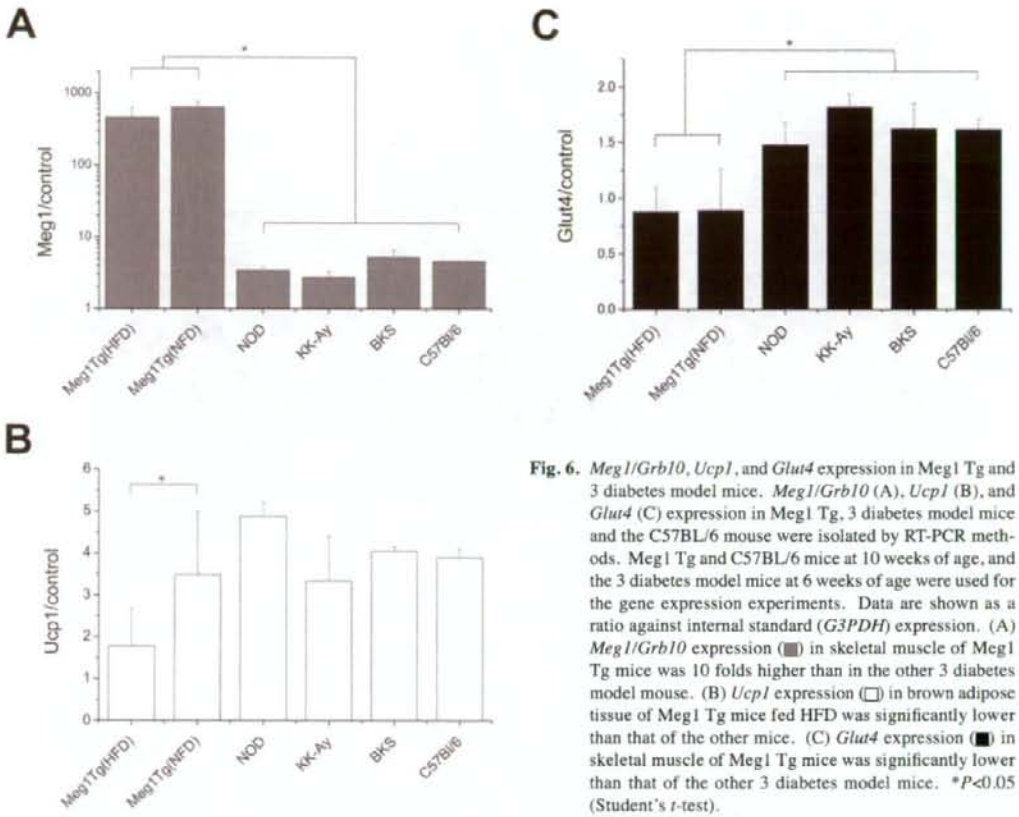

Fig. 6. *Meg1/Grb10*, *Ucp1*, and *Glut4* expression in Meg1 Tg and 3 diabetes model mice. *Meg1/Grb10* (A), *Ucp1* (B), and *Glut4* (C) expression in Meg1 Tg, 3 diabetes model mice and the C57BL/6 mouse were isolated by RT-PCR methods. Meg1 Tg and C57BL/6 mice at 10 weeks of age, and the 3 diabetes model mice at 6 weeks of age were used for the gene expression experiments. Data are shown as a ratio against internal standard (*G3PDH*) expression. (A) *Meg1/Grb10* expression (■) in skeletal muscle of Meg1 Tg mice was 10 folds higher than in the other 3 diabetes model mouse. (B) *Ucp1* expression (□) in brown adipose tissue of Meg1 Tg mice fed HFD was significantly lower than that of the other mice. (C) *Glut4* expression (■) in skeletal muscle of Meg1 Tg mice was significantly lower than that of the other 3 diabetes model mice. $^*P<0.05$ (Student's *t*-test).

ance (Fig. 3). The plasma insulin concentration of Meg1 Tg mice was significantly higher than in control mouse (Table 2). These data demonstrate that the dysregulation of plasma glucose was caused by insulin resistance. Our results provide support for Meg1 Tg mouse as a 2DM mouse model. Moreover, the body weights of Meg1 Tg neonates were slightly lower than controls, and this difference increased with growth up to 12 to 15% of body weight. BMI of Meg1 Tg mice was also smaller than control mice (Figs. 1 and 2). Body, visceral fat weight and liver weights were also slightly lower in Meg1 Tg mice than in control mice. However, the visceral fat/body weight and liver/body weight ratios of Meg1 Tg mice were similar to those of control mice (Table 1). Overall, the Meg1 Tg mouse showed a non-obese mouse character.

There are several spontaneous polygenic models of 2DM, such as the OLETF rat, the KK-A^y mouse, the NSY mouse, and the BKS mouse, which develop overt obesity and hyperinsulinemia prior to the onset of diabetes [7, 14, 34]. The KK-A^y mouse and the BKS mouse showed obesity compared with controls [33, 35]. However, the Meg1 Tg mouse model showed a non-obese character. Only limited data exist for 2DM in Japanese subjects, probably because Japanese people are relatively lean. Some Japanese 2DM patients are non-obese patients and the causal factors of 2DM onset is being analyze by many laboratories [15, 28]. The Meg1 Tg diabetes mouse model may provide a useful tool for these researches.

Biochemical analysis data of the Meg1 Tg mouse demonstrated metabolic abnormality. Plasma BUN in

Meg1 Tg mice was significantly lower than in control mice. Plasma TG, insulin, adiponectin, and resistin levels of HFD-fed Meg1 Tg mice were significantly higher than those of control mice. The mean concentration of TG in Meg1 Tg mice, especially with HFD feeding, was over 200 mg/dl, a condition that is called high-fat plasma. High-fat plasma is said to be a precursor of diabetes [19]. A high level of plasma adiponectin and low BMI were found in Meg1 Tg mice fed HFD (Fig. 2B). There was an inverse correlation between plasma adiponectin and BMI in the Meg1 Tg mouse (data not shown). This shows that the Meg1 Tg mouse has characteristics similar to human 2DM. The circulating IGF-1 level in Meg1 Tg mice tended to be lower than that of control mice (Table 2). A lower IGF-1 level could explain the lower body weight of the Meg1 Tg mouse due to the role of IGF-1 signaling in postnatal growth [10]. Overall, the biochemical data suggest that HFD feeding in the Meg1 Tg mouse induces human 2DM related characteristics. In a comparison of the biochemical data between the Meg1 Tg mouse and other 2DM model mice, such as KK-A^y and BKS mouse, the Meg1 Tg mouse did not show plasma insulin and a remarkable rise of plasma glucose levels like KK-A^y and BKS mice, but showed a property that is characteristic of human 2DM, a rise of adiponectin level [5, 16, 18]. In addition, it is a very unique characteristic that this property is caused by environmental factors such as diet.

In mRNA expression analysis, diabetes related genes, such as *Grb10*, *Ucp1*, and *Glut4*, in the Meg1 Tg mouse were compared with 3 famous diabetes mouse models (Fig. 6). Regarding the relationship between the *Meg1/Grb10* gene and the non-obese character of the Meg1 Tg mouse, overexpression of the *Meg1/Grb10* gene may hold the IGF-1 acceptor signal transduction system in check during development in the embryonic stage and the holding effect would be maintained until long after birth [31]. It is reported that maternal duplication of proximal chromosome 11 retards embryonic growth, whereas paternal duplication promotes growth [2]. A recent *Grb10* knockout study has clearly demonstrated that *Meg1/Grb10* is the gene responsible for embryonic overgrowth observed in paternal duplication of the chromosome 11 region [3], although the effect of *Meg1/Grb10* overproduction remains to be addressed. Our data may provide answers to these puzzling issues. Grb10 regulates the insulin signaling and sensitivity *in vivo* [36]. This suggests that a high level of the *Grb10* gene could affect insulin resistance in the Meg1 Tg mouse. The reduction in intracellular lipid by constitutive expression of *Ucp1* reflects down-regulation of fat synthesis rather than up-regulation of fatty acid oxidation [32]. The down-regulation of *Ucp1* in Meg1Tg mice fed HFD could explain the non-obese character of the Meg1 Tg mouse. The quantity of expression of *Glut4* mRNA in skeletal muscle decreased significantly in Meg1 Tg mice (Fig. 6C). GLUT4 translocation can be activated by insulin, to which the Meg1 Tg mouse has resistance, and may be related to the GLUT4 decrease in skeletal muscle.

Recently, association of hGrb10 genetic variations with 2DM in Caucasian subjects has been reported [2]. There is no evidence of such a correlation in Japanese subjects. However, it has been revealed that the expression of hGrb10 was influenced by epigenetic alterations. It is important to investigate hGrb10 expression and the relationship to environmental conditions including diet in 2DM model mice.

Acknowledgment(s)

We thank Ms. Tomoko Hata and Naho Takekawa for technical assistance.

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