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ORIGINAL ARTICLE

IGF-I overexpression does not promote compensatory islet cell growth in diet-induced obesity

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Abstract Although IGF-I was known to stimulate the growth of pancreatic islet cells from early in vitro experiments and in vivo reports on rodents, recent gene targeting experiments have indicated that IGF-I and its receptor do not play a major role in normal islet cell growth. In our previous reports, liver- or pancreatic-specific IGF-I deficiency caused no decrease in β-cell mass; a general and β-cell-enriched IGF-I overexpression caused no change in normal islet cell growth. On the other hand, increased metabolic demands (such as in obesity and insulin resistance) result in β-cell compensation in cell number and insulin secretion. In order to test whether IGF-I could promote islet cell growth and facilitate islet compensation due to obesity-induced insulin resistance, we have challenged MT-IGF mice to a high-fat diet. After 28 weeks, both MT-IGF mice and wild-type littermates gained comparable 40–57% of body weight, with similar increases in fat masses; all mice maintained a normal sensitivity to insulin and did not become severely hyperglycemic. Nevertheless, compared to wild-type littermates, the equally obese MT-IGF mice maintained improved glucose tolerance and a diminished insulin level; similar to when fed a normal chow diet. More importantly, under IGF-I overexpression, there was no further increase in β-cell mass caused by obesity. Thus, IGF-I overexpression had no significant effect on weight gain and islet cell compensation in response to high-fat diet-induced obesity.

Keywords Insulin-like growth factor I · Transgenic mice · Pancreatic islets · High-fat diet · Obesity · Insulin resistance

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Introduction

Insulin-like growth factor (IGF)-I plays an important role in normal cell proliferation, differentiation, thus is crucial for intrauterine development and postnatal growth. Most of the IGF-I effects are mediated by the IGF-I receptor (IGF-IR) which is ubiquitously expressed [1]. Deficiency in IGF-I or IGF-IR severely compromises mouse development, results in dwarfism, and neonatal lethality [2–4]. As a potent growth factor, IGF-I was known to stimulate the growth of pancreatic islet cells from early in vitro experiments and subsequent in vivo reports on rodents [5–7]. However, more recent cell-specific gene targeting has indicated that IGF-I and IGF-IR do not play a major role in normal islet cell growth [8–10]; we have reported that liver- or pancreatic-specific IGF-I deficiency caused no decrease in β-cell mass [11, 12]; and a general and β-cell-enriched IGF-I overexpression (in MT-IGF mice) caused no change in normal islet cell growth [13]. These in vivo results convincingly propose that normally IGF-I does not promote islet cell growth.

Pancreatic islet β-cells secrete the essential hormone insulin, which controls fuel homeostasis in health, and defects in its action/production result in diabetes mellitus.

The β-cell mass can be regulated not only during normal growth and development, but also in response to specific metabolic challenges in adult life such as obesity-induced insulin resistance or during pregnancy. Type 2 diabetes mellitus (T2DM) is caused by the imbalance of insulin resistance and islet cell compensation. In response to diminished insulin effects and the resulting increase in blood glucose level, the islet cells increase insulin release and undergo expansion through hyperplasia and/or hypertrophy. Normally islet cell growth is stimulated by a host of growth factors including glucose, glucagon-like peptide 1, hepatocyte growth factor, and growth hormone [14–16]. Compared to a clear stimulation of islet cell growth by prolactin during pregnancy, it is relatively unclear on which signals stimulate islet cell growth in response to insulin resistance [17, 18]. Previously we have demonstrated that without growth hormone signals, islet cell compensation can still occur [19]. Although IGF-I is unlikely involved in normal islet cell growth; as a potent growth factor, it might still have an effect on compensatory islet cell growth when supplemented exogenously.

Thus, in order to test whether elevated IGF-I level could increase islet cell growth and facilitate islet compensation in response to obesity-induced insulin resistance, we have challenged MT-IGF mice to a high-fat diet for an extended 28 weeks.

Materials and methods

The MT-IGF mice

MT-IGF mice have germline integration of a human IGF-I cDNA driven by the mouse metallothionein 1 promoter, and were studied on a mixed C57BL/6 background [20, 21]. They were maintained in 12 h dark/light cycles at room temperature with free access to food and water. To determine genotype, genomic DNA from tail clips, primers MT-1 and MT-2 were used in PCR reactions, which yield a 0.5-kb band for the transgenic allele [13]. Serum or plasma concentrations of insulin were determined using RIA kits (Linco Research, St. Charles, MO). Blood glucose levels were measured using the OneTouch blood glucose meter (LifeScan Canada, Burnaby, BC). The McGill University Animal Care Committee approved all animal-handling procedures.

High-fat diet-induced obesity

Male MT-IGF mice and wild-type littermates (3 month of age) were fed for 28 weeks with a high-fat diet (HFD: fat 34.9%, protein 26.2%, carbohydrate 26.3%; Research Diets, New Brunswick, NJ). Their body weight was measured once a week, and blood glucose levels every 3 week for the first 12 weeks then every week for the remainder of the study, using an OneTouch blood glucose meter. At 9 and 16 weeks, insulin tolerance tests were performed. Animals were injected with recombinant human insulin (0.75 IU/kg ip; Roche), and blood glucose levels were measured at 0, 20, 40, and 60 min afterward. At 10, 17, and 26 week, glucose tolerance test was performed to mice fasted for 24 h and injected with glucose (1 g/kg i.p.). At 28 weeks, the mice were anesthetized by intraperitoneal administration of a cocktail of ketamine-xylazine-acepromazine, body length and weight were measured and designated fat pads were removed and weighed. The mice were killed by cervical dislocation, blood was collected for serum preparation, and pancreata were rapidly removed for histochemical analysis. Similar experiments were performed in female MT-IGF and wild-type mice which were fed high-fat diet for a shortened 16 weeks.

Serum IGF-I assay

The MT-IGF mice produce two forms of IGF-I, i.e., human IGF-I derived from the transgene and endogenous murine IGF-I, that cannot be measured by a single assay. Instead, the levels of total serum IGF-I released by extraction were measured by using a mouse/rat IGF-I RIA kit (no cross-reaction to human IGF-I; DSL-2900) and a human IGF-I quantikine ELISA kit (no cross-reaction to mouse IGF-I; DG100, R&D Systems) consecutively.

Immunohistochemistry and islet β-cell mass

Pancreatic sections were stained with insulin (Monosan, Uden, The Netherlands) using diaminobenzidine substrate. Microscopic images were captured with a Retiga 1300 digital camera (Q Imaging, Burnaby, BC) using Northern Eclipse software version 6.0 (Empix Imaging, Mississauga, ON). The β-cell mass was determined as previously reported [22].

Statistical analysis

Data were expressed as mean $\pm$ SE. The graphs and the calculation of Area under Curve were prepared using SigmaPlot software, version 11 (Systat, San Jose, CA). The Student's *t*-test (unpaired and paired) and one-way analysis of variance (ANOVA) were performed using InStat software version 3 (GraphPad Software, San Diego, CA).

Results

High-fat diet-induced obesity in MT-IGF mice

In response to high-fat diet, wild-type mice exhibited a continuous and steady elevation in body weight, which resulted in a 57% gain by 28 weeks (Fig. 1a and Table 1); illustrating the effectiveness of the diet strategy. MT-IGF mice were heavier from the beginning because of IGF-I-induced weight gain unrelated to the diet; during the first half of the experiment, up to 15 weeks on the high-fat diet, they also gained weight further and steadily in a comparable rate; after that, however, the weight acceleration was somehow halted in the latter half of the treatment. As a result, MT-IGF mice only gained 40% of body weight in 28 weeks, although not significantly less than wild-type littermates (Table 1 and Fig. 1a). There was no difference in body length and body mass index in MT-IGF vs. wild-type littermates, after diet-induced obesity (Table 1).

To further confirm the obese phenotype, mice were killed after 28 weeks on the diet and various fat pads were excised, weighed, and corrected for total body weight. As shown in Fig. 1b, both the wild-type and MT-IGF mice increased the weight of each fat pad to similar extents (from baseline values not included but reported previously) [12, 19], indicating that MT-IGF mice were not significantly resistant to diet-induced increase in adiposity. The amounts of weight gain in various fat pads were similar to our previous report after 17 weeks of high-fat diet, using GHR−/− mice [19]. Similar results in body and fat weight gains were also obtained from female MT-IGF mice vs. wild-type littermates, fed the high-fat diet for a shortened 16 week (data not shown).

With diet-induced obesity, we had expected insulin resistance, hyperglycemia, or even type 2 diabetes in wild-type mice; thus random fed glucose levels were measured every two or three weeks. In wild-type mice, there seemed to be a tendency of elevated glucose level especially toward the latter half of the diet treatment, from 150 to 180 mg/dl; but this trend was largely over shadowed by the variability of the glucose values in the course of the treatment (Fig. 1c). MT-IGF mice were hypoglycemic to start with, caused by the excess IGF-I as we have recently reported [13]; the decreased glucose level exhibited similar fluctuations as in the wild-type mice and was not significantly elevated by the mice becoming increasingly obese. Similarly decreased glucose levels in female MT-IGF mice vs. wild-type littermates were also observed, which were fed with the high-fat diet for 16 weeks (data not shown). Thus, in this particular study, high-fat diet-induced obesity after 28 weeks did not cause significant increase in blood glucose level in either group of littermate mice. In Table 2,

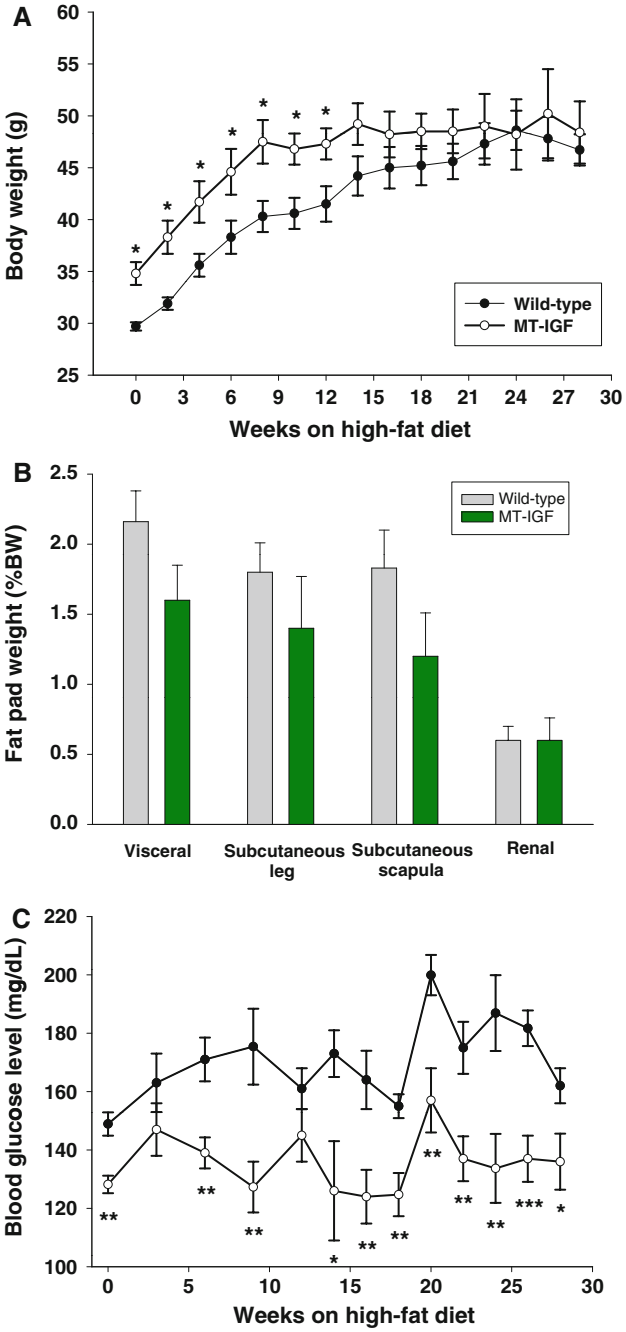

Fig. 1 Changes in body weight, fat mass, and blood glucose level in MT-IGF mice in response to high-fat diet-induced obesity. **a** High-fat diet induced a steady weight gain in both wild-type and MT-IGF mice. Male MT-IGF and wild-type littermates (3-month-old) were fed with a high-fat diet for 28 weeks. Their body weight was measured once a week and plotted against the number of weeks on the diet. **b** Comparison of relative fat mass (per total body weight) in MT-IGF and wild-type mice. After 28 week on the diet, four major fat pads were excised and measured in wet weight. *BW* body weight. **c** Changes in blood glucose level in MT-IGF mice vs. wild-type littermates during the 28 weeks on high-fat diet. Glucose level was measured in random fed status in the afternoon hours every three weeks. $N = 7$–8. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. wild-type littermates

Table 1 Changes in body and pancreas weights and β-cell mass in MT-IGF mice after high-fat diet-induced obesity

Parameter	Wild-type (8)	MT-IGF (7)	*P* value
Body weight (g)	46.7 ± 1.5	48.4 ± 3.0	NS
Relative weight (vs. before HFD)	1.57 ± 0.05	1.40 ± 0.12	NS
Body length (cm)	11.2 ± 0.1	11.6 ± 0.3	NS
Body mass index	3.9 ± 0.1	3.9 ± 0.2	NS
Pancreas weight (g)	1.1 ± 0.1	1.5 ± 0.2	0.04
% Pancreas weight per BW	2.2 ± 0.2	2.9 ± 0.3	NS
β-Cell mass (mg)	18.3 ± 5.3	7.7 ± 2.0	NS
β-Cell mass per BW (mg/kg)	368 ± 102	167 ± 37	NS

Male littermate mice of 3-month-old were fed a high-fat diet for 28 weeks and killed. Values were listed as means ± SE. Number of mice were given in parentheses. *P* values were derived from unpaired *t*-test vs. wild-type mice. *NS* not significant

Table 2 Serum IGF-I levels measured at the end of the high-fat diet

	Number	Endogenous	Human IGF-I	Combined
Female WT	5	448 ± 27	0	448 ± 27
Female MT-IGF	7	50 ± 3***	378 ± 10	428 ± 9
Male WT	9	415 ± 43	0	415 ± 43
Male MT-IGF	8	54 ± 9***	347 ± 16	401 ± 13

*** $P < 0.001$ vs. wild-type littermates

IGF-I overexpression in MT-IGF mice was confirmed by measuring human IGF-I level in mouse serum.

Changes in insulin sensitivity and glucose tolerance

We have reported improved glucose tolerance in MT-IGF mice vs. wild-type littermates under normal diet, probably due to decreased glucose production and/or increased glucose uptake imposed by IGF-I overexpression [13]. To study whether diet-induced obesity will cause insulin resistance therefore glucose intolerance, we performed glucose tolerance tests to these mice after 10, 17, and 26 weeks on the diet (Fig. 2a–c, respectively). Clearly, MT-IGF mice on high-fat diet still maintained improved glucose tolerance vs. their wild-type littermates, which was more significant at 10 and 26 week (Fig. 2a, c). In the meantime, compared to mice fed normal chow diet, both wild-type and MT-IGF mice exhibited a clear elevation in the tolerance curves at 26 weeks, indicating significant glucose intolerance induced by obesity (Fig. 2c, d). The *Area under Curve* calculated from Fig. 3c was increased by 43% in wild-type mice fed high-fat diet vs. chow diet (columns 1 vs. 2), and by 94% in MT-IGF mice (columns 3 vs. 4; Fig. 2d). After the same 26-week on high-fat diet, the *Area under Curve* value in MT-IGF mice was only 60% of wild-type littermates (columns 2 vs. 4), indicating improved glucose tolerance due to IGF-I overexpression either in lean or obese mice. Thus, although MT-IGF mice maintained a clear advantage in glucose clearance vs. wild-type littermates under high-fat diet-induced obesity, IGF-I overexpression in those mice did not prevent the worsening of glucose intolerance imposed by the onset of obesity itself.

Insulin tolerance tests were performed at 9 and 16 weeks on the diet; both MT-IGF mice and their wild-type littermates exhibited normal insulin sensitivity (Fig. 2e), which was unchanged from what we have reported for these mice fed normal chow diet [13]. Moreover, in diet-induced obesity, fasting serum insulin level at 9 and 26 weeks was unaffected by IGF-I overexpression (Fig. 2f); however, fed insulin level was significantly decreased at 17 weeks; both aspects, again, were similar (i.e., relatively unchanged) to what we have reported under normal chow diet [13]. The insulin tolerance test and measurement of serum insulin level in fasted mice thus indicate that IGF-I overexpression in lean or obese mice caused no major change in insulin sensitivity.

Evaluation of islet cell overgrowth in response to diet-induced obesity

We have recently reported that IGF-I overexpression in MT-IGF mice results in enlarged pancreas but normal β-cell percentage (i.e., proportional increase in β-cell mass) under normal chow diet [13, 23]. In order to characterize possible changes in compensatory islet cell growth caused by diet-induced obesity, we measured β-cell mass in insulin-stained pancreatic sections and compared the weight of the pancreas at 28 weeks (Table 1). Consistent with previous observation under normal chow diet, the wet tissue weight in obese MT-IGF mice was increased 36% vs. wild-type littermates ($P = 0.04$), indicating that IGF-I overexpression caused pancreatic enlargement. However, when we measured β-cell mass, not only we did not see a possible increase, MT-IGF mice actually exhibited a tendency of reduction, albeit insignificantly due to high variability in individual values of β-cell mass. Thus, when corrected by their body weight, the β-cell mass in MT-IGF mice was only 45% of their wild-type littermates (NS). Except for seemingly diminished insulin staining on β-cells, there was no other abnormality in pancreatic histology, especially in β-cell morphology in the face of obesity, in MT-IGF mice vs. wild-type littermates (Fig. 3). Regardless the precise change, our result indicated that IGF-I overexpression did not *increase* islet cell compensation in response to obesity.

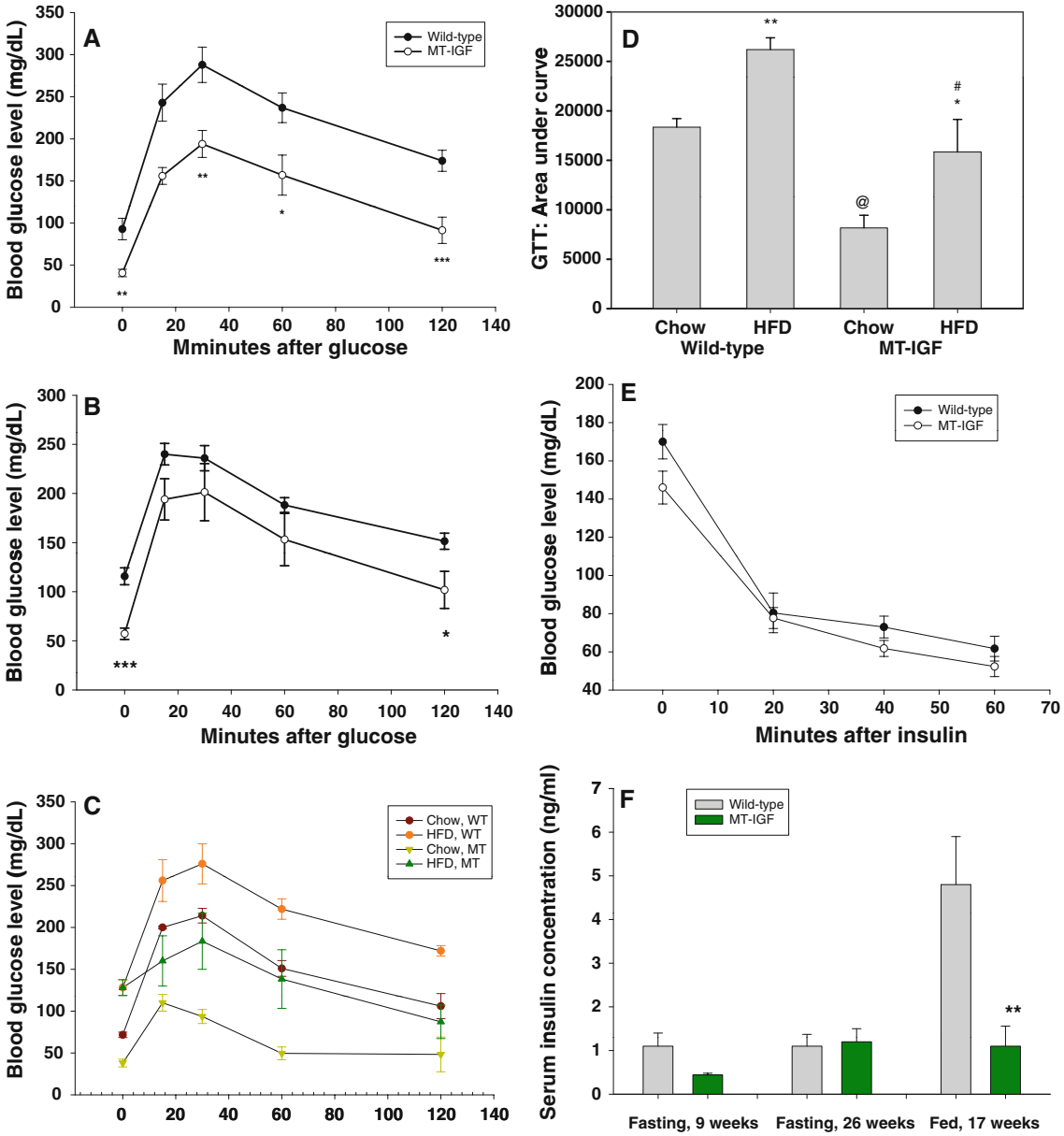

Fig. 2 Changes in glucose tolerance, insulin sensitivity, and serum insulin level in MT-IGF mice in response to high-fat diet-induced obesity. **a–c** Glucose tolerance tests performed at 10, 17, and 26 weeks, respectively, after high-fat diet. Mice were fasted for 24 h and injected with glucose (1.0 g/kg ip). Blood glucose levels were measured at 0, 15, 30, 60, and 120 min after the injection. Values were illustrated as Mean ± SE; $N = 9$. In panel **c**, the responses in a group of mice fed normal chow were also illustrated; $N = 5–9$. **d** Area under curve (arbitrary unit) was calculated based on the four graphs contained in Fig. 3c. Result of ANOVA: $P = 0.0001$; variation among column means was significantly greater than expected by chance. Result of unpaired *t*-test: * $P < 0.05$, ** $P < 0.01$ vs. same genotype mice after normal chow; @ $P = 0.004$ vs. wild-type littermates fed chow diet; # $P = 0.0093$ vs. wild-type littermates fed high-fat diet. **e** Insulin tolerance test performed at 16 week after the diet. Wild-type and MT-IGF mice in random fed state were injected with insulin (0.75 IU/kg ip). Blood glucose levels were measured at 0, 20, 40, and 60 min. Similar result was obtained after 9 weeks (data not shown). $N = 9$. **f** Serum insulin levels measured at different weeks on the diet and under either fasted or fed status. Mice were fasted for 24 h at 9 and 26 weeks and fed at 17 weeks and blood was collected to measure insulin level. $N = 7–9$; ** $P < 0.01$ vs. wild-type littermates

Discussion

As we and others have reported, a general and yet islet cell-enriched IGF-I overexpression (in MT-IGF mice) increased body weight and pancreatic growth, decreased blood glucose and insulin levels, improved glucose tolerance but did not affect normal β-cell mass [13, 23, 24]. In this study, following a prolonged high-fat diet, MT-IGF mice

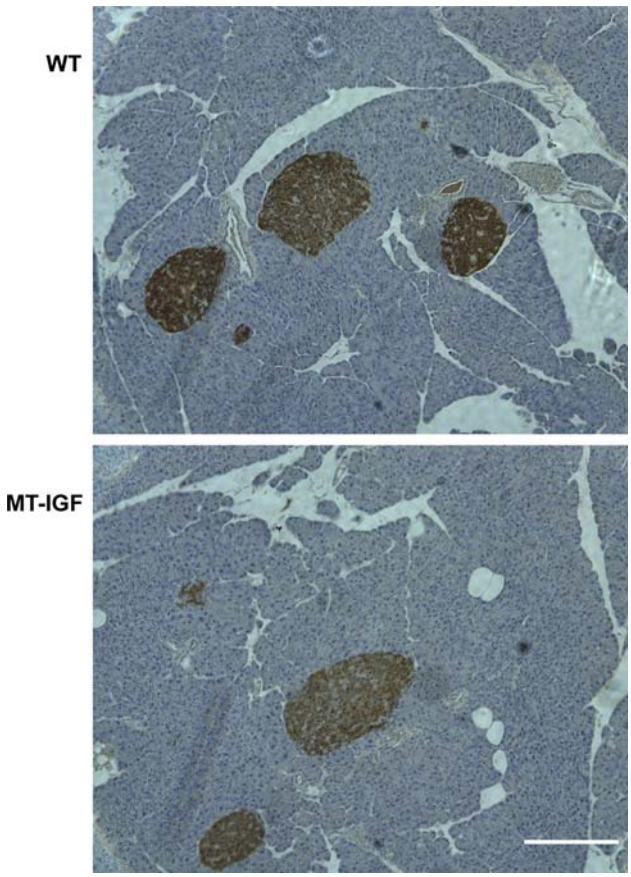

Fig. 3 Pancreatic islet morphology in MT-IGF mice after fed a high-fat diet for 28 weeks. Pancreatic sections were prepared from animals listed in Table 1, stained for insulin (*dark brown*) and with hematoxylin (*blue* for cell nuclei). Representative images were taken at ×100 for wild-type (*top*) and MT-IGF mice (*bottom*). The bar indicates 0.5 mm. (Color figure online)

exhibited no difference from wild-type littermates, i.e., gained significant weight thus became obese, maintained normal insulin sensitivity, and more importantly exhibited no accelerated islet cell expansion. Even under obesity, MT-IGF mice maintained relatively improved glucose tolerance than wild-type littermates. Thus, IGF-I overexpression had no significant effect on weight gain and islet cell compensation in response to high-fat diet.

This finding is consistent to a recent report using β-cell-specific IGF-IR gene deletion that endogenous IGFs were not involved in islet cell compensation in response to insulin resistance [25].

In contrast to earlier claims that IGF-I stimulate islet cell growth based on several in vitro and in vivo studies [5–7], this observation extended our previous reports and indicated that even in compensatory islet cell growth, overexpressed IGF-I did not seem to have a significant contribution.

A multitude of relationships exist for IGF-I and adiposity, e.g., IGF-I promotes adipocyte growth (lipogenic); nutrient intake and adiposity significantly affect (mostly boost) plasma IGF-I level; and IGF-I deficiency might cause reduced adiposity. The development of obesity is caused by the expansion of the adipose tissue involving terminal differentiation of preadipocytes, proliferation, and enlargement of existing adipocytes [26, 27]. IGF-I is highly expressed by adipose tissues, second only to liver, its primary source of production; in turn, IGF-I would stimulate adipose differentiation and proliferation thus increase adiposity, as demonstrated in primary preadipocytes and cell lines [28–31]. Conversely, the plasma level of IGF-I is clearly affected by nutrition status, i.e., normal IGF-I secretion depends on adequate caloric intake; the average IGF-I concentration increases with body mass index (BMI) up to a value of 28. However, further increases in BMI lead to decreased IGF-I level; in very obese individuals, total IGF-I level is often significantly decreased compared to people of normal weight [32]. Intriguingly, the serum concentration of free IGF-I increases steadily as BMI does, and is elevated in obese subjects, reflecting increased active IGF-I in the circulation [33]. Further, reduced adiposity has been reported in mice with targeted inactivation of hepatic IGF-I expression, although it is uncertain whether this was attributable to reduced IGF-I production or to secondary changes in growth hormone and leptin levels [34]. In this study, IGF-I overexpression in MT-IGF mice caused no further increase in adiposity and even exhibited a slight tendency of reduced obesity when fed a high-fat diet, which cannot be directly reconciled with above mentioned reports. A possible effect of decreased growth hormone release, in response to elevated IGF-I, in adiposity cannot explain the result either. Because it is lipolytic, decreased level of growth hormone tends to increase adiposity [35]; MT-IGF mice were expected to be more sensitive to high-fat diet in gaining weight and therefore becoming more insulin resistant, which was not confirmed by this study. More study is clearly required to better understand IGF-I and adiposity. Also, we need to be careful when translating results obtained from mouse models to human metabolic responses.

IGF-I is an insulin-like hormone which acts directly by stimulating glucose uptake in muscle and adipose tissues and by inhibiting glucose production in the liver and indirectly by improving insulin sensitivity in key target tissues. Consequently, in our previous report, IGF-I overexpression in MT-IGF mice caused improved glucose tolerance, decreased glucose production, and diminished glucose and insulin levels when the animals were fed a normal diet; and significantly protected mice from streptozotocin-induced diabetes [13]. In this study, MT-IGF mice and wild-type littermates were fed a high-fat diet, became obese and slightly resistant to insulin, judged by impaired glucose tolerance (Fig. 2c) and elevated glucose and insulin levels (Figs. 1c, 2f). However, the level of the insulin resistance was insufficient to cause the onset of diabetes mellitus, even

after a prolonged 28-week on the diet. This was in contrast to our previous success in creating type 2 diabetes only after 4–8 week, clearly due to strain-specific variability [12].

Under this obesity-induced partial insulin resistance, MT-IGF mice were able to maintain improved glucose tolerance (Fig. 2a–d) and diminished insulin level (Fig. 2f), likely caused by the same insulin-like effects including decreased glucose and insulin production and increased glucose uptake, under chow or high-fat diets.

After demonstrating that IGF-I normally does not stimulate islet cell growth in a previous report, a primary goal of this study was to test whether IGF-I overexpression contribute to β-cell compensation against obesity-induced insulin resistance. In wild-type mice, we established a dramatic increase in β-cell mass (to 368 mg/kg body weight), compared to the range of 38–53 mg/kg under chow diet in our previous reports [12, 22, 36]. The level of random fed insulin ($\sim$4.7 ng/ml after 17 week of high-fat diet; Fig. 2f) compared to our previous reports of 0.6–0.9 ng/ml also indicated significant islet hyperplasia if not only caused by insulin resistance [12, 13, 22, 36]. Under this condition, IGF-I overexpression resulted in no sign of further increase in β-cell mass, if not a slight reduction (NS; Table 1). One explanation for the reduced β-cell mass would be that the presence of excessive IGF-I, acting in an insulin-like fashion, may have reduced the demand for islet compensation. Since obesity causes reduced IGF-I level, we did not expect to see increased islet compensation caused by increased IGF-I production. Rather, this study simply tested whether elevated IGF-I level in obese mice (via exogenous expression) plays a role in islet growth; the result was clearly negative. As reported previously, the islet overexpression of IGF-I in MT-IGF mice is truly excessive by more than a thousand fold; although we have confirmed the IGF-I level in this study (Table 2), it is likely to have been enhanced under obesity-induced oxidative stress which is known to activate the metallothionein promoter used in our transgenic mice [37]. Thus, the negative effect on β-cell compensation cannot be attributed to reduced IGF-I overexpression.

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