma) at 2 g/kg body weight, and blood glucose was measured at 0, 15, 30, 45, 60, and 120 min after injection.

Glucose-stimulated insulin secretion (GSIS)—For the GSIS test, glucose was injected (2 g/kg) i.p. after overnight (14–16 h) fasting. Blood was collected from tail veins at 0, 5, 15, and 30 min and serum was separated. Serum insulin levels were subsequently measured by an ultrasensitive mouse insulin enzyme-linked immunosorbent assay (ELISA) (Crystal Chem, Downers Grove, IL, USA).

Statistics

Values are given as mean±SD. Fasting and *ad libitum*-fed baseline data were compared in transgenics and controls using the unpaired Student's *t*-test. For the various tolerance tests, analysis of variance (ANOVA) for repeated measures was performed to identify significant differences in mean concentration, both at the different time points of a specified experiment [relative to the initial (0 min) values] and between the two different groups of mice (SPSS Statistics 17.0, SPSS, Chicago, IL, USA). A *p*-value <0.05 was considered statistically significant.

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Figure 1.

Glucose tolerance test (GTT) in GH transgenic and control mice after receiving an i.p. injection of glucose (2 g/kg body weight). Data are expressed as mean±SD. (A) Three-month-old male PEPCK-bGH mice; n=5 in each group, $p=0.015$; (B) 12-month-old male PEPCK-hGH mice; n=15 (N), n=10 (Tg), $p=0.001$; (C) 6-8-month-old female PEPCK-bGH mice; n=18 (N), n=15 (Tg), $p=0.032$; (D) 9-month-old male PEPCK-bGH mice; n=12 (N), n=3 (Tg), $p=0.016$. Filled diamonds represent mean±SD of normal mice and filled squares represent mean±SD in transgenic mice.

Figure 2.

Blood glucose levels during a pyruvate tolerance test (PTT) in male mice after receiving an i.p. injection of sodium pyruvate (2 g/kg body weight).

Data are expressed as mean $\pm$ SD, n=12 (N), n=3 (Tg). Repeated measures ANOVA revealed a significant effect of genotype ($p=0.003$). Filled diamonds represent mean $\pm$ SD of normal mice and filled squares represent mean $\pm$ SD of transgenic mice.

Figure 3.

Insulin tolerance test (ITT) in PEPCK-bGH and control mice after receiving an i.p. injection of insulin (dose indicated). Data are expressed as mean $\pm$ SD. (A) Six-month-old female PEPCK-bGH mice; n=16 (N), n=13 (Tg), p =NS; (B) 6-8-month-old male PEPCK-bGH mice; n=5 (N), n=5 (Tg), p =0.047 at 15 min; p =0.043 at 30 min; p =0.05 at 45 min; (C) 6-8-month-old female PEPCK-bGH mice; n=7 (N), n=5 (Tg), p =NS; (D) 9-month-old male PEPCK-bGH mice; n=12 (N), n=3 (Tg), p =0.021. Filled diamonds represent mean $\pm$ SD of normal mice and filled squares represent mean $\pm$ SD of transgenic mice.

Figure 4.

Glucose-stimulated insulin secretion (GSIS) in male PEPCK-bGH mice after receiving an i.p. injection of glucose (2 g/kg body weight).

Data are expressed as mean $\pm$ SD, n=12 (N), n=3 (Tg). Repeated measures ANOVA revealed a significant effect of genotype ($p=0.007$). Filled diamonds represent mean $\pm$ SD of normal mice and filled squares represent mean $\pm$ SD of transgenic mice.