Increased adiposity and insulin correlates with the progressive suppression of pulsatile GH secretion during weight gain

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Abstract
Pathological changes associated with obesity are thought to contribute to GH deficiency. However, recent observations suggest that impaired GH secretion relative to excess calorie consumption contributes to progressive weight gain and thus may contribute to the development of obesity. To clarify this association between adiposity and GH secretion, we investigated the relationship between pulsatile GH secretion and body weight; epididymal fat mass; and circulating levels of leptin, insulin, non-esterified free fatty acids (NEFAs), and glucose. Data were obtained from male mice maintained on a standard or high-fat diet. We confirm the suppression of pulsatile GH secretion following dietary-induced weight gain. Correlation analyses reveal an inverse relationship between measures of pulsatile GH secretion, body weight, and epididymal fat mass. Moreover, we demonstrate an inverse relationship between measures of pulsatile GH secretion and circulating levels of leptin and insulin. The secretion of GH did not change relative to circulating levels of NEFAs or glucose. We conclude that impaired pulsatile GH secretion in the mouse occurs alongside progressive weight gain and thus precedes the development of obesity. Moreover, data illustrate key interactions between GH secretion and circulating levels of insulin and reflect the potential physiological role of GH in modulation of insulin-induced lipogenesis throughout positive energy balance.

Key Words
- pulsatile GH secretion
- dietary-induced weight gain
- insulin
- non-esterified free fatty acids
- adiposity

Introduction
Growth hormone (GH) defines a key anabolic hormone that regulates body composition and energy homeostasis. Attainment of peak GH secretion in early adulthood (Balercia et al. 2011), and sustained GH secretion throughout adulthood, is associated with improved metabolism, maintenance of healthy body composition, and improved quality of life (Jorgensen et al. 2011, Park et al. 2011). Conversely, GH deficiency is associated with increased adiposity, reduced bone mass, and reduced lean muscle mass (de Boer et al. 1995). Both dietary-

Factors produced in proportion to adipose mass or changes in endocrine function that accompany increased adiposity are thought to contribute to impaired GH secretion in obesity. In line with this, impaired GH secretion is reversed following weight loss (Williams et al. 1984, Rasmussen et al. 1995). Correlative studies assessing the relationship between adiposity and the secretion of GH provide essential information to identify potential factors that suppress GH secretion in obesity (Gill et al. 1997, Vahl et al. 1997, Veldhuis et al. 2009, 2011). Recent measures demonstrate that impairments in pulsatile GH secretion in humans precede dietary-induced weight gain (Cornford et al. 2011, 2012) and that the suppression of GH secretion with increased food consumption improves meal tolerance (Cornford et al. 2012). Accordingly, the progressive decline in GH secretion may exacerbate dietary-induced weight gain and ultimately contribute to the development of obesity.

Based on the premise that pulsatile GH secretion is impaired in mice during dietary-induced weight gain (Huang et al. 2012), we have assessed the relationship between pulsatile GH secretion and body weight; epididymal fat mass; and circulating levels of leptin, insulin, non-esterified free fatty acids (NEFAs), and glucose in mice. Measures were collected from mice maintained on a standard diet or high-fat diet (HFD). Correlation analyses confirmed an inverse relationship between measures of pulsatile GH secretion and body weight, epididymal fat mass, and circulating levels of leptin and insulin. Parameters of pulsatile GH secretion did not change relative to circulating levels of NEFAs or glucose. We conclude that impaired pulsatile GH secretion in the mouse occurs alongside progressive weight gain and thus precedes the development of obesity. Moreover, our findings are in agreement with the recent clinical findings by Cornford et al. (2012), demonstrating a potential role for the GH/insulin axis to sustain NEFA and glucose homeostasis. Thus, we anticipate that impaired pulsatile GH secretion relative to increased dietary intake contributes to increased adiposity and the eventual development of obesity.

Materials and methods

Animals

Eight-week-old wild-type C57BL/6J male mice were obtained from the University of Queensland Biological Resources (UQBR). Mice were pair-housed in a 12 h light:12 h darkness cycle (lights on at 0630 h and off at 1830 h). Room temperature was maintained at 24 ± 2 °C. Starting at 8 weeks of age, mice were randomly assigned and maintained on either a standard diet (n = 16; 19.6% protein and 4.6% fat; Specialty Feeds, Glen Forrest, WA, Australia) or a commercial HFD (n = 12; HFD, 22.6% protein and 23.5% fat; SF04-001, Specialty Feeds). Mice had free access to food and water for the duration of all experiments. Experimental procedures were performed in accordance with and approved by the University of Queensland Animal Ethics Committee.

Assessment of pulsatile GH secretion relative to dietary intervention

At 16 weeks of age (following 8 weeks of dietary intervention), pulsatile GH secretion was assessed as described previously (Steyn et al. 2011). For this, 36 sequential tail-tip blood samples (4 μl/sample) were collected at 10-min intervals over a 6-h sampling period. Sampling commenced at 0700 h. Given an approximate total blood volume of 2.1 ml/mouse (based on an estimated 7% blood volume relative to body mass, NHMRC (2008)), blood loss was restricted to <5.5% of total blood volume over the 6-h sampling period. This is well below the amount of blood loss known to have a physiological impact on the well-being of mice (Raabe et al. 2011). Following collection, samples were immediately placed on dry ice and transferred to −80 °C for future analysis.

Collection of adipose tissue and plasma samples

Following assessment of pulsatile GH secretion, mice were allowed to recover for 2–5 days before killing. At the time of killing, epididymal fat pads and plasma were collected. Given ultradian patterns of secretion and variations in circulating levels of many of the factors assessed, collection of blood and tissue was strictly restricted between 0900 and 1100 h. Mice were weighed and anesthetized with a single i.p. injection of sodium pentobarbitone (32.5 mg/kg, 1PO643-1; Virbac Animal Health, Sydney, NSW, Australia). Epididymal fat pads were isolated and weighed as a representative depot for total
body adipose mass (Rogers & Webb 1980). Terminal cardiac blood samples were collected using a heparinized syringe (100 IU/ml). Plasma and red blood cell contents were immediately separated via centrifugation (2000 g for 3 min at room temperature), placed on dry ice, and stored at −80 °C for future analysis.

Hormone, NEFAs, and glucose analysis

Analysis for GH was performed using an in-house mouse GH ELISA (Steyn et al. 2011). Circulating levels of leptin and insulin were determined using commercial ELISA kits (EZML-82K, Mouse Leptin ELISA; EZRMI-13K, Rat/Mouse Insulin ELISA, Millipore, Billerica, MA, USA). Circulating levels of glucose and NEFAs were determined by commercial colorimetric assay (Item No. 10009582, Glucose Assay Kit, Cayman Chemical Company, Ann Arbor, MI, USA; 279-75401, NEFA C assay; Wako, Osaka, Japan). The within- and between-assay coefficients of variation (CVs) for all ELISA assays were below 9 and 3% respectively.

Data and statistical analysis

The kinetics and secretory patterns of pulsatile GH secretion were determined by deconvolution analysis following parameters established previously (Steyn et al. 2011, 2012a,b). A series of correlation analyses were then performed to assess the relationship between body weight and adiposity relative to circulating levels of leptin, NEFAs, glucose, and insulin and measures of pulsatile GH secretion, as well as correlations between measures of GH secretion and circulating levels of leptin, NEFAs, glucose, and insulin. Correlation analyses were limited to data collected from all mice, irrespective of dietary intervention (compiled data, n=28), or from mice maintained on the standard diet only (control, n=16). Correlations were determined by linear regression and Spearman correlation coefficient. Data are presented as mean ± S.E.M. Differences between groups were determined by unpaired Student's t-test. All measures (excluding deconvolution analysis) were performed using GraphPad Prism version 6.0a (GraphPad, Inc., La Jolla, CA, USA). For all measures, a P value <0.05 denotes statistical significance.

Table 1

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control (n=16)</th>
<th>HFD (n=12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>30.0±0.53</td>
<td>34.1±1.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Epididymal fat weight (g)</td>
<td>0.63±0.05</td>
<td>1.29±0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>9.78±1.03</td>
<td>26.2±3.27</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NEFAs (mEq/l)</td>
<td>0.16±0.01</td>
<td>0.17±0.02</td>
<td>0.74</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>229±7.60</td>
<td>219±12.7</td>
<td>0.50</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.91±0.10</td>
<td>1.73±0.25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total GH secretion rate (ng/ml per 6 h)</td>
<td>387±37.7</td>
<td>246±23.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pulsatile GH secretion rate (ng/ml per 6 h)</td>
<td>361±38.1</td>
<td>225±19.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mass of GH secreted/burst (MPP, ng/ml)</td>
<td>118±12.3</td>
<td>69.0±8.54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Basal GH secretion rate (ng/ml per 6 h)</td>
<td>26.7±6.82</td>
<td>20.7±5.16</td>
<td>0.51</td>
</tr>
<tr>
<td>Number of pulses/6 h</td>
<td>3.31±0.29</td>
<td>3.58±0.31</td>
<td>0.53</td>
</tr>
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</table>

MPP, mass per pulse; NEFAs, non-esterified fatty acids; HFD, high-fat diet. P values <0.05 were considered significant.

Results

Measures of circulating leptin and insulin increase relative to body weight and epididymal fat mass

As shown in Table 1, high-fat feeding resulted in significant weight gain (P<0.01). This was accompanied by a significant increase in epididymal fat mass (P<0.01) and an elevation in circulating levels of leptin (P<0.01) and insulin (P<0.01). Circulating levels of NEFAs (P=0.74) and glucose (P=0.50) were not significantly different between control and HFD-fed mice.

Regression with Spearman CV analysis was performed to assess the relationship between body weight and adiposity (epididymal fat) relative to circulating levels of leptin, NEFAs, glucose, and insulin in 16-week-old mice maintained on a standard mouse diet (open circles) and a HFD (closed circles) and are illustrated in Fig. 1. Assessment was done for compiled data sets (compiled data, control, and HFD, n=28) and for control data independent of HFD-fed mice (control, n=16) and the
results are summarized in Table 2. Circulating levels of leptin (Fig. 1A) and insulin (Fig. 1D) increased relative to an increase in body weight for compiled and control data sets ($P<0.01$ for all measures, Table 2). As with body weight, circulating levels of leptin and insulin increased relative to epididymal fat mass (Fig. 1E and H respectively), although for the control population alone, the relationship with insulin did not reach significance ($P=0.10$, Table 2). Circulating levels of NEFAs (Fig. 1B and F) and glucose (Fig. 1C and G) did not change relative to body weight or epididymal fat mass in any analysis. Overall observations confirm that measures of body weight and epididymal fat mass in mice predict circulating levels of leptin and insulin, while circulating levels of NEFAs and glucose in these data sets remain stable, regardless of weight gain or adiposity.

High-fat feeding contributes to the suppression of pulsatile GH secretion in mice

Total, pulsatile, and basal GH secretion rates, the mass of GH secreted per burst (mass per pulse (MPP)), and the number of GH pulses observed over the 6-h sampling period in mice maintained on a standard diet (control; $n=16$) and HFD ($n=12$) are summarized in Table 1. In accordance with the previous observations (Huang et al. 2012), dietary-induced weight gain was associated with a significant decline in pulsatile GH secretion. Relative to controls (Fig. 2A), we observed a significant decline in total and pulsatile GH secretion rate, as well as the MPP in HFD-fed mice (Fig. 2B). Dietary intervention did not impact basal GH secretion rate or the number of pulses observed over the 6-h sampling period.

Pulsatile GH secretion in mice decreases relative to increased body weight, adiposity, and circulating levels of leptin regardless of dietary intervention

The relationship between total GH and the MPP relative to body weight, epididymal fat mass, and circulating levels of leptin is illustrated in Fig. 3. The relationship between parameters of pulsatile GH secretion and circulating levels of NEFAs, glucose, and insulin is presented in Fig. 4. Regression with Spearman CV analysis was performed to assess the relationship between parameters of pulsatile GH secretion relative to body weight; epididymal fat mass; and circulating levels of leptin, NEFAs, glucose, and insulin. Assessment was done for compiled data sets and control data independent of HFD-fed mice (Tables 3 and 4).

Total GH secretion rate, pulsatile GH secretion rate, and the MPP over the 6-h sampling period varied considerably among 16-week-old mice maintained on a standard diet or HFD (Table 1). Total and pulsatile GH secretion rates and the MPP decreased relative to an increase in body weight (Fig. 3A and D), epididymal fat
mass (Fig. 3B and E), and circulating levels of leptin (Fig. 3C and F) and insulin (Fig. 4C and F). These relationships were observed regardless of dietary intervention; however, they reached greater statistical significance in compiled data sets. Pulsatile measures of GH secretion did not change relative to circulating levels of NEFAs (Fig. 4A and D) or glucose (Fig. 4B and E) in control or compiled data sets. These observations show that the decline in pulsatile measures of GH secretion relative to an increase in body weight and adiposity is evident even in control non-obese mice.

**Discussion**

Impaired GH secretion in obesity is characterized by a reduction in the number of GH secretory bursts and GH secretion rate (Veldhuis et al. 1991). Given that GH secretion is recovered after significant weight loss, it is thought that impaired GH secretion is a consequence of obesity (Rasmussen et al. 1995, Nam & Marcus 2000). Recent data, however, demonstrate the rapid suppression of GH secretion relative to excess calorie consumption (Cornford et al. 2011, 2012). In this context, impairments to GH secretion may precede obesity, while contributing to normal endocrine adaptations that minimize the physiological impact of sustained positive energy balance.

GH secretion declines relative to an increase in BMI, suggesting that BMI determines GH secretory dynamics (Savastano et al. 2006, Veldhuis et al. 2011). Our data confirm this inverse relationship and are in agreement with previous measures where GH output significantly decreased relative to increased body weight in rats (De Schepper et al. 1998). We extend these observations to show that body weight in C57Bl/6J mice predicts parameters of pulsatile GH secretion regardless of dietary intervention. As C57Bl/6J mice are prone to weight gain (as fat) regardless of dietary intervention (Collins et al. 1997), the change in GH secretion relative to body weight in C57Bl/6J mice presumably reflects measures of GH secretion relative to adiposity. In line with this, impairment of GH secretion exists before dietary intervention in rats that are prone to weight gain (as fat) (Lauterio et al. 1998).

In humans, fat mass is a dominant negative correlate of pulsatile GH secretion in obese (Savastano et al. 2006) and lean populations (Vahl et al. 1997). This correlation is

<table>
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<tr>
<th>Compiled data (control and HFD, n=28)</th>
<th>Control data (control, n=16)</th>
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<tr>
<td><strong>Body weight (g)</strong></td>
<td><strong>Epididymal fat weight (g)</strong></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>0.81</td>
</tr>
<tr>
<td>NEFAs (mEq/l)</td>
<td>-0.28</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>-0.04</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.61</td>
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NEFAs, non-esterified fatty acids; HFD, high-fat diet, animals were maintained on the HFD for 8 weeks, starting at 8 weeks of age.

![Figure 2](http://joe.endocrinology-journals.org)  
**Figure 2**: Representative examples of pulsatile GH secretion in 16-week-old C57Bl/6J mice maintained on a standard diet (A) or high-fat diet (HFD) (B). Left panels demonstrate profiles of circulating levels of whole blood GH measured in 36 consecutively collected blood samples (4 h). Blood samples were collected at 10-min intervals, starting at 0700 h. Right panels illustrate output figures of GH secretion profiles following deconvolution analysis. Output parameters demonstrate the secretion rate of GH throughout the 6-h sampling period (ng/ml). Arrows denote the onset of peak periods of GH secretion. Parameters relating to pulsatile GH secretion between control and HFD-fed mice following deconvolution analysis are summarized in Table 1.
metabolic syndrome than subcutaneous mass and is an independent predictor of mortality in overweight men (Kuk et al. 2006a,b). Thus, impaired GH secretion in obesity may directly contribute to the aggregation of VAT mass and consequently obesity-associated pathologies. Genetic modifications in mice resulting in extremes of GH signaling alter whole-body fat mass. Over-expression of bGH in mice results in a decrease in body fat percentage (Li et al. 2003, Berryman et al. 2004) and a near significant 40% reduction in epididymal fat mass relative to body weight (Berryman et al. 2004). Accordingly, increased GH secretion in mice during adulthood results in a decrease in

stronger when isolating observations to specific fat depots.

For example, the amount of visceral adipose tissue (VAT) in humans better reflects parameters of pulsatile GH secretion when compared with subcutaneous or total body fat mass percentage (Vahl et al. 1997). Conversely, GH treatment in GH-deficient individuals (Jørgensen et al. 1996) and in acromegaly (Brummer et al. 1993) markedly reduced VAT mass. Of particular interest, an increase in VAT mass in humans is more closely associated with
were collected at 10-min intervals between 0700 and 1300 h to assess the relationship between pulsatile GH secretion levels of GH predict visceral adiposity in mice. Measures confirm that, as in humans (Vahl et al. 1997), prevailing levels of GH predict visceral adiposity in mice. Measures that assess the relationship between pulsatile GH secretion and different adipose depots are needed to further clarify depot-specific effects of GH secretion in adult mice.

Table 3  Spearman correlation analysis of deconvolution parameters of pulsatile GH secretion with body weight; epididymal fat weight; and circulating levels of leptin, non-esterified fatty acids (NEFAs), glucose, and insulin. Regression analysis was performed on compiled data sets collected from 16-week-old C57Bl/6J mice maintained on a standard diet and a high-fat diet. For GH, samples were collected at 10-min intervals between 0700 and 1300 h.

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Epididymal fat weight (g)</th>
<th>Leptin (ng/ml)</th>
<th>NEFAs (mEq/l)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (ng/ml)</th>
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<tbody>
<tr>
<td><strong>Total GH secretion rate</strong> (ng/ml per 6 h)</td>
<td>$r = -0.58$</td>
<td>$P = 0.02$</td>
<td>$r = -0.83$</td>
<td>$P &lt; 0.01$</td>
<td>$r = -0.87$</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td><strong>Pulsatile GH secretion rate</strong> (ng/ml per 6 h)</td>
<td>$r = -0.59$</td>
<td>$P = 0.03$</td>
<td>$r = -0.80$</td>
<td>$P &lt; 0.01$</td>
<td>$r = -0.85$</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td><strong>Mass of GH secreted/burst</strong> (MPP, ng/ml)</td>
<td>$r = -0.51$</td>
<td>$P = 0.04$</td>
<td>$r = -0.59$</td>
<td>$P = 0.02$</td>
<td>$r = -0.63$</td>
<td>$P = 0.01$</td>
</tr>
<tr>
<td><strong>Basal GH secretion rate</strong> (ng/ml per 6 h)</td>
<td>$r = -0.09$</td>
<td>$P = 0.73$</td>
<td>$r = -0.06$</td>
<td>$P = 0.82$</td>
<td>$r = -0.03$</td>
<td>$P = 0.93$</td>
</tr>
<tr>
<td><strong>Number of pulses/6 h</strong></td>
<td>$r = 0.14$</td>
<td>$P = 0.61$</td>
<td>$r = -0.05$</td>
<td>$P = 0.75$</td>
<td>$r = -0.15$</td>
<td>$P = 0.48$</td>
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$P$ values $< 0.05$ were considered significant. $n = 28$.

*Animals were maintained on the high-fat diet for 8 weeks, starting at 8 weeks of age.

Leptin circulates in proportion to fat mass (Frederich et al. 1995, Maffei et al. 1995), and obesity is associated with an elevation in circulating levels of leptin (Liu et al. 1999). As for humans (Gill et al. 1997), we found that circulating levels of leptin increased specific to epididymal fat mass in mice. This is in agreement with previous observations showing a close correlation between epididymal expression of leptin mRNA and circulating levels of leptin (Villafuerte et al. 2000). As leptin acts to maintain GH secretion (Tannenbaum et al. 1998), circulating levels of leptin should directly predict GH secretion in mice. However, we demonstrate that circulating levels of leptin in mice increased relative to a decrease in total and

Table 4  Spearman correlation analysis of deconvolution parameters of pulsatile GH secretion with body weight; epididymal fat weight; and circulating levels of leptin, non-esterified fatty acids (NEFAs), glucose, and insulin. Regression analysis was limited to data sets collected from 16-week-old C57Bl/6J mice maintained on a standard diet. For GH, samples were collected at 10-min intervals between 0700 and 1300 h.

<table>
<thead>
<tr>
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<th>Epididymal fat weight (g)</th>
<th>Leptin (ng/ml)</th>
<th>NEFAs (mEq/l)</th>
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</tr>
<tr>
<td><strong>Mass of GH secreted/burst</strong> (MPP, ng/ml)</td>
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<td>$P = 0.04$</td>
<td>$r = -0.59$</td>
<td>$P = 0.02$</td>
<td>$r = -0.63$</td>
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<td>$P = 0.75$</td>
<td>$r = -0.15$</td>
<td>$P = 0.48$</td>
</tr>
</tbody>
</table>

$P$ values $< 0.05$ were considered significant. $n = 16$.
pulsatile GH secretion rate and the MPP. As with epididymal fat mass, these predictions were observed independent of dietary-induced weight gain. Accordingly, elevated levels of leptin in obesity do not directly contribute to the suppression of GH secretion (Ozata et al. 2003). Rather, impaired leptin signaling, and/or the development of central leptin resistance (as is characteristic of obesity, Munzberg et al. (2005) and Myers et al. (2008)), may contribute to reduced GH secretion (Furuhata et al. 2000). Thus, the observed correlations between circulating levels of leptin and parameters of pulsatile GH secretion better reflect the role of leptin as a marker of adiposity. In agreement with this, reduced adiposity as a consequence of GH treatment occurs alongside a decrease in circulating levels of leptin (Janssen et al. 1997).

It is often stated that obesity is associated with an increase in circulating levels of NEFAs (Boden & Shulman 2002, Bays 2005, Langin et al. 2005). Recent comprehensive assessments of NEFA kinetics in obese humans, however, revealed that the rate of NEFA release into circulation is reduced (Mittendorfer et al. 2009, McQuaid et al. 2011), while the rate of clearance of NEFAs from plasma is increased (Mittendorfer et al. 2009). As a consequence, circulating levels of NEFAs are maintained within a relatively narrow range, regardless of an increase in adiposity. This relationship was verified in a recent meta-analysis of 1064 publications showing relatively stable circulating levels of NEFAs regardless of a change in BMI (Karpe et al. 2011). In this regard, adipose tissue provides a storage site for excess dietary fat and may act as a buffer for fatty acid flux (Frayn 2002). Ultimately, this prevents adverse metabolic consequences associated with elevated circulating levels of NEFAs (Zimmermann et al. 2004, Boden & Zhang 2006). Accordingly, we demonstrate that circulating levels of NEFAs in mice throughout progressive weight gain do not change significantly, regardless of increased adiposity. This is in agreement with observations from humans (Mittendorfer et al. 2009) and provides further evidence to suggest that an elevation of circulating levels of NEFAs is not a dominant feature during the early development of obesity (McQuaid et al. 2011). Presumably, an increase in circulating levels of NEFAs should only be observed following a complete disruption to the mechanisms that sustain NEFA balance.

Acting through lipoprotein lipase and hormone-sensitive lipase, circulating GH and insulin are thought to sustain the balance between stored and circulating fatty acids (Lafontan & Langin 2009). To our knowledge, potential direct interactions between GH and adipose triglyceride lipase (ATGL, the key enzyme that initiates triglyceride hydrolysis, Zimmermann et al. (2004)) are yet to be confirmed. Thus, it remains unknown whether the progressive suppression of GH secretion following weight gain would directly contribute to the overall slowing of ATGL-mediated NEFA flux. Regardless of the mechanisms of action, the inverse relationship between GH and circulating levels of NEFAs is well defined. GH treatment in humans results in an increase in lipolysis and a subsequent rise in plasma NEFAs (Gravholt et al. 1999, Moller et al. 2003), while GH-deficient patients have reduced lipolysis and an associated reduction in plasma NEFA concentrations (Boyle et al. 1992). Conversely, an elevation of circulating levels of NEFAs is known to suppress circulating levels of GH, presumably via the direct suppression of pituitary GH release (Casanueva et al. 1987, Alvarez et al. 1991, Briard et al. 1998) or hypothalamic (Briard et al. 1998)-stimulated GH secretion. Accordingly, it is thought that the suppression of GH secretion in obesity occurs in response to an elevation of circulating NEFAs (Girod & Brotman 2003). As discussed earlier, we did not observe a significant rise in circulating levels of NEFAs in response to increased adiposity. Rather, data demonstrate that the suppression of GH secretion occurred in the absence of a change in circulating levels of NEFAs. We thus conclude that it is unlikely that the progressive suppression of GH secretion following an increase in adiposity, and presumably in obesity, occurs as a direct consequence of an elevation in circulating levels of NEFAs. Rather, given the opposing actions of GH and insulin in sustaining NEFA exchange, we propose that impairments in GH secretion that accompany an increase in adiposity is a physiological adaptation that facilitates the homeostatic balance of circulating NEFAs. Given this, one would anticipate an inverse relationship between circulating levels of GH and the secretion of insulin.

Clasey et al. (2001) reported an inverse correlation between insulin and pulsatile GH secretion in non-obese healthy humans, whereas a study in rats revealed no significant correlation (De Schepper et al. 1998). The validity of measures in the rat was, however, limited by the use of a small sample size. In our study, we demonstrate a significant inverse relationship between measures of GH secretion and circulating levels of insulin in mice. This relationship was strengthened by inclusion of high-fat-fed animals and demonstrates the opposing effect of dietary-induced weight gain on circulating levels of insulin and GH. Overeating (Brons et al. 2009) and elevated GH (Barbour et al. 2005) impairs insulin signaling. Accordingly, it was found that the suppression of GH secretion following excess food consumption in humans...
ameliorates insulin resistance and sustains optimal meal tolerance (Cornford et al. 2012). Thus, the suppression of GH secretion during periods of excess food consumption appears to be a pivotal physiological adaptation to promote insulin-driven lipogenesis, thereby preventing hyperlipidemia (Cornford et al. 2012). Based on our observations, we propose an extension of the findings by Cornford et al. (2012) and anticipate that the maintenance of GH/insulin balance throughout extended periods of excess calorie consumption provides a key physiological mechanism to cope with prolonged positive energy balance. Given the eventual development of insulin resistance characteristic of type 2 diabetes (T2D), one would presume that the ‘protective’ actions of suppressed GH secretion would wane following the complete suppression of GH signaling. This would be further accentuated by the reduction in GH receptor expression seen in adipose tissue of obese subjects (Erman et al. 2011a,b).

Given the proposed interaction between GH and insulin in sustaining meal tolerance (Cornford et al. 2011), one may speculate that insulin contributes to the suppression of GH secretion following weight gain and obesity. Within the hypothalamus, insulin may impact GH secretion by promoting the release of catecholamines (Sauter et al. 1983), which in turn would stimulate the production of somatostatin to inhibit GH-releasing hormone (GHRH)-induced GH secretion. Involvement of the hypothalamus in the progressive decline of GH secretion relative to weight gain has, however, not been directly assessed. Moreover, human and animal studies provide compelling evidence to suggest that the hypothalamus may not be the principle site for altered GH secretion in obesity. Hypothalamic gene expression for GHRH and somatostatin does not change in dietary-induced obese rats, regardless of pituitary insensitivity to GHRH (Cattaneo et al. 1996), while chronic GHRH treatment coupled with arginine (to suppress endogenous SRIF release) does not recover GH secretion in dietary-induced obese patients (Ghigo et al. 1993). Consequently, it seems that impairments of GH secretion in obesity occur independent of changes in hypothalamic control of GH secretion. Rather, suppressed GH secretion in obesity is thought to occur predominantly in response to systemic signals acting directly on somatotrophs. Insulin suppresses GH release from isolated somatotrophs (Melmed 1984, Melmed & Slana 1985, Melmed et al. 1985, Luque & Kineman 2006) by acting on pituitary insulin receptors (Luque & Kineman 2006). These effects persist regardless of the development of systemic insulin resistance (Luque & Kineman 2006), confirming that somatotrophs remain insulin sensitive in obesity. In this context, it is likely that the progressive rise in insulin in response to dietary-induced weight gain promotes the progressive suppression of GH secretion. Moreover, these effects are sustained via continual pituitary insulin responsiveness. Anecdotal evidence from T1D patients further demonstrates the inverse relationship between endogenous GH secretion and insulin, wherein GH secretion is markedly elevated relative to a matched non-diabetic control population (Hansen & Johansen 1970, Hayford et al. 1980, Amiel et al. 1984, Asplin et al. 1989). Moreover, intensive insulin treatment markedly reduces GH secretion in juvenile T1D patients (Amiel et al. 1984). Of interest, the suppression of GH secretion following insulin treatment in T1D patients might occur as a consequence of a change in circulating levels of insulin-like growth factor 1 (IGF1). Circulating measures of IGF1 are reduced in T1D patients (Amiel et al. 1984, Asplin et al. 1989) and recover following intensive insulin treatment (Amiel et al. 1984). Accordingly, insulin-induced IGF1 may feed back to normalize GH secretion in these patients. It should be noted, however, that the assessment and accurate interpretation IGF1 measures require careful consideration of the bioavailability of IGF1, and in particular co-assessment of IGF1 binding proteins (IGFBPs). To this extent, a major caveat of the current study is the lack of data demonstrating interactions between pulsatile GH secretion and measures of free, bound, and tissue-specific IGF1 expression and measures of circulating IGFBPs. Future studies specifically addressing the role of IGF1 as a key inhibitor of GH secretion following progressive weight gain are of critical importance and must be completed alongside careful consideration of potential direct and indirect interactions with insulin.

In summary, our data confirm that dietary-induced weight gain results in a progressive reduction in total GH secretion rate, pulsatile GH secretion rate, and the mass of GH secreted per secretory event/pulse in mice. Thus, it is unlikely that GH deficiency observed in obesity occurs as a consequence of obesity-associated endocrine dysfunction. Rather, the progressive suppression of GH secretion is observed alongside developing weight gain and hyperinsulinemia. Presumably, this subverts the opposing physiological roles of GH and insulin in modulating NEFA flux and glucose homeostasis, where the suppression of GH secretion relative to an elevation in insulin is an essential physiological adaptation to sustain NEFA and glucose balance. Given our observations, future investigations need to address the mechanisms that account for the progressive reduction in GH secretion relative to weight gain.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
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