Suppression of hyperinsulinemia restores growth hormone secretion and metabolism in obese mice

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Abstract

The well-balanced secretion between insulin and growth hormone (GH) is essential in regulating substrate metabolism, energy metabolism, and body composition. High insulin and low GH levels are often observed in obesity, contributing to reduced energy expenditure and further fat accumulation. Although suppression of hyperinsulinemia is proposed as a treatment for obesity, changes in GH secretion and energy metabolism following this treatment are not thoroughly studied. This leaves unexplained observations, such as unchanged lean mass following insulin reduction. In this study, high-fat diet-induced obese (DIO) and normal chow-fed lean mice on a C57BL/6J background were treated for 7 weeks with diazoxide (1250 mg/kg in food), a KATP channel opener that suppressed insulin secretion. Diazoxide treatment for 10 days was sufficient to increase pulsatile GH secretion in DIO mice before any significant body weight change. The restored insulin-GH balance in DIO mice was followed by improvement in substrate and energy metabolism in a prolonged treatment period (4–6 weeks), including reduced fat mass, increased lipid oxidation and energy expenditure, as well as improved insulin sensitivity and metabolic flexibility. These metabolic benefits occurred along with the changes in the expression level of genes regulated by the insulin-GH balance. When applying diazoxide to normal chow-fed normoinsulinemic lean mice, none of the above metabolic effects was observed, suggesting that the metabolic changes following diazoxide treatment were mediated through the suppression of hyperinsulinemia. These results suggest that suppression of hyperinsulinemia by diazoxide restores GH secretion and improves substrate and energy metabolism in DIO mice.

Key Words
- growth hormone
- diazoxide
- hyperinsulinemia
- obesity
- energy metabolism

Introduction

Obesity is one of the most common health problems in the contemporary world. It increases risks for some severe chronic diseases, such as type 2 diabetes (T2D), cardiovascular diseases, and cancers. The development of obesity is caused by the imbalance between energy intake and expenditure. Metabolic hormones, such as insulin and growth hormone (GH), play critical roles in the regulation of substrate and energy metabolism (Huang et al. 2020a).
Both insulin and GH play an anabolic role in protein metabolism but act differently in other substrate and energy metabolism. Insulin promotes energy storage (i.e. lipogenesis) whereas GH stimulates the usage of the stored energy (i.e. lipolysis and lipid oxidation) (Huang et al. 2020a). In terms of glucose metabolism, insulin facilitates glucose uptake and oxidation (Kelley et al. 1990) whereas GH promotes hepatic glucose production (Chia 2014). Obese individuals display hyperinsulinemia and reduced GH secretion (Berryman et al. 2013), which may lead to reduced energy expenditure and increased fat accumulation, according to insulin-GH balance theory (Huang et al. 2020a). In addition, the carbohydrate-insulin model of obesity also suggests that postprandial hyperinsulinemia led to fat accumulation (Ludwig & Ebbeling 2018), although whether it is through reduced food intake remains controversial (Ludwig & Ebbeling 2018, Hall et al. 2021). Therefore, restoring the insulin-GH balance could be beneficial for the treatment of obesity.

Mild suppression of hyperinsulinemia is an emerging treatment for obesity (Page & Johnson 2018). Diazoxide, an ATP-sensitive potassium channel (K_{ATP} channel) opener, acts on pancreatic beta-cells to suppress insulin secretion (Ashcroft & Gribble 1999). By applying diazoxide to obese rodents and patients, both preclinical (Alemzadeh et al. 1993, 1996, 2002, 2008, Aizawa et al. 1995, Alemzadeh & Holshouser 1999, Standridge et al. 2000, Surwit et al. 2000, Hensley et al. 2001, Alemzadeh & Tushaus 2004, Guo et al. 2008) and clinical (Alemzadeh et al. 1998, Van Boekel et al. 2008, Loves et al. 2018b, Huang et al. 2020b) studies showed reduced fat mass and body weight without changing lean mass. However, the change of GH following the reduction of hyperinsulinemia by diazoxide treatment remains undefined. Addressing this knowledge gap is essential to understand and untangle some of the phenomena in previous studies. For example, the preserved lean mass following diazoxide treatment regardless of a reduction in insulin levels may actually be associated with the protein-sparing effect of GH. In addition, the failure of diazoxide in reducing fat mass in obese children with hypothalamic-pituitary lesions (Brauner et al. 2016) may be due to the inability to recover the lipolytic hormone GH.

In this study, we applied diazoxide to both diet-induced obese (DIO) mice and normal chow-fed lean mice and focused on the alterations in GH secretion profile, as well as the following changes in substrate and energy metabolism.

### Materials and methods

#### Animals

Male C57BL/6j mice were single-housed under a temperature of 22 ± 2°C with a 12 h light:12 h darkness cycle (lights on at 08:00 h, off at 20:00 h) in the animal facility of the Institute for Bioengineering and Nanotechnology, the University of Queensland. All mice had free access to water and food (details below). All experiments were approved by the University of Queensland Animal Ethics Committee.

#### Experimental design

The flow chart of the experimental design was shown in Supplementary Fig. 1 (see section on supplementary materials given at the end of this article). DIO mice were induced by high-fat diet (HFD) feeding (43% energy from fat) (SF04-001, Specialty Feeds, Glen Forrest, WA, Australia) from 7-week-old for 5 weeks before the initiation of the treatment, while the normal chow-fed lean mice started treatment at 12-week-old. Both DIO and lean mice were randomly divided into two subgroups: control group – mice were continued with the previous diet (DIO, n=10; lean, n=5); treatment group – mice received 1250 mg/kg diazoxide added into the previous diet (DIO+DZX, n=12; lean+DZX, n=6). The concentration of diazoxide mixed in the food was calculated from previous publications (Gray et al. 2010, Bischof & Wevrick 2018) where 150 mg/kg-day diazoxide was able to suppress insulin secretion in obese mouse models and adjusted to the food intake in our current mouse model by previous measurement (a 30 g mouse consumed approximately 3.6 g food per day). There was no significant difference in the baseline body weight at 12-week-old between control and treatment groups in both lean and DIO mice (Supplementary Table 1). To eliminate the impact of body weight changes on GH secretion, measurement of GH profiles started as early as 10 days of treatment (defined as short-term in this study), prior to any significant changes of body weight between treatment and control groups, using half of the DIO and DIO+DZX mice. The rest of the mice underwent indirect calorimeter measurement during the first week and following 5 weeks of the treatment for the short-term and long-term effects, respectively. To assess the prolonged effect of diazoxide treatment on glucose metabolism, insulin tolerance test (ITT) and glucose tolerance test (GTT) were performed after 4 weeks of the treatment (defined as a long-term effect in this study) with a 4-day interval between two tests. Following 7 weeks of the diazoxide treatment, all mice were sacrificed by sodium pentobarbital (32.5 mg/kg, intraperitoneal injection) during pentobarbital (32.5 mg/kg, intraperitoneal injection) during

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10:30–12:30 h underfed state. Terminal plasma was collected by cardiac puncture and centrifugation. Fat tissues from interscapular, inguinal, epididymal depots were dissected and weighted to represent the weight of brown, s.c. and visceral depots, respectively. Tissues were snap-frozen for gene expression measurement.

Indirect calorimeter measurement
Mice were individually housed in the indirect calorimeter chamber (TSE PhenoMaster, Germany) for 3–5 days to assess the effects of diazoxide on energy metabolism. The instrument recorded food intake, locomotor activity, oxygen consumption, and respiratory exchange ratio (RER) every hour. The metabolic flexibility was assessed by removing all the food at 20:00 h when the light was turned off. Body fat mass and lean mass were measured by an NMR device (Bruker, USA).

Tests for glucose metabolism
GTT and ITT were performed to assess the whole-body glucose tolerance and insulin sensitivity, respectively. Prior to GTT and ITT, the mice underwent overnight (14 h) and morning (5 h) fasting, respectively. During the experiments, mice were treated with glucose (2 g/kg, oral gavage) for GTT or human insulin solution (0.75 U/kg for DIO mice, 0.5 U/kg for lean mice, intraperitoneal injection) for ITT at time point 0 min. Glucose levels were measured by a glucose ketone meter (Nova Stat Strip Xpress Glucose Hospital Meter, Nova Biomedical, UK) and glucose-test strips (42214, Nova Biomedical UK) following glucose or insulin administration in various time points (15, 30, 60, 120 min for GTT; 15, 30, 45, 60, 90 min for ITT). To assess the direct effect of diazoxide on hepatic glucose production, a pyruvate tolerance test was performed on lean mice. Briefly, mice underwent an overnight fast (14 h) before receiving sodium pyruvate (2 g/kg, intraperitoneal injection). Blood glucose levels were recorded as described above at 15, 30, 60, 90, 120 min after pyruvate injection. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the equation: HOMA-IR = fasting blood glucose (mmol/L) × fasting plasma insulin (μU/L)/22.5.

Serial blood collection for GH measurement
Pulsatile GH secretion profile was determined according to an established method (Steyn et al. 2011). Briefly, 2 μL of the whole blood was collected from tail tips at a 10-min interval from 09:30 to 15:30 h. The blood samples were immediately mixed with 58 μL 0.01 M PBS supplemented with 0.05% Tween 20 and snap-frozen on dry ice. The samples were stored at −80°C before measured by in-house GH ELISA (Steyn et al. 2011). The intra- and inter-assay coefficients of variation for the GH ELISA assay ranged from 3.3% to 7.5%.

Hormones and metabolites measurement
Plasma insulin and free fatty acids (FFA) levels were measured by the Rat/Mouse Insulin ELISA Kit (Millipore) and NEFA C kit (Wako), respectively.

Gene expression measurement
Total RNA of the liver and s.c. fat was extracted by TRizol before purified by a NucleoSpin RNA Kit (Scientifix, Australia). The cDNA was synthesized by iScript® RT Supermix (Bio-Rad). Quantitative PCR was performed by SYBR® Green PCR Master Mix (Thermo Fisher Scientific) under QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific). Primer pairs were shown in Supplementary data (Supplementary Table 2). The expression level of the target gene was shown as fold change to that of the housekeeping gene (beta-actin) by the 2−ΔΔCT method. No significant difference was observed in the CT values of beta-actin between diazoxide and control groups in both Lean and DIO mice (Supplementary Table 3).

Statistics
Statistical analysis was carried out by GraphPad Prism 8 software (GraphPad Software) except for the pulse amplitude and the deconvolution analysis for GH. The results were presented by scatter plot with mean or mean ± s.e.m. for XY graphs. Differences between groups were identified by two-tailed Student’s t-test. The difference was considered to be statistically significant when P-value is < 0.05. Analysis of GH pulse amplitude was performed using the non-commercial Pulse_XP hormone cluster analysis software (Michael L. Johnson, Keswick, VA). The quantitative features underlying GH secretion associated with observed GH levels were determined by deconvolution analysis using established parameters (Steyn et al. 2011).

Results
Diazoxide restores insulin-GH balance in DIO mice in the short-term
Following diazoxide treatment, the morning fasting insulin levels in DIO mice were significantly reduced (Fig. 1A),
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Figure 1
Insulin and GH secretion change following diazoxide treatment. (A and B) Insulin levels in DIO (A) and lean (B) mice treated by diazoxide. (C) Representative examples of circulating GH levels (left) and the GH secretion pulse onset (right) in each group during the 6-h measurement period. (D and E) the mean GH level (D) and mean GH pulse amplitude (E) during the 6-h measurement period calculated by the circulating GH concentrations. (F, G, H, I, J and K) Deconvolution analysis showed the changes of total GH secretion (F), pulsatile GH secretion (G), mass of GH secretion per burst (H), basal GH secretion (I), the number of secretory bursts per 6 h (J), and approximate entropy (K). Data were presented by scatter plot with mean, \( n = 4–5 \) each group. *\( P < 0.05 \), **\( P < 0.01 \); ns, non-significant.

along with the significantly decreased HOMA-IR (Supplementary Fig. 2A). The GH profile (Fig. 1C) was measured following 10 days of the treatment, where the bodyweight of the DIO mice was not significantly different between control and diazoxide groups yet (Supplementary Fig. 2B). At this time point, diazoxide significantly increased the mean GH level (Fig. 1D) and the mean GH pulse amplitude (Fig. 1E) during the 6-h measurement period. The elevated [insulin]:[GH] ratio in obesity (Huang et al. 2012, 2020a, Steyn et al. 2013) was significantly decreased following diazoxide treatment as calculated by dividing fasting insulin level by the mean GH level (Supplementary Fig. 2C) or mean GH pulse amplitude (Supplementary Fig. 2D). The parameters for GH secretion generated from the deconvolution analysis showed that diazoxide treatment increased total GH secretion (Fig. 1F), pulsatile GH secretion (Fig. 1G), and mass of GH secretion per burst (Fig. 1H), whereas no significant change was observed in basal GH secretion (Fig. 1I). The diazoxide treatment did not alter the GH secretion pattern, as indicated by the unchanged number of secretory bursts per 6 h (Fig. 1J) and approximate entropy (Fig. 1K) (a parameter that reflects the regularity of GH pulses). Although GH secretion was increased, the total IGF-1 level remained unchanged in DIO mice following diazoxide treatment (Supplementary Fig. 1E). In control lean mice, diazoxide did not significantly alter fasting insulin levels (Fig. 1B).
The short-term effects of diazoxide on substrate and energy metabolism

To determine the instant effects of diazoxide treatment on substrate and energy metabolism, mice were recorded in the indirect calorimeter during the first 5 days after the initiation of the diazoxide treatment. Following the diazoxide treatment in DIO mice, the food intake was slightly but significantly suppressed (Fig. 2A), and the RER was reduced during the dark phase (Fig. 2B and E). Despite reduced food intake, the energy expenditure, indicated by oxygen consumption, in DIO mice treated by diazoxide was sustained (Fig. 2C and F). No change in locomotor activity was observed (Fig. 2D and G). In lean mice treated by diazoxide, all of the above parameters remained unchanged during the first 5-day treatment (Supplementary Fig. 3).

The long-term effects of diazoxide on substrate and energy metabolism

To determine the long-term effects of diazoxide treatment on substrate and energy metabolism, tests for glucose metabolism were performed following 4 weeks of diazoxide treatment; body composition and parameters in the indirect calorimeter were assessed after 5–7 weeks of the treatment. In DIO mice, diazoxide treatment improved insulin sensitivity and glucose tolerance in the long term, as indicated by ITT (Fig. 3A and B) and GTT (Fig. 3E and F), respectively. In lean mice, diazoxide treatment did not change insulin sensitivity (Fig. 3C and D), but unexpectedly reduced blood glucose levels during GTT (Fig. 3G and H). It may be attributed to the direct effect of diazoxide in reducing hepatic glucose production (Kishore et al. 2011). To test this, a supplemental pyruvate tolerance test was performed only in lean mice. The result showed that diazoxide significantly reduced blood glucose levels following the injection of pyruvate (Fig. 3I), which was the substrate for gluconeogenesis. This indicated that the reduced glucose levels in lean mice with diazoxide treatment might be caused by the inhibition of hepatic gluconeogenesis.

Regarding the long-term effects on body composition and energy metabolism, diazoxide treatment reduced body weight gain in DIO mice (Fig. 4A), of which fat mass was significantly reduced whereas lean mass was maintained (Fig. 4B and C). The reductions in food intake (Fig. 4I) and RER (Fig. 4J and N) by diazoxide in DIO mice were similar to the changes observed in the first 5-day treatment period (Fig. 2) and sustained in the long-term treatment period. However, if food intake was corrected by body weight, there was no significant change between diazoxide and control groups in DIO mice (Supplementary Fig. 4A), indicating that the reduction in food intake may be more related to the change in body weight than the direct effect of diazoxide in the long-term treatment condition. Besides, the energy expenditure, indicated by oxygen consumption, was increased in DIO mice after long-term diazoxide treatment (Fig. 4K and O). These changes possibly led to the reduced plasma FFA level (Fig. 4D). Moreover, diazoxide-treated DIO mice showed reduced RER after the initiation of fasting (Fig. 4M),
indicating a prompt switch to lipid usage during fasting, thus suggesting improved metabolic flexibility. Similar to the short-term, diazoxide did not change the locomotor activity in DIO mice in the long-term treatment (Fig. 4L and P). In lean mice, diazoxide did not change any parameters regarding the body composition (Fig. 4E and G) and energy metabolism in the long-term treatment (Supplementary Fig. 5). However, the FFA levels were increased following diazoxide treatment in lean mice (Fig. 4H).

**The gene expression changes in substrate metabolism following the long-term diazoxide treatment**

To determine the changes of glucose and lipid metabolism on the molecular level, gene expression analysis was performed on the liver and s.c. fat tissues. Expectedly, diazoxide reduced the expression levels of genes involved in gluconeogenesis in both DIO (G6p and Pepck) and lean (G6p) mice (Fig. 5A and B). In terms of lipid metabolism, diazoxide-treated DIO mice showed increased lipid oxidation genes (Ppara and Cpt1) expression in the liver (Fig. 5A), as well as the increased expression level of genes involved in lipolysis (Hsl and Atgl) and white adipose tissue (WAT) browning (Ucp1, Pgc1α, Pparγ, and Cidea) in the s.c. fat (Fig. 5C). These changes were indiscernible in lean mice with or without diazoxide treatment (Fig. 5B and D).

**Discussion**

The insulin-GH balance plays an important role in regulating substrate and energy metabolism (Huang et al. 2020a). With high insulin and low GH levels, obese individuals display disrupted insulin-GH balance, which in turn may hamper energy homeostasis and deteriorate the obese condition (Huang et al. 2020a). Although reducing hyperinsulinemia by diazoxide has been shown to reduce fat mass in both preclinical and clinical studies, the accompanied GH secretion profile following the
Reduction of hyperinsulinemia was unknown. This study sought to define the GH secretion profile, as well as the following changes in substrate and energy metabolism after the reduction of hyperinsulinemia by diazoxide. Previous studies showed that DIO increased insulin and decreased pulsatile GH levels in mouse (Huang et al. 2012, Steyn et al. 2013), leading to increased [insulin]:[GH] ratio. Here, we showed that diazoxide restored pulsatile GH secretion without altering the secretion pattern in DIO mice. With the reduced insulin and increased GH levels, the insulin-GH balance shifted toward normal. Following the restoration of the insulin-GH balance, the substrate metabolism switched toward lipid utilization, resulting in increased lipid oxidation in both liver and s.c. adipose tissues. In addition, the energy expenditure was increased relative to food intake in DIO mice following diazoxide treatment in both the short- and long-term, leading to reduced fat mass and improved insulin sensitivity. Weakened protein anabolism via reduced insulin secretion by diazoxide treatment in DIO mice may be recompensed by the recovered GH. None of the above metabolic effects was observed in diazoxide-treated normoinsulinemic normal chow-fed lean mice. It strongly suggested that these effects of diazoxide seen in DIO mice were mediated through reducing hyperinsulinemia and recovering insulin-GH balance.
The suppressed GH secretion in obesity has been studied for a few decades; however, the underlying mechanism is not clear yet. Many studies showed that GH secretion was reduced after weight gain (Rasmussen et al. 2010, Huang et al. 2012, Steyn et al. 2013) or recovered GH secretion after weight loss in obesity (Rasmussen et al. 1995, 2007). Further, studies found that adiposity was a negative determinant of GH secretion in both humans (Iranmanesh et al. 1991, Clasey et al. 2001) and rodents (Huang et al. 2012, Tan et al. 2016). However, all these above studies were observational. None of them had dissected out a single factor that was solely responsible for the suppressed GH secretion in general obesity. Although some candidates, such as increased insulin, leptin, or FFA, were proposed as contributors to the suppression of GH secretion in obesity (Maccario et al. 2000), their role in general obesity remained unclear. For instance, studies found that reducing plasma FFA by acipimox led to restored spontaneous GH secretion (Kok et al. 2004) or the response to GH-releasing hormone (GHRH) (Maccario et al. 1996) in obese individuals, it could not fully explain the low GH secretion in general obesity because FFA was not always elevated in obesity (Karpe et al. 2011). The other study showed that 3-day overeating resulted in suppressed GH secretion, possibly through hyperinsulinemia, but was independent of fat mass, blood glucose, FFA, cortisol, and IGF-1 levels (Cornford et al. 2011). However, the causal role of hyperinsulinemia in the suppressed GH in a general obese population was not thoroughly established in that study because (1) obesity was not always caused by short-term overeating; (2) overeating might cause other changes which affected GH secretion, for example, increased circulating leptin level (Kolaczynski et al. 1996). To our knowledge, there was no study which investigated the GH secretion profile following...
the modification of insulin secretion in obese individuals so far. Our results showed that reducing hyperinsulinemia by diazoxide led to GH recovery in the short term prior to any significant changes in body weight (adiposity) in DIO mice. This provided direct evidence that hyperinsulinemia played a major role in suppressing GH secretion in obesity. In terms of the mechanisms, previous studies suggested that hyperinsulinemia might directly inhibit GH secretion through insulin receptors in somatotrophs both in vivo and in vitro (Luque & Kineman 2006, Gahete et al. 2013). Besides, hyperinsulinemia may reduce the production of insulin-like growth factor-binding protein 1 in the liver (Powell et al. 1991), leading to increased free IGF-1 levels, and thus inhibits GH secretion through IGF-1 negative feedback effect on somatotrophs (Powell et al. 1991). Although the varied activity of some hypothalamic neurons (i.e. GHRH and somatostatin) may alter GH secretion, they were unlikely to play a major role in this case because the GH secretion pattern was unchanged following diazoxide treatment in DIO mice.

In terms of the substrate and energy metabolism following diazoxide treatment, previous studies showed that diazoxide reduced food intake (Aizawa et al. 1995, Alemzadeh & Holshouser 1999, Alemzadeh et al. 2002, 2008, Alemzadeh & Tushaus 2004, Guo et al. 2008) and increased basal metabolic rate (Alemzadeh et al. 2008) in obese Zucker Fatty Rats and Otsuka Long–Evans Tokushima Fatty Rats. In the DIO mice, which reflected general human obesity, we observed similar results (Fig. 4I, K and O). Both reduced food intake and increased energy expenditure facilitated the reduction of body weight and fat mass in DIO mice treated by diazoxide. Moreover, decreased RER following diazoxide treatment (Figs 2B, E and 4J, N) indicated that the substrate metabolism was switched toward lipid utilization. This increased fat utilization might come from increased lipolysis in WAT, as well as increased lipid oxidation in the liver and browning WAT, evidenced by the increase in the expression level of genes involved in lipolysis and lipid oxidation (Fig. 5). Importantly, these enzymes were regulated by insulin-GH balance (Huang et al. 2020a). Once the FFA released from WAT were taken up and oxidized, the net effect was reduced fat mass (Fig. 4B and C) and circulating FFA levels (Fig. 4D). Moreover, we showed improvement in metabolic flexibility (Fig. 4M) following diazoxide treatment in DIO mice. Metabolic inflexibility in obese individuals was associated with insulin resistance (Goodpaster & Sparks 2017) and the susceptibility to weight gain upon environmental challenges, such as HFD (Astrup 2011). The improved metabolic flexibility by diazoxide in DIO mice could be attributed to the improved insulin sensitivity and might contribute to weight reduction.

The effects of diazoxide on the overall glucose metabolism depend on three factors: (1) the suppressed insulin secretion, which tends to increase blood glucose levels; (2) the improved insulin sensitivity, which may decrease blood glucose levels and (3) the direct effect of diazoxide in reducing glucose production (Kishore et al. 2011) and increasing glycogen synthesis (Jansen et al. 1967, Alemzadeh et al. 2002) in the liver. Clinical trials showed impaired (Due et al. 2007, Van Boekel et al. 2008, Brauner et al. 2016, Loves et al. 2018a) or unchanged (Alemzadeh et al. 1998) glucose tolerance following diazoxide treatment, whereas nearly all diazoxide studies in rodents showed either improved glucose tolerance or reduced fasting blood glucose levels (Alemzadeh et al. 1993, 1996, 2002, 2008, Aizawa et al. 1995, Alemzadeh & Holshouser 1999, Surwit et al. 2000, Alemzadeh & Tushaus 2004, Guo et al. 2008). The contradictory results may come from species differences, as the major organ for glucose clearance is the liver in rodents but skeletal muscle in humans (Chandrasekera & Pippin 2014). Also, the blood glucose levels of rodents are heavily reliant on gluconeogenesis, compared to humans (Kowalski & Bruce 2014). Therefore, the reduced hepatic glucose production as seen in both DIO and lean mice in this experiment (Fig. 5A and B), and possibly increased glycogen synthesis, may override the other two factors and become the determinant factor for the overall glucose metabolism, whereas the suppressed insulin secretion may play a major role in regulating skeletal muscle glucose metabolism in humans. Therefore, rodents may not be an ideal model to study the pharmacological effects of diazoxide on glucose levels. Nevertheless, diazoxide improved insulin sensitivity in DIO mice (Fig. 3A, B and Supplementary Fig. 2B), but not lean mice (Fig. 3C and D), indicating that the hormonal changes may contribute to the improved insulin sensitivity. It was also supported by previous studies showing improved insulin sensitivity following the reduction of hyperinsulinemia by streptozotocin in obese mouse models (Ning et al. 2011, Pedersen et al. 2015). In addition, it was suggested that hyperinsulinemia per se led to insulin resistance (Shanik et al. 2008) and reduction of hyperinsulinemia alleviated insulin resistance (Page & Johnson 2018). These previous studies and our results all support that hyperinsulinemia may play a causal role in the development of insulin resistance in the absence of hyperglycemia and may be targeted to treat insulin resistance.

There are a few limitations of this study. First, diazoxide exerts effects other than insulin suppression, such as
inhibition of food intake and hepatic glucose production. Whether these effects also contribute to the recovered GH secretion following diazoxide treatment in DIO mice are not clear yet. Future experiments may use pair-feeding to abolish the effects of diazoxide on food intake. However, diazoxide did not alter food intake in lean mice, suggesting that the reduced food intake by diazoxide in DIO mice may be secondary to the reduction of hyperinsulinemia. Secondly, diazoxide treatment in the current study was only for 7 weeks. Any effects of the extended period of treatment are yet to know. Thirdly, due to the species difference between rodents and humans, some of the results in glucose metabolism may not fully apply to humans. In the future, a more specific approach to suppress insulin secretion is needed to understand better the role of insulin, as well as the regulation of GH secretion, in obesity. Such an approach may also be applied to treat obese patients. Mouse models with disrupted GH signaling may also be used to investigate the role of GH in the metabolic effects of diazoxide.

In conclusion, this study demonstrated that suppression of hyperinsulinemia by diazoxide restores pulsatile GH secretion in DIO mice independent of body weight change. The restored insulin-GH balance in DIO mice treated by diazoxide may contribute to the subsequent beneficial effects in substrate and energy metabolism, including improvement in body composition, insulin sensitivity, and metabolic flexibility. This study highlights the suppression of insulin secretion as a potential therapy for obesity in the future.

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