Aqueous Extract of Kotahla Himbutu (*Salacia reticulata*) Stems Promotes Oxygen Comsumption and Supresses Body Fat Accumulation in Mice

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Kothala himbutu (KT) is a traditional medicinal plant used in treating diabetes in Ayurvedic medicine. We investigated the effect of the aqueous extract of KT stems (KTE) on energy expenditure in normal mice. Male C57BL/6J mice (n = 28) were divided into 4 groups depending on the type of diet they were fed for 9 weeks: normal (N) diet group (N: 13.8% energy in the form of fat), high-fat (HF) diet group (HF: 53.0% energy in the form of fat), 0.1% freeze-dried KTE (KTED)-supplemented N diet group (N+KTED), and 0.1% KTED-supplemented HF diet group (HF+KTED). KTED intake significantly reduced body weight gain in mice in the N and HF groups. Although it did not affect the plasma levels of glucose, triglyceride, and nonesterified fatty acid, KTED significantly decreased the HF diet-induced increased plasma insulin level. The epididymal and perirenal white adipose tissue (WAT) weights were significantly lower in the HF+KTED group than in the HF group. The oxygen consumption (VO_2), measured by indirect calorimetry, of the mice in the KTED-supplemented groups was significantly higher than that of the mice in the N and HF control groups. Moreover, KTED significantly reduced the size of epididymal WAT adipocytes in the N and HF groups. Thus, KTED promoted VO_2 and suppressed WAT accumulation in the mice on the N and HF diets. Therefore, KTE is beneficial in reducing N diet- and HF diet-induced obesity, which may be partly attributable to the stimulation of whole body energy metabolism.

Key words —— *Salacia reticulata*, energy expenditure, obesity, high-fat diet, mice

INTRODUCTION

Obesity is a metabolic disorder resulting from an imbalance between the intake and expenditure of metabolisable energy. It is known to be a strong risk factor for lifestyle-related diseases, including diabetes, hypertension, and atherosclerosis.[1–3] The prevalence of obesity is increasing globally, and high-fat (HF) diet-induced caloric hyperphagia is considered to be one of the important environmental factors contributing to the obesity epidemic.[4,5]

Salacia reticulata (Hippocrateaceae), referred to as Kothala himbutu (KT) in Singhalese, is a woody climber native to Sri Lanka and a traditional medicinal plant. In Indian and Sri Lankan traditional Ayurvedic medicine, it is extensively used in the treatment of rheumatism, gonorrhoea, and skin diseases, and in particular, it is used as a specific remedy for diabetes in the initial stages.[6] The aqueous extract of KT stems (KTE) or roots is currently used as a herbal therapy for diabetes mellitus in Sri Lanka.[7]

The boundary between food and medicine has been explored, and functional foods have recently emerged as one of the key concepts in the food industry worldwide.[8,9] Hence, many people in the United States, Japan, and other industrial countries are consuming traditional medicinal plants as dietary supplements and herbal tea for preventing diseases and maintaining good health. KT is commercially available and is being consumed as a food supplement for preventing diabetes and obesity in countries such as the United States and Japan. However, the mechanism underlying the effect of KT on diabetes and obesity remains considerably unclear.

The aqueous extracts of KT roots and stems have been reported to prevent postprandial hy-

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perglycaemia in man and decrease the fasting plasma level of glucose, haemoglobin A_1c, and body mass index (BMI) of patients with mild type 2 diabetes.[10, 11] Further, the aqueous extract of KT roots has been reported to suppress body weight gain and perirenal fat accumulation in female Zucker fatty rats; the KT aqueous extract and cyclodextrin also suppress visceral fat accumulation in male Sprague-Dawley rats via oral administration of an HF diet.[12, 13]

Salacinol and kotalanol have been isolated from the water-soluble fraction of KT as compounds that decrease the plasma glucose levels by alpha-glucosidase inhibitory activity.[14, 15] Moreover, previous studies conducted in our laboratory using type 2 diabetic KK-Ay mice have revealed the mechanism(s) by which KTE decreases the fasting blood glucose level. An active compound present in KTE—mangiferin—directly acts on liver cells and suppresses the gluconeogenic pathway by downregulating fructose-1,6-bisphosphatase (FBP) expression, resulting in a decrease in the fasting blood glucose level in diabetic mice.[16]

Till date, the association of the mechanism underlying the regulation of liver glucose production by KTE with the whole body energy metabolism has remained unclear. Further, thus far, in most of the animal studies reporting the anti-obesity effect of KTE, the measurements were performed after the animals were killed, because of which the effect of KTE on the metabolic rate, which is attributed to energy expenditure, was not clearly investigated and established. Thus, the effect of KTE on oxygen consumption (VO_2) has not been studied previously in mice.

In this study, we demonstrated that the mechanism underlying the anti-obesity effect of KTE was related to energy metabolism. We examined the effect of KTE on the inhibition of obesity in C57BL/6J mice, which can be used as a model of the HF diet-induced obesity. VO_2 was measured using indirect calorimetry. Moreover, we measured the plasma levels of glucose, insulin, triglyceride (TG), nonesterified fatty acid (NEFA), and the weight of white adipose tissue (WAT) and the size of brown adipose tissue (BAT) and WAT adipocytes.

MATERIALS AND METHODS

Plant Material —— Whole stems (excluding the leaves and roots) of KT, harvested in Sri Lanka, were purchased from the Ayurveda Kothalahimbutu Association (Tokyo, Japan). The plant was identified by Dr. G. A. S. Premakumara from the Herbal Technology Division of the Industrial Technology Institute, Colombo, Sri Lanka. A voucher specimen of KT stems was deposited in our laboratory (Department of Clinical Dietetics and Human Nutrition, Faculty of Pharmaceutical Sciences, Josai University, Japan).

Preparation of the Freeze-dried Aqueous Extract of KT —— The KT stems were dried and ground to a fine powder, and 100 g of the powder was boiled in 12.5 l distilled water for 2 hr. The solvent was filtered and centrifuged for 10 min at $3000 \times g$ in order to remove particulate matter. The supernatant was designated as KTE. KTE was dried with a freeze dryer (Labconco Corp., Kansas City, MO, U.S.A.) to yield the freeze-dried aqueous extract of the Kothala himbutu stem (KTED, 9 g). The KTED yield (w/w) from the KT stems was approximately 3.8%.

Animals and Diets —— We purchased 28 four-week-old male C57BL/6J mice from CLEA Japan (Tokyo, Japan). The mice were individually housed in an environmentally controlled room under a constant temperature of 25 ± 3°C and a 12-hr light/dark cycle. The experiments were performed after acclimatization for 1 week. Under the standardised conditions, the mice (5-week-old at that time) were divided into the following 4 groups on the basis of the type of diet they were fed: the normal (N) diet group (N group) whose diet contained 6% (w/w) fat (13.8% energy in the form of fat), HF diet group (HF group) whose diet contained 35% (w/w) fat (53.0% energy in the form of fat), 0.1% freeze-dried KTE (KTED)-supplemented N diet group (N+KTED group), and 0.1% KTED-supplemented HF diet group (HF+KTED group). The compositions of the diets and energy densities are shown in Table 1. The animals were maintained on these diets for 9 weeks. In order to accurately evaluate the effect of KTED intake on HF diet-induced obesity, we closely monitored the food intake and body weights in all the diet groups and adjusted the food provisions accordingly. All the mice had free access to distilled water (for drinking purpose). The food intake and body weights were measured daily throughout the study. After 9 weeks, the mice were euthanized by cervical dislocation. Animal care was in conformance with the Standards Relating to the Care and Management of Experimental Animals laid down by the Ministry of Education, Culture,

Table 1. Composition of Experimental Diets

Ingredients	N	N+KTED	HF	HF+KTED
		g/kg		
Casein	200	200	200	200
Sucrose	650	649	357	356
Cellulose powder	40	40	40	40
Corn oil	9	9	50	50
Lard	51	51	300	300
Vitamin mixture[a)]	10	10	10	10
Mineral mixture[b)]	35	35	35	35
Choline chlorede	2	2	2	2
DL-Methionine	3	3	3	3
KTED	–	1	–	1
Energy, kJ/g	16.5	16.5	22.5	22.5
Fat, % of energy	13.8	13.8	53.0	53.0

KTED: freeze-dried aqueous extract from the stem of Kothala himbutu. *a*) Vitamin mixture: composition of AIN-93-VX. *b*) Mineral mixture: composition of AIN-93G-MX.

Sports, Science and Technology of Japan and the Institutional Animal Care and Use Committee of Josai University.

Collection of Plasma, Liver, and Adipose Tissues —— Blood was collected from the postcaval vein. The plasma was separated and stored at −80°C until analysis. The liver and adipose tissues (epididymal and perirenal WAT and interscapular BAT) were immediately removed according to defined anatomical landmarks. The tissues were weighed, following which they were immediately frozen with the use of liquid nitrogen and maintained at −80°C until analysis.

Blood Analysis —— The plasma concentrations of glucose, TG, and NEFA were determined by the glucose C2 test Wako, TG E-test Wako, and NEFA C-test Wako assay kits (Wako, Osaka, Japan), respectively. The plasma insulin levels were measured using the mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi, Gunma, Japan) according to the manufacturer's instructions.

Indirect Calorimetry —— Energy metabolism studies were conducted during the last week of the experiment. VO_2 was measured by indirect calorimetry, using the O_2/CO_2 metabolism measuring system for small animals (Muromachi, Tokyo, Japan). The mice were placed in chambers and had free access to water and their respective diets during the measurement. The measurements were performed every 3 min for 24 hr under a constant airflow rate of 400 ml/min. On an average, 3 consecutive measurements were performed for the mice in each group. The average VO_2 in the 24-hr period was calculated during the dark (1900–0700 hr) and light (0700–1900 hr) cycles.

Measurement of Adipocyte Size —— Samples of epididymal WAT and interscapular BAT from each mouse were fixed with 4% paraformaldehyde, and stained with oil red O. The oil red O working solution was prepared as described by Ramirez-Zacarias *et al.*[17] The cell diameters of 100 adipocytes were measured with a micrometric scale.

Statistical Analysis —— Data are expressed as means ± S.Ds. A comparison of the mean values between the groups was performed by one-way analysis of variance (ANOVA) with Tukey's least significant difference test. Differences were considered significant at a p value of < 0.05. These tests were performed using SPSS version 7.5.1J (SPSS Japan Inc., Tokyo, Japan).

RESULTS AND DISCUSSION

Effect of KTED on Food Intake, Body Weight Gain, Liver Weight, and Weight of WAT

Food intake among the groups remained the same throughout the experimental period (Table 2). In the HF group, a marked increase was observed in the body weight, which was in contrast to that observed in the N group (Table 2). In addition, the body weight gain induced by the HF diet (as observed in the HF group) was observed to be significantly reduced with the KTED supplementation in the HF+KTED group. Furthermore, the body weight gain in the mice that were on the N+KTED diet (N+KTED group) was significantly lower than that in the mice that were on the N diet alone (N group). These data showed that KTED inhibited body weight gain in mice that were fed the N and HF diets. The liver weight did not differ in the groups, in comparison to the N control group (Table 2).

To examine the effects of KTED on visceral fat accumulation, the epididymal and perirenal WATs were weighed. The epididymal and perirenal WAT weights in the HF group were markedly greater than those in the N group (epididymal WAT, 250%; perirenal WAT, 420%, Table 2). The epididymal WAT weights in the N+KTED and HF+KTED groups were 60% and 70% lower than those in the N and HF groups, respectively. Further, the perirenal WAT weights in the N+KTED and HF+KTED groups were 70% and 65% lower than those in the N and HF groups, respectively. These data showed that KTED decreased the epididymal and perirenal

Table 2. Effect of KTED on Food Intake, Body Weight Gain, and Liver and Adipose Tissue Weight

	N	N+KTED	HF	HF+KTED
Food intake (g/day)	2.32 ± 0.40	2.23 ± 0.15	2.24 ± 0.24	2.25 ± 0.17
Body weight gain (g)	5.16 ± 1.46^{b}	2.17 ± 0.93^{c}	7.33 ± 1.96^{a}	3.40 ± 1.10^{bc}
Liver weight (g/100 g body weight)	5.54 ± 0.51	5.49 ± 0.31	4.75 ± 0.34	4.87 ± 0.11
Adipose tissue weight				
Epididymal fat weight (g/100 g body weight)	1.59 ± 0.18^{b}	0.65 ± 0.12^{b}	3.99 ± 0.70^{a}	1.20 ± 0.27^{b}
Perirenal fat weight (g/100 g body weight)	0.40 ± 0.12^{b}	0.12 ± 0.03^{c}	1.68 ± 0.37^{a}	0.24 ± 0.06^{bc}

Values are ± S.D., $n = 7$. Values in a row with different superscript letters are significantly different by Tukey's least significant test at $p < 0.05$.

Table 3. Effect of KTED on Plasma Glucose, Insulin, Triglyceride and Free Fatty Acid

	N	N+KTED	HF	HF+KTED
Glucose (mg/dl)	232.7 ± 38.2^{b}	196.0 ± 91.3^{b}	454.2 ± 99.4^{a}	382.3 ± 85.9^{a}
Insulin (pg/ml)	298.3 ± 81.1^{c}	290.2 ± 72.2^{c}	1722.3 ± 505.5^{a}	815.3 ± 197.1^{b}
Triglyceride (mg/dl)	64.9 ± 7.6^{b}	60.1 ± 11.8^{b}	137.9 ± 26.4^{a}	136.6 ± 30.4^{a}
NEFA (μEq/ml)	1.10 ± 0.27^{b}	0.98 ± 0.16^{b}	1.72 ± 0.35^{a}	1.79 ± 0.28^{a}

Values are ± S.D., $n = 7$. Values in a row with different superscript letters are significantly different by Tukey's least significant test at $p < 0.05$.

WAT weights in the mice that were on the N and HF diets. Because the boundary of the tissue was indistinct during sampling, BAT was not weighed.

Kishino *et al*. reported that a mixture of KTE and cyclodextrin reduces the body weight gain and visceral fat accumulation in HF diet-fed C57BL/6J mice.[13] Our data are consistent with those of this report. Our study results indicated that KTED inhibited visceral WAT (epididymal and perirenal fat) accumulation, resulting in a decrease in the body weight gain in mice that were on the N and HF diets. The inhibition of visceral WAT accumulation influences the plasma adipocytokine concentration and insulin sensitivity. The insulin sensitivity is affected not only by the visceral WAT mass but also by the size of the fat cells.

Effect of KTED on the Plasma Levels of Glucose, Insulin, TG, and NEFA

The plasma glucose level in the HF group (454.2 ± 99.4) was 95% more than that in the N group (232.7 ± 38.2, Table 3). KTED did not affect the plasma glucose levels in the N and HF groups (N+KTED, 196.0 ± 91.3; HF+KTED, 382.3 ± 85.9). The plasma insulin level in the HF group (1722.3 ± 505.5) was 5.8-fold higher than that in the N group (298.3 ± 88.1), while this level in the HF+KTED group (815.3 ± 197.1) was 50% lower than that in the HF group (Table 3). KTED significantly suppressed the HF diet-induced increase in the plasma insulin level.

Hyperinsulinemia due to insulin resistance is another characteristic of HF diet-induced obesity.[18] It has been reported that some nutrients and natural products reduced obesity and hyperinsulinemia in some animals fed an HF diet.[19,20] Our study results showed that KTED might be effective in inhibiting hyperinsulinemia caused by the intake of an HF diet. The plasma TG and NEFA levels in the HF group (137.9 ± 26.4 and 1.72 ± 0.35) were 110% and 320% greater than those in the N group (64.9 ± 7.6 and 1.10 ± 0.27), respectively (Table 3). However, KTED did not affect the plasma TG levels in the N and HF groups (N+KTED, 60.1 ± 11.8; HF+KTED, 136.6 ± 30.4), and it did not affect the plasma NEFA levels in the N and HF groups (N+KTED, 0.98 ± 0.16; HF+KTED, 1.79 ± 0.28). Moreover, no significant differences were observed in the aspartate aminotransferase and alanine aminotransferase activities between the N and N+KTED groups (data not shown). These results suggested that KTED did not directly affect the plasma levels of the abovementioned metabolites.

Effect of KTED on VO_2

KTED decreased the epididymal and perirenal WAT weights in both the N and HF groups. Moreover, KTED significantly suppressed the HF diet-induced increase in the plasma insulin level. To investigate the effect of KTED administration on en-

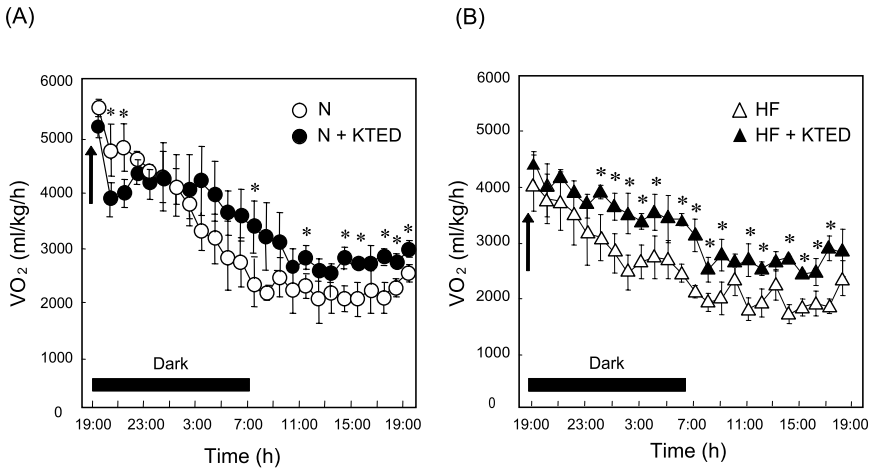

Fig. 1. Direct Calorimetry Results for the C57BL/6J Mice Fed Each Diet for 9 Weeks.

A: Oxygen consumption (VO_2) during 24 hr on the N and N+KTED diets. B: Oxygen consumption (VO_2) during 24 hr on the HF and HF+KTED diets. Food was provided at 1900 hr (arrow) and left for the remainder of the day. The light and dark periods are shown. Data are expressed as means ± S.D.; $n = 3$. $^{*}p < 0.05$ vs. the controls.

ergy metabolism, we examined the energy expenditure in mice by monitoring the VO_2. The VO_2 of the mice in the N, N+KTED, HF, and HF+KTED groups was measured by indirect calorimetry. In all the diet groups, the diurnal VO_2 of the mice was observed to be lower than their nocturnal VO_2. The VO_2 was measured after every meal at 1900 hr, and its values in the N+KTED and HF+KTED groups were found to be significantly higher than those in the N and HF groups, respectively (Fig. 1). As shown in Fig. 1B, at all time points, the VO_2 measured at 24 hr after meals appeared to be particularly greater in the HF+KTED group than in the HF group. In this study, the total energy intake through the N and HF diets was almost the same (Table 1). This indicated that the anti-obesity effect of KTED was not due to decreased energy intake. Our results indicated that an increase in energy expenditure was one of the main causes of the reduced body fat accumulation.

Ohnuki *et al*. reported that capsaicin and capsiate have the ability to release catecholamines and increase the VO_2.[21] Catecholamines released from sympathetic nerves promote lipid mobilization and thermogenesis via β3-adrenoreceptors.[22, 23] Therefore, physical and pharmacological approaches that lead to sympathetic activation have been recommended for the management of obesity.[24]

High doses of tea catechins are reported to inhibit catechol-*o*-methyltransferase (the enzyme that degrades catecholamines) *in vitro*[25] and increase VO_2 in HF diet-fed C57BL/6J mice,[26] but it remains unclear as to whether a KTE component is involved in the catechol pathway. In our previous study, KTE upregulated the expression levels of the genes associated with catecholamine metabolism, thus increasing the expression of catechol-*o*-methyltransferase and monoamine oxidase A (enzymes that degrade catecholamines) in C57BL/6J mice.[27] Thus, our results revealed that KTE might be involved in catecholamine metabolism via gene regulation. However, the role of the catecholamine-mediated mechanisms of energy metabolism by KTE requires further investigation.

It has been reported that certain nutrients and phytochemicals isolated from soya bean, such as dietary proteins and phytoestrogens, respectively, promote energy expenditure in mice.[28, 29] Some experiments have focused on the regulation of the gene expression of uncoupling proteins (UCPs) by dietary proteins, which could be important for studying energy expenditure.[30] Dietary supplementation of epigallocatechin gallate—a green tea bioactive polyphenol—reduces body fat accumulation and upregulates liver UCP2 mRNA in mice with diet-induced obesity.[31] Another report states that fenofibrate—a peroxisome proliferator-activated receptor α (PPAR-α) activator—profoundly reduces epididymal and mesenteric fat accumulation, and that reduction in body weight by fenofibrate is associated with the upregulation of hepatic UCP2 mRNA in low-density lipoprotein receptor-deficient mice.[32] In our previous study, KTE downregulated the gene expression level of UCP2 in the liver of C57BL/6J mice.[27] Therefore, KTE might influence energy metabolism through gene regulation of UCP2. In the future, we need to measure UCP in

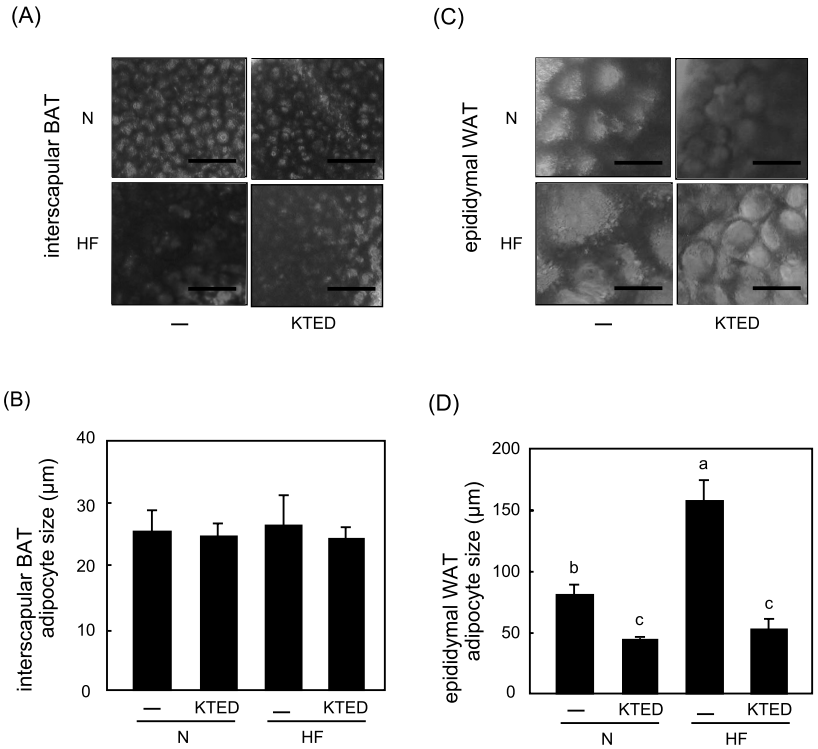

Fig. 2. Effect of KTED on Adipocyte Size

A: Micrographs of interscapular BAT adipose cells. The cells were stained with oil red O. The scale bars indicate 100 μm. B: Average size of the adipocytes (n = 100) from the interscapular BAT of C57BL/6J mice on the N diet, N+KTED diet, HF diet, or HF+KTED diet. C: Micrographs of epididymal WAT adipose cells. The cells were stained with oil red O. Scale bars indicate 100 μm. D: Average size of the adipocytes (n = 100) from the epididymal WAT of C57BL/6J mice on the N diet, N+KTED diet, HF diet, or HF+KTED diet. Data are expressed as means ± S.D.; n = 7. Data with different superscript letters are considered to be significantly different by Tukey's least significant test at $p < 0.05$.

BAT, β-oxidase enzymes in the liver or muscle, and body (rectal) temperature in order to clarify the specific mechanisms underlying the anti-obesity effect of KTE.

Mangiferin, one of the main components of KTE, specifically activates PPAR-α luciferase activity in human embryonic kidney 293 cells and enhances PPAR-α-dependent lipoprotein lipase expression and activity in the THP-1 derived macrophage cell line.[33] We have previously shown that KTE upregulates the gene expression of hepatic lipase in the liver of KK-Ay mice.[16] These data indicated that an active compound contained in KTE, such as mangiferin, might influence lipid metabolism, and that this might be one of the causes of reduced body fat accumulation.

Effect of KTED on Adipocyte Size

KTED inhibited WAT (epididymal and perirenal fat) accumulation, and promoted VO_2 in both the N and HF groups. In order to clarify the specific mechanisms underlying the anti-obesity effect and amelioration of insulin resistance, the size of the adipocytes (interscapular BAT and epididymal WAT) was measured.

The size of interscapular BAT adipocytes did not differ in the groups when compared to the N control group (Fig. 2A and 2B). However, the size of epididymal WAT adipocytes in the HF group was significantly increased compared to that of the N group. The epididymal WAT adipocytes of the N+KTED and HF+KTED groups were significantly smaller than those of the N and HF groups, respectively (Fig. 2C and 2D). These data indicated that KTED might reduce the N and HF diet-induced increase in the size of epididymal WAT adipocytes, although KTED did not affect the size of interscapular BAT adipocytes.

WAT and BAT both play an important role in the mammalian energy equilibrium because they are important for energy storage (WAT) as well as energy dissipation (BAT). BAT is a powerful tool for survival due to its thermogenic function, whereas WAT is very important for energy storage and metabolic activity. Recent studies have shown that fat tissue (WAT) is not simply an energy storage organ but exerts important endocrine and immune functions. These are achieved predominantly

through the release of adipocytokines, which include leptin, resistin, adiponectin, tumour necrosis factor-α, interleukin (IL)-6, or IL-1.[34] Among the others, leptin and adiponectin have been proposed to be major insulin-sensitizing adipocytokines.[35]

Kishino *et al*. reported that a mixture of KTE and cyclodextrin reduced HF diet-induced increase in visceral fat mass and adipocytokine (leptin and adiponectin) concentration in C57BL/6J mice and Sprague-Dawley rats.[13] The increase in visceral fat mass and the concentration of plasma adipocytokine in obesity reduced the sensitivity of adipocytokine. KTED might influence the sensitivity of adipocytokine, because KTED inhibited HF diet-induced WAT (epididymal and perirenal fat) accumulation.

It has been suggested that increased adipocyte size is associated with insulin resistance.[36] Therefore, preventing the generation of enlarged adipocytes might lead to inhibition of the development of insulin resistance. The adipocytokine release within adipose tissue also appears to be correlated with adipocyte size.[37,38] Adiponectin may mediate thiazolidinedione (TZD)-induced prevention of insulin resistance.[39] TZD-mediated PPAR γ activation increases the number of small adipocytes, thereby increasing the production of adiponectin.[40] In this study, KTED significantly reduced the size of epididymal WAT adipocytes. Further, KTED suppressed the HF diet-induced increase in the plasma insulin level. These results indicated that KTED might inhibit the insulin resistance in normal mice on an HF diet. Moreover, in our previous study, it was presumed that the compound in KTE acted as a ligand of PPAR or retinoid X receptor (RXR).[27]

In the previous study with KK-Ay diabetic mice, DNA microarray analysis showed that KTE regulates liver gene expression. Moreover, mangiferin, an active compound present in KTE, acts directly on liver cells and suppresses the gluconeogenic pathway by downregulating FBP expression.[16] Differential display analysis revealed that KTE did not affect gene expression in the muscle of C57BL/6J mice administered KTE for 4 weeks. Furthermore, reverse transcriptase-polymerase chain reaction (RT-PCR) analysis revealed that KTE did not affect the mRNA levels of certain genes, such as FBP type 2 (muscle type) and UCP2 (data not shown). Thus, we concluded that in mice, the liver is likely to be one of the target tissues of KTE. However, it is unclear as to whether the effects of KTE on WAT accumulation and the size of adipocytes are responsible for the modulation or are a result of modulation.

In this study, it was observed that KTED affects WAT accumulation and the size of WAT adipocytes. It will be necessary to examine its effect on adiponectin and leptin mRNA expression in adipocytes *in vitro* in order to clarify the detailed mechanisms of whole body energy metabolism.

In summary, KTED promotes the VO_2 and suppresses WAT accumulation in normal mice on the N and HF diets. Moreover, KTED reduced the size of WAT adipocytes in both the N and HF groups. Therefore, KTED is beneficial in reducing N diet- and HF diet-induced obesity, and this effect might be attributed, at least in part, to the stimulation of whole body energy metabolism. Our results also emphasize the importance of monitoring normal mice on an N diet, and suggest that KTE might prevent or reduce obesity and could potentially reduce the risk of associated diseases, including type 2 diabetes.

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