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RESEARCH ARTICLE

Evaluation of Glucose Uptake and Uncoupling Protein 1 Activity in Adipose Tissue of Diabetic Mice upon β-Adrenergic Stimulation

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Abstract

Purpose: Regulation of metabolic activity in adipose tissue is of great concern for treating obesity. This study aimed to evaluate the adrenergic regulation of glucose uptake and the thermogenic program in adipose tissues in mouse models of both type 1 and 2 diabetes mellitus (DM).

Procedures: Male mice were treated with streptozotocin to induce type 1 (T1) DM, and obese ob/ob mice were used for the type 2 (T2) DM model. After selective β_3-adrenoreceptor stimulation by CL 316,243 (CL) treatment, 2-deoxy-D-[^{14}C]glucose ([^{14}C]DG) was administered to DM and corresponding control mice. Radioactivity and uncoupling protein 1 (UCP1) expression were measured and analyzed in adipose tissues.

Results: In T1DM, [^{14}C]DG uptake in brown adipose tissue (BAT) decreased both at rest and upon CL stimulation, and UCP1 expression was preserved. However, CL treatment enhanced [^{14}C]DG uptake without impairing UCP1 expression in inguinal white adipose tissue (iWAT). In this model, CL could not alter blood glucose levels. In T2DM mice, the blood glucose level was significantly lowered by CL treatment. There was no decrease in CL-induced [^{14}C]DG uptake in BAT, and UCP1 expression was maintained. However, [^{14}C]DG uptake was not increased in iWAT and no UCP1 expression was observed in iWAT (browning).

Conclusions: The metabolic response against adrenergic stimulation varied depending on the type of adipose tissue and DM. This could be important for the therapeutic activation of adipose tissue metabolism in obese diabetic patients.

Key words: Adipose tissue, Diabetes mellitus, Glucose, Uncoupling protein

Introduction

Two types of adipose tissue, white adipose tissue (WAT) and brown adipose tissue (BAT), are known to exist in mammals [1]. WAT exists subcutaneously and around internal organs, comprising the so-called "fat cells," and accumulates excess energy in the form of triglycerides [1]. On the other hand, BAT is present in the cervical, supraclavicular, and paravertebral regions. BAT contains abundant mitochondria and shows high expression of uncoupling protein 1 (UCP1), which uncouples oxidative phosphorylation from ATP synthesis to increase heat energy dissipation [2]. This heat generation is involved in the metabolic pathway of fatty acids liberated from triglycerides

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and glucose and is regulated by the adrenergic pathway, including sympathetic nerves, norepinephrine, and the β_3-adrenergic receptor [3, 4]. In this pathway, glucose is the major metabolic substrate of BAT. Studies using positron emission tomography (PET)-computed tomography with 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) have revealed a high prevalence of metabolically active regions around the supraclavicular areas in adult human subjects, and biopsies from these regions are enriched with UCP1-positive cells [5].

Recently, WAT was discovered to express UCP1 when subjected to chronic cold exposure or β_3-adrenergic stimulation in rodents, and this is referred to as "beige adipocytes" or "browning of WAT" [1]. Activating these UCP1-positive cells is a potential therapeutic strategy to combat obesity [6, 7]. Obesity is thought to be caused by an imbalance between energy intake and energy consumption and is the leading risk factor for diabetes mellitus (DM). Although reducing weight through exercise is one of the first steps in treating obese diabetic patients, some patients cannot perform physical exercise sufficiently. On the other hand, autonomic disturbance and sympathetic nerve dysfunction are closely related to diabetes status and are important for stimulating BAT and browning of WAT [8]. However, little has been investigated about the relationship between adrenergic stimulation and diabetic metabolic disorder of adipose tissue.

Thus, this study aimed to evaluate the effect of adrenergic stimulation on energy metabolism in adipose tissues from a mouse model of DM by assessing radiolabeled glucose uptake and UCP1 expression.

Materials and Methods

Animal Preparation

All experimental procedures and animal care were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the institutional committee.

For type 1 DM (T1DM), a streptozotocin (STZ)-induced model was used. Male ICR mice (Japan SLC, Inc., Shizuoka, Japan), aged 8 weeks, were housed at a constant temperature of 24 °C with food and water given *ad libitum* and were fed a standard diet. They were randomly divided into two groups: T1DM mice and non-DM mice (control). T1DM mice received intraperitoneal injections of 100 mg/kg STZ (Wako Pure Chemical Industries, Osaka, Japan) dissolved in saline [9]. The control mice received only saline as the vehicle. After 3 days, mice received a booster of STZ (150 mg/kg) or vehicle intraperitoneally. In the STZ-treated group, mice with blood glucose concentrations > 400 mg/dl in the fed state were defined as T1DM positive and were used for experiments 11 days after the first STZ injection.

Genetically obese diabetic ob/ob mice, aged 8 weeks, were used as the type 2 DM (T2DM) model [10]. As the non-DM group (control), +/+ mice were used. These mice were purchased from the same company that provided ICR mice.

To stimulate the adrenergic pathway, mice were injected subcutaneously with 1 mg/kg of the β_3-adrenoceptor agonist, 5-[(2*R*)-2-[[(2*R*)-2-(3-chlorophenyl)-2-hydroxyethyl]amino] propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243; Tocris Bioscience, Bristol, UK) ($n = 6$–8), or with saline (controls; $n = 6$–8) at the same time daily for 4 days. Body weight was measured daily just before CL 316,243 (CL) injection. In addition, blood glucose levels were measured before treatment and at 5 h after the third injection of CL under free feeding. We compared these data because these animals were fasted from 5 h after the third injection of CL as described below, and the fourth CL injection was administered after overnight fasting.

Experimental Protocol

All mice were fasted for 18 h before 2-deoxy-D-[^{14}C]glucose ([^{14}C]DG (American Radiolabeled Chemicals, St. Louis, MO, USA) administration. [^{14}C]DG (37 kBq) was injected through the tail vein. After 30 min, mice were anesthetized with isoflurane and euthanized; several organs, including the interscapular BAT and inguinal WAT (iWAT), were dissected and weighed. Tissues were dissolved in 1 ml of tissue solubilizer (Soluene-350; PerkinElmer, Inc., Waltham, MA, USA). After solubilization, the radioactivity of sample tissues was measured using a Beckman LS6500 liquid scintillation counter (Beckman Instruments, Brea, CA, USA).

Radioactivity was converted into tracer dose and radioactive concentration per wet weight (% dose/g). It was also converted into the differential absorption ratio (DAR) to account for body weight [11, 12]. DAR was defined as

$$DAR = \frac{Tissue\ radioactivity\ (Bq/g)}{Applied\ dose\ (Bq)/Body\ weight\ (g)}$$

Histological Analysis

For immunohistochemical staining, tissue specimens from BAT and iWAT were fixed in 10 % formalin, embedded in paraffin, and cut into 4-μm-thick sections. These slices were then deparaffinized and incubated with 0.3 % hydrogen peroxide in methanol to inhibit endogenous peroxidase activity. After washing with phosphate-buffered saline, the slides were incubated with 10 % normal goat serum for 1 h and then with rabbit antiserum against rat UCP1 overnight at 4 °C. Slides were washed and incubated with biotinylated goat anti-rabbit IgG

Table 1.. Effect of CL 316,243 treatment on body weight in mice from diabetes mellitus models

	Body weight (g)		
	Before administration	At the third administration	Just before [^{14}C]DG injection
T1DM mice			
Control (vehicle)	36.2 ± 0.83	36.20 ± 0.67	32.50 ± 0.86
CL 316,243	38.58 ± 2.17	36.89 ± 1.86	32.11 ± 1.77
T2DM mice			
Control (vehicle)	47.23 ± 1.86	48.03 ± 1.95	44.37 ± 1.81
CL 316,243	49.60 ± 2.66	48.50 ± 1.85	44.63 ± 2.52

(Nichirei Bioscience, Tokyo, Japan) for 1 h and finally with the avidin-biotin-peroxidase complex (Nichirei Bioscience), followed by visualization with 3,3′-diaminobenzidine (Thermo Fisher Scientific, Gaithersburg, MD, USA) according to the conventional avidin-biotin complex method.

Statistical Analyses

Data are expressed as means ± SD. Comparisons between two groups were performed using Student's *t* test. DAR of adipose tissues in mice was analyzed with a two-way analysis of variance (ANOVA) with the Holm method *post hoc* analysis.

Statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [13].

Results

Effect of Adrenergic Stimulation in the T1DM Model

The changes in body weight during CL treatment are shown in Table 1. There was no significant difference in body weight between the CL-treated group (DM with CL) and non-treated group (DM with saline). The blood glucose level at the third CL injection was not significantly different from that before injection (576.6 ± 28.0 *vs.* 560.4 ± 45.2 mg/dl, respectively) and was similar to that in the DM with saline group (538.6 ± 60.1 *vs.* 590.6 ± 18.2 mg/dl, respectively) (Fig. 1a).

[^{14}C]DG uptake in BAT is shown in Fig. 2a. Under CL treatment, [^{14}C]DG uptake was significantly greater in both control and T1DM groups than in saline-treated mice ($p < 0.001$ and $p < 0.01$, respectively). However, [^{14}C]DG uptake in CL-stimulated T1DM was significantly lower than that in the CL-stimulated control ($p < 0.05$). Histologically,

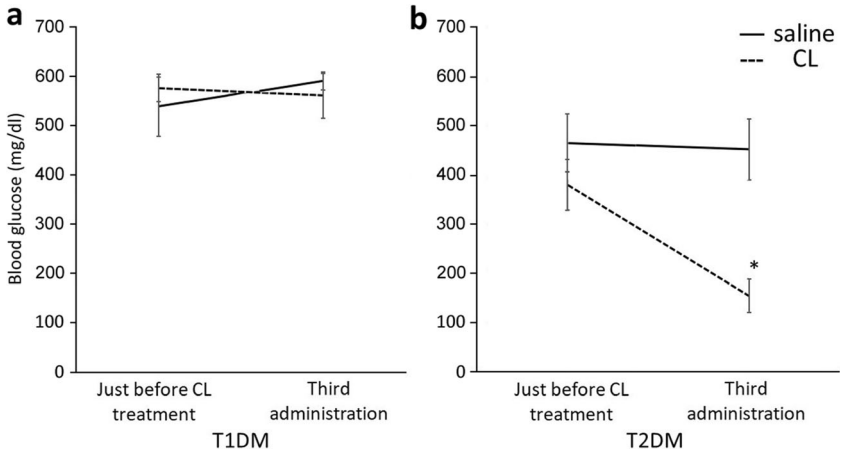

Fig. 1. Effect of adrenergic stimulation on blood glucose levels in both **a** type 1 and **b** type 2 diabetes mellitus mice. Blood glucose levels in streptozotocin-induced type 1 diabetes mellitus (T1DM) and genetic ob/ob obese and diabetes mellitus (T2DM) with saline or CL 316,243 (CL) treatment ($n = 6$–8 per group). Blood glucose levels after CL injection are significantly lower than those before CL treatment in T2DM (*$p < 0.05$).

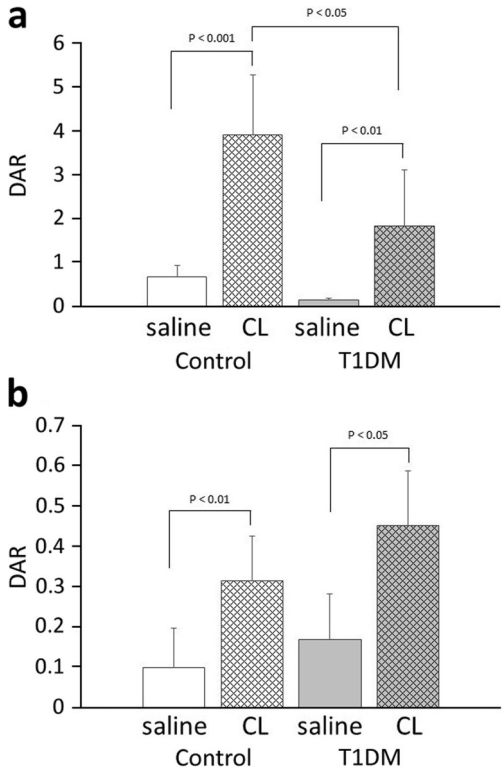

Fig. 2. Effect of CL 316,243 on glucose uptake of adipose tissue in T1DM mice. The differential absorption ratio (DAR) of [^{14}C]DG uptake in **a** the interscapular brown adipose tissue (BAT) and **b** the inguinal white adipose tissue (iWAT) of T1DM model mice and non-diabetic (control) mice injected with CL 316,243 (CL) or saline ($n = 6$–8 per group).

the size of lipid droplets was decreased and multilocular adipocytes and UCP1 expression was induced throughout BAT by CL treatment in T1DM mice as observed (Fig. 3a). These data suggest that metabolic activity of BAT in T1DM mice was impaired but could be stimulated by CL treatment compared to the control mice.

Figure 2b shows the [^{14}C]DG uptake in iWAT of T1DM. CL treatment significantly enhanced [^{14}C]DG uptake in both control and T1DM mice compared to the saline treatment ($p < 0.01$ and $p < 0.05$, respectively), but there was no significant difference between the control and T1DM under CL stimulation. This tendency differed from uptake in BAT. Immunohistochemical staining revealed multilocular adipocytes positively stained with the anti-UCP1 antibody in iWAT after CL treatment, but not in iWAT with saline (Fig. 3b). These data suggest that browning of iWAT was not impaired in T1DM mice compared to the control mice.

Effect of Adrenergic Stimulation in the T2DM Model

Body weight measurements showed no significant difference before and after CL treatment in T2DM mice (Table 1). Surprisingly, the blood glucose levels measured before and after CL treatment showed a significant difference (379.0 ± 51.6 *vs.* 154.1 ± 34.1 mg/dl, respectively, $p < 0.05$). In contrast, there was no significant difference in the saline-treated controls (465.0 ± 59.2 *vs.* 452.0 ± 62.5 mg/dl) (Fig. 1b).

Figure 4a presents the [^{14}C]DG uptake in BAT. There was a tendency that DG uptake was enhanced after CL treatment in the control, but no significant difference was observed between saline and CL treatments in the control mice. On the other hand, there was a significant difference between saline and CL treatments in T2DM mice ($p < 0.001$). We found that CL led to higher [^{14}C]DG uptake in T1DM mice than in the control ($p < 0.01$). Under these circumstances, an obvious increase in UCP1 expression was observed (Fig. 5a).

CL treatment did not enhance [^{14}C]DG uptake in iWAT of either the control or the T2DM group significantly (Fig. 4b), and histological examination showed no change in the size of lipid droplets and no

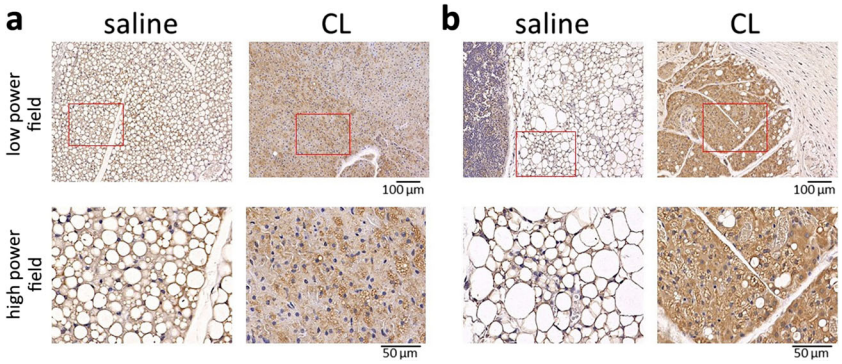

Fig. 3. Immunohistochemistry of **a** interscapular brown adipose tissue (BAT) and **b** inguinal white adipose tissue (iWAT) slices of type 1 diabetes mellitus (T1DM) mice stained for uncoupling protein 1.

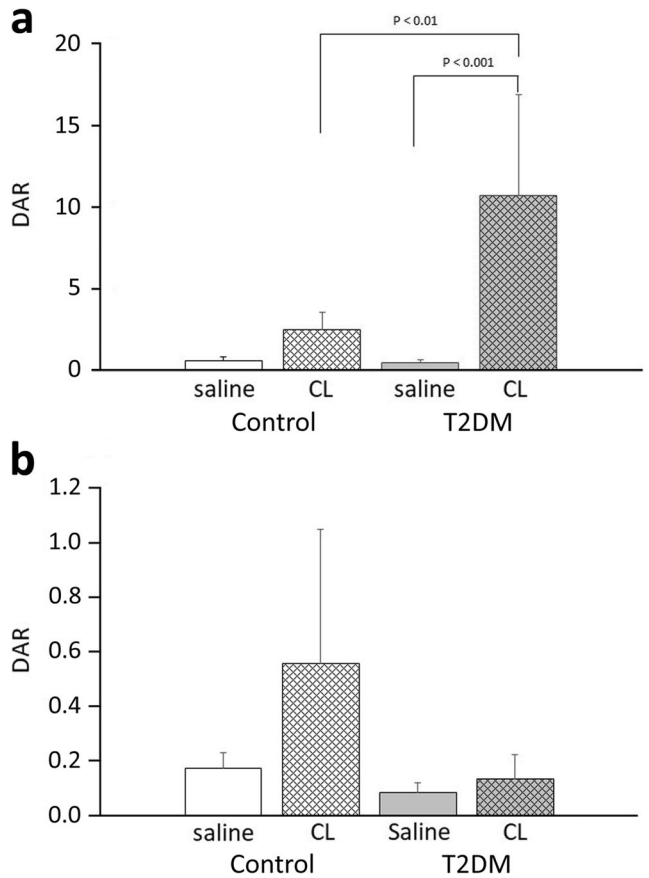

Fig. 4. Effect of CL 316,243 on glucose uptake of adipose tissue in genetic T2DM mice. The differential absorption ratio (DAR) of [^{14}C]DG uptake in **a** the interscapular brown adipose tissue (BAT) and **b** the inguinal white adipose tissue (iWAT) of T2DM model mice and non-T2DM (control) mice injected with CL 316,243 (CL) or saline ($n = 5–6$ per group).

apparent UCP1 expression in iWAT tissue upon CL administration in T2DM mice (Fig. 5b).

Discussion

We observed that [^{14}C]DG uptake into BAT declined in T1DM mice under adrenergically stimulated conditions. This tendency is similar to a previous study by Baranwal et al. [14]. However, the ratio of [^{14}C]DG uptake under stimulation to that of resting conditions was not significantly decreased. Histological study confirmed that adrenergic stimulation increases UCP1 expression in the BAT of T1DM mice. This might indicate that β-adrenergic agonists could be used to stimulate glucose metabolism in BAT, even when resting metabolism is impaired. This shows that β-adrenergic agonists can enhance calorie consumption to promote weight loss in patients with T1DM.

In our study on iWAT, adrenergically stimulated [^{14}C]DG uptake was not impaired in T1DM mice, even though [^{14}C]DG uptake in BAT was reduced. A recent study reported that sympathetic nerve stimulation could raise glucose uptake into iWAT [15] and increase the number of cells with abundant UCP1 gene expression in iWAT [16]. Thus, it is possible that the metabolic reactivity of adipose tissue against adrenergic stimulation differs between BAT and iWAT in T1DM. Mössenböck et al. reported that insulin signaling is not essential for iWAT browning in T1DM mice [17]. They indicated that glucose transporter 1, which is independent of insulin, might be related to this phenomenon. Our data showing preserved adrenergically stimulated [^{14}C]DG uptake and UCP1 expression in the iWAT of STZ-induced T1DM mice are consistent with their result. β-Adrenergic agonists could thus be used as enhancers of metabolic activity in iWAT for treating T1DM.

In this study, we did not observe a significant effect of CL treatment on body weight in T1DM mice. Wu et al. reported that a β-adrenergic agonist induced weight loss in an STZ-induced DM model with obesity [18]. In this study, we used normal-weight mice, and not obese mice, for the STZ-induced T1DM model. Thus, adrenergic stimulation might have resulted in weight loss in obese T1DM mice and further studies will be necessary to clarify this.

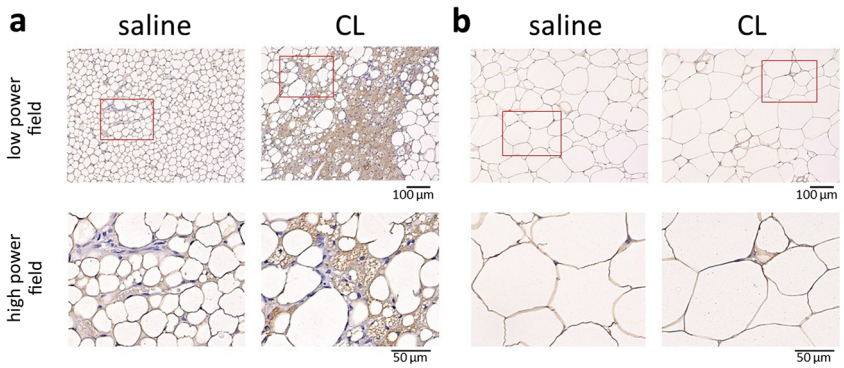

Fig. 5. Immunohistochemistry of UCP1 expression in **a** brown adipose tissue (BAT) and **b** inguinal white adipose tissue (iWAT) slices of type 2 diabetes mellitus (T2DM) mice stained for uncoupling protein 1.

We found reduced blood glucose levels upon CL treatment in genetic T2DM mice but not in STZ-induced T1DM mice. A previous paper reported that CL treatment increases insulin secretion and serous free fatty acids that play a role in lowering blood glucose [19–21]. T2DM consists of an array of dysfunctions characterized by insulin resistance and inadequate insulin secretion. Thus, the insulin secretion ability is probably slightly preserved and CL can further enhance insulin secretion, thereby improving glucose tolerance. On the other hand, insulin was not secreted at all in T1DM mice because STZ exerts preferential toxicity toward pancreatic β-cells. Therefore, it was considered that the blood glucose levels were not altered upon CL stimulation in T1DM mice. The biopharmaceutical relationship between CL and insulin is still unclear. Yoshitomi et al. reported that a β_3-adrenergic receptor agonist acted as a hormone-sensitive lipase to reduce plasma lipid concentrations and blood glucose levels [22]. Thus, additional research is required to clarify this issue.

Surprisingly, adrenergically stimulated [^{14}C]DG uptake in the BAT of T2DM mice was higher than that of the control mice, and even UCP1 expression showed a lower tendency than that in T1DM mice. This discrepancy between the degree of glucose uptake and UCP1 activity in BAT might depend on the type of DM. Recently, some researchers found that CL administration increased the uptake of radio-labeled glucose in the BAT of UCP1 knockout mice [23, 24]. Thus, CL-induced glucose uptake in BAT is thought to be independent of UCP1 activity. The result of the present study showing that CL-induced [^{14}C]DG uptake in BAT was not disturbed in T2DM also supports this phenomenon. Enhanced BAT metabolic activity may affect blood glucose levels regardless of BAT thermogenesis. However, the reason for this finding is not fully understood because other researchers found impaired glucose uptake in BAT using genetically different T2DM rat models [25]. We assume that this finding could be driven by certain factors, such as different degrees of insulin resistance, which depend on the disease model animals and/or the difference between rats and mice. Further research is needed on this aspect, as this may have potential in T2DM management.

Previous reports suggest that glucose uptake in BAT is affected by insulin resistance [26] and blood glucose levels [27]. Additionally, these factors are also involved in the type of diabetic animal model and their age [26]. The ob/ob mouse is a model of T2DM with obesity and relatively mild hyperglycemia [10]. Thus, these factors were considered to be involved in the unimpaired [^{14}C]DG uptake in BAT of ob/ob mice. The age of the animals used in the model might be related to the metabolic status in these mice.

Currently, the development of new treatments against obesity that target BAT and WAT is being attempted, including BAT transplantation [28] or induction of WAT browning. Our study shows the difference in glucose uptake between BAT and WAT and that between T1DM and T2DM after adrenergic stimulation. To our knowledge, no study has yet examined this issue. Browning of WAT has become a “hot topic” because it might be able to facilitate weight loss and improve metabolic health. However, our results indicated that targeting BAT might be more effective than targeting WAT when treating obesity, regardless of the DM type. A recent study showed that browning was associated with adverse outcomes [29]. Thus, our findings will contribute knowledge regarding the relationship between obesity and adipose tissue.

There have been many investigations about the characteristics of activated BAT using different nuclear medicine and molecular imaging techniques [30–32]. Some studies found that obese individuals have lower BAT content as detected by PET and [^{18}F]FDG [33, 34]. Thus, it seems to be difficult that such imaging techniques will apply to obesity research. However, Vijgen et al. reported that cold stress-induced [^{18}F]FDG accumulation in BAT was seen in approximately half of the morbidly obese subjects after bariatric surgery [35]. According to their results, stimulated metabolic activity in BAT can be seen even in morbidly obese subjects, which help in evaluating the effect of obesity treatment. They used a tent and air conditioner to generate cooling conditions to stimulate BAT in their study. Compared to their protocol, the drug-induced adrenergic stimulation used in the present study is thought to be a simple and reproducible method. Our technique and several pieces of evidence that were obtained here about metabolic characteristics of adipose tissue might be useful for further clinical research in patients with DM and obesity.

We rarely encounter untreated patients with T1DM (such as the present study on mice) in clinical [^{18}F]FDG-PET studies because, generally, this disease has been controlled with insulin for many years. However, immune checkpoint inhibitor therapy, which has recently emerged as a new cancer treatment, is known to be associated with acute T1DM as a side effect [36]. Thus, in the near future, the findings of the present study would be helpful because of the increased possibility of T1DM conditions in cancer PET patients undergoing such treatment.

On the other hand, T2DM is rarely encountered in cancer PET studies. Cold exposure (adrenergic stimulation) during winter season may lead to the increased frequency of visualization of adipocytes in such patients. When BAT is visualized in cancer patients, they may present false-positive findings in the diagnosis of supraclavicular lymph node metastases, leading to poor diagnostic performance. Since adrenergic stimulation also has a hypoglycemic effect, it is possible that changes in glucose metabolism in the whole body, such as enhancement of muscle accumulation, may occur, and careful interpretation for PET scanning may be required.

Limitation

In this study, we could not control the body temperature of mice during the experiment. As mentioned above, body temperature affects glucose uptake into BAT and WAT; thus, its effect is not negligible in our results. However, room temperature affects all mice equally, so these influences of body temperature might be canceled out among the groups.

Conclusion

It is important to consider targeting adipocytes and the type of DM when treating patients with obesity and DM using β-adrenergic agonists. In T1DM, adrenergic stimulation is effective in increasing glucose uptake and WAT browning. In contrast, in T2DM, CL 316,243 induces high glucose uptake in BAT but has little effect on WAT browning. Such differences in adrenergic stimulation for DM models should be considered to manage DM effectively.

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Compliance with Ethical Standards

Conflict of Interest

The authors have indicated that they have no financial conflict of interest.

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