Summary

Vinegar is generally believed to be good for health. A mash consisting of 35% ethanolic extract from bitter melon malt vinegar-water (8:50:42) was subjected to further acetate fermentation and the resulting vinegar was converted to dried vinegar powder by spray drying after adsorption on dextrin, which was mixed with a commercial rat chow (CRF-1) in the ratio of 1:19 so as to prepare an experimental diet. Male 12-wk old rats of LETO and OLETF strains were fed this experimental diet in parallel with CRF-1 (control) and examined for respiratory quotient (RQ) and blood or plasma parameters associated with diabetes mellitus. Administration of the experimental diet increased daily food intake as well as daily energy expenditure in both strains. RQ significantly lessened in the vinegar diet-fed group of LETO strain, which was reflected not only in the increased energy consumption from fat but also in the decreased energy consumption from carbohydrate, while no significant difference was observed between both dietary groups of OLETF strain in this respect. The profiles of diurnal energy expenditure in both dietary groups of LETO strain exerted two peaks before lights-on and lights-off. Nevertheless, there was a clear difference between both dietary groups of OLETF strain; interestingly the reproduction of the two peaks became conspicuous in the vinegar diet-fed group despite the lack of such peaks in the control. As a consequence of blood or plasma inspection, it turned out that there was no change in HbA1c but a significant increase in plasma cholesterol in the vinegar diet-fed OLETF rats. From these results, a long-term administration of bitter melon malt vinegar can be expected to suppress a lowering of energy turnover inherent with aging and thereby improve anorexia rather than to bring about a preventive effect against the manifestation of NIDDM.

Key Words

bitter melon malt vinegar, energy expenditure, non-insulin dependent diabetes mellitus, aging, health

People living in Okinawa prefecture in Japan are known to enjoy notable longevity. A dietary factor supposed to be linked to their longevity is ingestion of bitter melon (Momordica charantia). Bitter melon, a specialty called 'gohya' produced in Okinawa prefecture, is believed to be good for the health, to increase appetite, and to prevent susceptibility to summer heat. In this connection, it is suggested that the bitter melon extract affects an improvement in blood glucose level as well as gastric emptying (1). Based on this proposal, we hypothesized that a diet containing vinegar fermented from bitter melon extract might improve diabetic condition.
in this respect was observed between 05:00 and 08:00 as well as 20:00 and 22:00 in both strains of 8 wk age. However, the two peaks disappeared in the OLETF rats of 24 wk age after manifestation of NIDDM, whereas they remained unchangeable in the control LETO of the same age (3, 4). Such a change in the daily profile of energy expenditure was linked to the diabetic condition of the OLETF rats. In practice, the two peaks could be reproduced by improvement in DM and/or insulin resistance (5, 6). The controlled body weight gain postponed the worsening of NIDDM (5, 7, 8), and the administration of a PPARγ agonist lowered insulin resistance by decreasing plasma TNF-α level in OLETF rats (6).

In the present study, the effects of a diet containing bitter melon malt vinegar on the daily profile of energy expenditure were examined with both the OLETF and LETO rats. The sources of energy used for combustion were estimated on the basis of respiratory quotient (RQ). In addition, the plasma or blood parameters for glucose and fat metabolism were measured in the usual way.

**MATERIALS AND METHODS**

**Preparation of the vinegar powder and diet.** Bitter melon was obtained from a local grocery as dried slices, which were repeatedly extracted with 35% ethanol. A mash consisting of bitter melon extract–water–malt vinegar (8:42:50) was subjected to acetic acid fermentation by the surface culture method (9). Acidity of the resulting vinegar was 40 g/L (as acetic acid), and then the residual content was 13% (w/v). To this bitter melon malt vinegar mix was added the same amount of dextrin by weight, followed by preparation of spray- dried vinegar powder (Fig. 1). A 1:19 (w/w) mixture of the vinegar powder and CRF-1 (a commercial rat chow, Oriental Co., Inc., Tokyo, Japan) (Fig. 1) was employed as the routine experimental diet hereinafter.

**Animal feeding.** Male OLETF and LETO rats of 4 wk age were obtained from Otsuka Research Institute, Tokushima, Japan. The rats were allowed to house individually in cages in an air-conditioned facility (temp. 21 °C; humidity, 60%) with a 12-h light/dark cycle (lights-on, 08:00–20:00). The experimental protocol was reviewed and approved by the appropriate committee of the Tokyo Metropolitan Institute of Gerontology.

After preliminary feeding of 8 wk with the CRF-1 diet (control), all 8 rats of the OLETF and LETO groups were fed the experimental ‘vinegar’ diet and the control diet, respectively. During the feeding period, changes in body weight were recorded at intervals of 2 wk, the daily food intake being estimated by subtraction of diet consumption from the start to the close for each week at 12–13, 15–16, 19–20, and 23–24 wk age.

**Oral glucose tolerance test.** OGTT was conducted at 23 wk of age only in the OLETF rats, because the LETO rats did not develop any diabetic symptom (2). The test solution was infused via orogastric tubing at a dose of 2 g glucose/kg BW after overnight fasting; the blood glucose level was measured at 0, 30, 60, and 120 min by the use of tail vein blood and glucose assay kit (Yamanouchi Pharm. Co., Tokyo, Japan).

**Energy expenditure and its source during metabolic study.** A metabolic study was conducted at 24 wk of age, because NIDDM became manifest at 18 wk of age in OLETF rats given CRF-1 (2–4).

Oxygen consumption and carbon dioxide production were measured continuously by breath analysis with an automatic O2- CO2 analyzer (NEC Medical Systems Co. Ltd., Model IH26, Tokyo, Japan), followed by calculation of energy expenditure per hour or per day. The sources of energy used for combustion during energy metabolism were estimated on the basis of RQ as previously described (3, 4).

**Some biochemical parameters in blood or plasma.** After the completion of metabolic study, the rats of 26 wk age were sacrificed between 09:00 and 11:00 without food deprivation. Blood was withdrawn from the tail vein and immediately subjected to HbAlc measurement (10). Subsequently, blood was collected from the abdominal aorta under etherization, mixed with EDTA, and centrifuged at 1,500×g for 15 min at 4 °C. The resulting plasma was stored at −70 °C until the assay of biochemical parameters.

Parallel to this operation, visera were also weighed with respect to liver, pancreas and adipose tissue containing mesenteric, epididymal and perinephric fat.

In time, the frozen plasma was thawed and examined for cholesterol as well as triacylglycerol concentrations with the acid of their respective assay kits (11, 12). Additionally, plasma insulin was measured with rat insulin as a standard by radioimmunoassay (13).

**Statistical analysis.** Data are expressed as means±SE and analyzed by one-way or multiple ANOVA, followed by Fisher’s protected least significant difference test. The difference was considered significant at p<0.05.

**RESULTS**

**Changes in body weight, food intake, and organ weights.**

Body weight and organ weights (including that of visceral fat) were not influenced by administration of the
Table 1. Wet weights of whole body, liver, pancreas, and visceral fat in LETO and OLETF rats of 26 wk of age previously fed both control (CRF-1) and vinegar diets.

<table>
<thead>
<tr>
<th>Measured item</th>
<th>LETO strain</th>
<th>OLETF strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vinegar diet</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>504±11</td>
<td>532±14</td>
</tr>
<tr>
<td>Liver (mg/g BW)</td>
<td>28.6±1.0</td>
<td>30.6±0.9</td>
</tr>
<tr>
<td>Pancreas (mg/g BW)</td>
<td>2.66±0.74</td>
<td>2.58±0.06</td>
</tr>
<tr>
<td>Visceral fat (mg/BW)</td>
<td>40.7±2.4</td>
<td>41.5±2.6</td>
</tr>
</tbody>
</table>

Values are the means±SE (n=8). There was a significant difference (p<0.05) between LETO and OLETF strains with respect to all the measured items, but not between the control and vinegar diet groups of the same strain.

OGTT in OLETF rats

As shown in Fig. 3, there was no significant difference in OGTT between both dietary groups on the basis of multiple ANOVA (F=3.7, p>0.08 for treatment; F=95.0, p<0.0001 for time; and F=2.2, p>0.1 for interaction of strain and time). However, the fasting blood glucose level was significantly lower in the vinegar diet group than in the control group (namely, 4.1±0.5 mM in the former vs. 7.3±0.3 mM in the latter).

Daily profile of energy expenditure

Energy consumption profiles over a 24-h test period at 24 wk of age are shown in Figs. 4 and 5.

Administration of the diet containing bitter melon malt vinegar increased energy expenditure in both strains. The profiles in LETO rats were similar to each other in both dietary groups. In other words, there were two peaks: one peak appeared between 05:00 and 08:00, the other between 20:00 and 22:00 (Fig. 4). In contrast, such two peaks were not apparent in control group of OLETF rats (Fig. 5). The two peaks were reproduced during 05:00–08:00 and 16:00–20:00 in the OLETF rats given the diet containing the bitter melon
Fig. 4. Diurnal profiles of energy expenditure in LETO rats of 24 wk previously fed both control and vinegar diets. Each symbol and bar represents the means±SE (n=8); ○ control group, ● vinegar diet group; * significantly different from the control at p<0.05.

Table 2. Daily energy expenditure and respiratory quotient in LETO and OLETF rats of 24 wk of age previously fed both control and vinegar diets.

<table>
<thead>
<tr>
<th>Measured item</th>
<th>LETO strain</th>
<th>OLETF strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vinegar diet</td>
</tr>
<tr>
<td>Daily energy expenditure (kJ/kg)</td>
<td>204.3±7.5</td>
<td>241.1±7.5*</td>
</tr>
<tr>
<td>kJ/kg BW</td>
<td>410.9±18.0</td>
<td>485.6±13.8*</td>
</tr>
<tr>
<td>Respiratory quotient(%)</td>
<td>0.98±0.01</td>
<td>0.94±0.01*</td>
</tr>
<tr>
<td>Energy sources¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Derived from fat (%)</td>
<td>7.0±3.5</td>
<td>20.2±3.8*</td>
</tr>
<tr>
<td>Derived from carbohydrate (%)</td>
<td>93.0±3.5</td>
<td>79.8±3.8*</td>
</tr>
</tbody>
</table>

Values are the means±SE (n=8). * Significantly different from the control.
¹ Energy sources for 24-h energy expenditure were estimated from RQ.

Table 3. Blood or plasma levels of HbA1c, insulin, triacylglycerol, and cholesterol in LETO and OLETF rats of 26 wk of age previously fed both control and vinegar diets.

<table>
<thead>
<tr>
<th>Blood or plasma parameter</th>
<th>LETO strain</th>
<th>OLETF strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vinegar diet</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>2.65±0.03</td>
<td>2.78±0.05</td>
</tr>
<tr>
<td>Insulin (nmol/L)</td>
<td>1.92±0.21</td>
<td>0.94±0.08*</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>0.83±0.06</td>
<td>0.28±0.05*</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>2.96±0.07</td>
<td>2.75±0.05*</td>
</tr>
</tbody>
</table>

Values are the means±SE (n=8). There was a significant difference between LETO and OLETF strains with respect to all of the measured items at p<0.05. * Significantly different from the control in the same strain.

malt vinegar.

Energy metabolism per day

The total energy expenditure (kJ/kg body or kJ/rat) was significantly higher in the vinegar diet group than in the control group irrespective of LETO or OLETF (Table 2). The mean value of RQ became significantly lower in LETO rats given the vinegar diet, while it was already low in OLETF rats not given the vinegar diet because of the manifestation of NIDDM. In LETO rats, ingestion of the vinegar diet significantly increased energy derived from fat but rather decreased energy from carbohydrate. Nevertheless, these parameters were not significantly changeable in OLETF rats.
Inspection for blood and plasma

As a result of inspection, OLETF rats were at significantly higher blood or plasma levels for HbA1c, insulin, triacylglycerol and cholesterol than LETO rats in accord with the previous observations (3, 4). Administration of the vinegar diet significantly decreased the plasma levels of insulin, triacylglycerol, and cholesterol in LETO rats, although HbA1c did not vary thereby. Conversely, the plasma level of cholesterol was rather increasing in the OLETF rats, but other parameters were not significantly affected by administration of the vinegar diet (Table 3).

DISCUSSION

The present study showed that ingestion of the diet containing bitter melon malt vinegar caused a rise in daily food intake and daily energy expenditure in both normal and diabetic rats. Because OLETF rats lack cholecystokinin (CCK)-A receptors (14, 15) to signal for CCK-mediated satiety (16), the amount of daily food intake in the LETO rats was significantly higher than in the LETO rats. The OLETF rats ate still more bitter melon malt vinegar supplementation. The amount of daily food intake in the OLETF rats was low at 23–24 wk of age (Fig. 2). The OLETF rats were susceptible to stress (17, 18). OGTT conducted at 23 wk of age might have been stressful, resulting in a decrease in food intake, because the rats were grasped and forced to ingest glucose.

Although the daily energy expenditure in the normal LETO rats was also increased by the bitter melon malt vinegar supplementation, the daily profile of energy expenditure was not altered; the two peaks, one during 05:00–08:00 and the other during 20:00–22:00, were observed. With respect to energy metabolism, RQ values were significantly low in rats given bitter melon malt vinegar. Thus it follows that the energy consumption from fat was enhanced by ingestion of bitter melon malt vinegar. Similarly a decrease in plasma triacylglycerol and cholesterol concentrations might be related to the increased fat metabolism. Neither body weight nor organ weights were, however, influenced by administration of bitter melon malt vinegar. Taken altogether, it is reasonable to consider that both energy intake and energy expenditure were elevated by administration of bitter melon malt vinegar. A decrease in plasma insulin level in LETO rats given bitter melon malt vinegar was conspicuous, although no explanation for this mechanism can be offered yet. Since the HbA1c level was normal, at least insulin deficiency is not responsible.

Contrary to the expectation of anti-diabetic action of bitter melon malt vinegar, neither HbA1c nor plasma insulin level nor plasma triacylglycerol level in the OLETF rats was decreased significantly, but plasma cholesterol level was rather increased by administration of bitter melon malt vinegar. The distinct effects of administration of bitter melon malt vinegar were an increase in energy expenditure and the normalization of daily profile of energy expenditure. The two peaks (during 05:00–08:00 and 20:00–22:00, respectively) that had been observed at 8 wk of age for rats without NIDDM, were reproduced (3, 4). Although the level of blood glucose after glucose ingestion was not decreased, that of fasting blood glucose was normalized by treatment with bitter melon malt vinegar (Fig. 3). Thus, its administration exerted a remedial effect on diabetic condition in the OLETF rats despite no effect on the plasma insulin or blood HbA1c level. It has been reported that the prevention of obesity by exercise or food restriction effectively postpones the onset of NIDDM in this strain (5, 7, 8). A preventive against the manifestation of NIDDM can normalize the daily profile of energy expenditure (5). For this reason, the control of obesity is more recommended together with the intake of bitter melon extract to prevent the manifestation of NIDDM.

Biological actions of bitter melon have been referred to in various aspects (19). Bitter melon serves as an alternative therapy that has primarily been effective in lowering the blood glucose level in patients with DM, because it contains as ingredient analogous to insulin in a fashion of action (19). It has been more recently reported that bitter melon reduces adiposity and normalizes glucose tolerance in rats fed a high fat diet (20). However, there has been no report dealing with the effect of bitter melon on diurnal profiles of energy metabolism. The mash consisted of bitter melon extract—water—malt vinegar (8 : 42 : 50). We announced that black vinegar (Kurozu) had physiological functions similar to bitter melon malt vinegar (21), although being inferior to bitter melon malt vinegar in increment of energy expenditure. A moment's thought may make it possible that such a component as saponin in bitter melon is responsible for the biological effect on energy expenditure, after elucidation of the underlying mechanism (19, 20). The respective contributions of bitter melon, barley, and vinegar to energy metabolism remain to be further investigated.

Aging is associated with a progressive decrease in appetite and food intake, which has been termed the anorexia by aging (22). This phenomenon is caused by the decline in physical activity and resting metabolic rate that occurs with aging. The decline of food intake that often happens in healthy elderly persons also predisposes them to pathological weight loss and protein-energy malnutrition, thereby raising morbidity or mortality (22). Our previous studies (23, 24) demonstrated that proper food restriction and/or exercise in Wistar rats could decrease age-associated pathological changes and inhibit an age-associated decline in basal metabolic rate. Consumption of bitter melon malt vinegar could also serve as another tool useful to prevent age-associated decline in energy expenditure as well as basal metabolic rate and to maintain health.

In conclusion, the administration of bitter melon malt vinegar could not only cause an increase in both food intake and energy consumption in normal and diabetic rats, but also normalize the daily profile of energy expenditure in diabetic OLETF rats. Our findings suggest that its administration may possibly prevent age-associated appetite loss, decline in energy metabolism, and
malnutrition.

Acknowledgments
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