Effects of Okinawan Sugar Cane Wax and Fatty Alcohol on Serum and Liver Lipids in the Rat

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Summary Partially purified Okinawan sugar cane wax and fatty alcohol were fed to Wistar strain rats to examine the effects on serum and liver cholesterol (Chol), triglyceride (TG) and phospholipid (PL). The fecal excretion of neutral sterols in the rats was also determined. There were no significant differences found in the body weight gain, food intake and liver weight among the animals of experimental diet groups. An addition of 0.5% sugar cane wax to the diet significantly lowered the concentrations of serum and liver Chol in the rats. There were no significant differences observed in PL and TG levels either in serum or liver among the experimental groups. These results indicate that cane wax, one of the elements contained in sugar cane rind as well as in black sugar, may have a cholesterol-lowering effect on the serum and liver of the rats. The amount of feces excreted by the three experimental diet groups of rats were exactly the same and also no significant differences were found in the excretion of Chol.

Key Words sugar cane wax, cholesterol, lowering effect

Our previous experiment showed that Okinawan black sugar might contain some elements which lower the concentrations of serum lipid in male rats (1). The following experiments (2) were carried out to further study the effective element (or elements) which lowered the serum lipid levels in the rats.

We have shown recently that the addition of 2% Okinawan sugar cane rind to high Chol-containing diet has an effect on weight control of rats and reduces the concentrations of Chol and TG in serum (3). This observation prompted us to study the component analysis of the sugar cane rind materials, and the results revealed that there were lipids, crude fiber and water present at levels of about 30%, 19.6% and 14.6% respectively. It was also found that the main components of the lipids
were wax and fatty alcohol comprising about 63% and 30% of the total lipids respectively.

In another paper (4) the methods of separation and of partial purification of wax and fatty alcohol from sugar cane rind materials were reported. Although a number of reports have been made on the properties of sugar cane wax in the sugar industry (5-8), almost no attention has been paid to the biological effect of sugar cane wax on serum lipid concentration.

The purpose of the present study was to assess the effects of Okinawan sugar cane wax and fatty alcohol on serum and liver lipid levels in rats.

MATERIALS AND METHODS

Materials. The rind materials were collected during the harvesting season from Okinawan sugar cane (Kind, NCO-310; Brix, 21%) raised in the Attached Agricultural Experiment Farm, University of the Ryukyus. From those rind materials, the cane wax and fatty alcohol were prepared by the method described previously.

Animals. Male Wistar strain rats (Nikon Rat Co., Saitama), initially weighing 140-180g, were randomly divided into 3 groups of 6 rats each, and caged individually. The following three experimental diets were given ad libitum for 14 days. Procedures for obtaining the serum, liver and feces were the same as previously described (4).

Diets. The composition of the experimental diets is shown in Table 1. For group I, a control diet, containing 5% lard, 1% Chol and 0.3% sodium cholate (weight %) was used to produce hypercholesterolemia in rats. Group II and III animals were fed on the diets that were supplemented with 0.5% cane wax and 0.5%

| Table 1. Composition of the experimental diets (weight %). |
|----------------------------------------------------------|
| **Diet Groups** | **Control** (I) | **Wax** (II) | **Fatty alcohol** (III) |
|-----------------|----------------|--------------|------------------------|
| Sucrose         | 64.7           | 64.2         | 64.2                   |
| Casein*         | 20.0           | 20.0         | 20.0                   |
| Cellulose       | 4.0            | 4.0          | 4.0                    |
| Mineral Mix*    | 4.0            | 4.0          | 4.0                    |
| Vitamin Mix*    | 1.0            | 1.0          | 1.0                    |
| Lard            | 5.0            | 5.0          | 5.0                    |
| Cholesterol     | 1.0            | 1.0          | 1.0                    |
| Sodium cholate  | 0.3            | 0.3          | 0.3                    |
| Wax             | —              | 0.5          | —                      |
| Fatty alcohol   | —              | —            | 0.5                    |

*Oriental Kobo K. K.

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fatty alcohol, respectively.

**Lipid analysis.** Serum and liver lipids were extracted using chloroform and methanol (in the volume ratio 2:1) as solvent according to Folch et al. (9). The methods used for determining each lipid fraction were as follows: Chol, Sperry and Webb’s method (10); TG, Fletcher’s method (11); and PL, Gomori’s method (12).

**Sterol analysis.** Feces excreted for the last three days of the experiment were collected from each rat and were freeze-dry-pulverized. Fecal sterol analysis was carried out as described previously (3).

## RESULTS

**Growth and food consumption**

As shown in Table 2, there were no significant differences found in body weight gain, food intake, feed conversion and liver weight among the three diet groups of animals.

**Serum lipid concentrations**

Serum concentrations of Chol, PL and TG are shown in Table 3. The mean

| Groups | Diets   | Initial weight (g) | Weight gain (g) | Food intake (g/day) | Feed conversion (g) | Liver weight (g/100 g body wt.) |
|--------|---------|--------------------|-----------------|---------------------|---------------------|-------------------------------|
| I      | Control | 157 ± 15           | 93 ± 12         | 17 ± 2.1            | 2.4 ± 0.3           | 4.1 ± 0.1                     |
| II     | Wax     | 158 ± 12           | 98 ± 14         | 17 ± 2.2            | 2.4 ± 0.2           | 4.1 ± 0.2                     |
| III    | Fatty alcohol | 156 ± 14      | 102 ± 16        | 18 ± 2.4            | 2.4 ± 0.2           | 4.3 ± 0.3                     |

* Mean ± SD.

**Table 3.** Effect of sugar cane wax and fatty alcohol on serum lipids.

| Groups | Diets         | Cholesterol | Phospholipid | Triglyceride |
|--------|---------------|-------------|--------------|--------------|
|        |               | Total       | Free         | Ester (%)    | (mg/dl) | (mg/dl) | (mg/dl) |
| I      | Control       | 267 ± 12.0* | 41 ± 5.2     | 85 ± 1.3     | 153 ± 24.8 | 111 ± 30.2 |
| II     | Wax           | 154 ± 45.6* | 29 ± 8.9b    | 81 ± 1.4     | 133 ± 30.5 | 139 ± 14.7 |
| III    | Fatty alcohol | 220 ± 43.4  | 36 ± 7.4     | 84 ± 0.7     | 147 ± 11.9 | 108 ± 20.3 |

* Mean ± SD. *a*Significantly different from the control diet (p < 0.005). *b*Significantly different from the control diet (p < 0.05).
Table 4. Effect of sugar cane wax and fatty alcohol on liver lipids.

| Groups | Diets   | Cholesterol | Phospholipid | Triglyceride |
|--------|---------|-------------|--------------|--------------|
|        |         | Total (mg/g liver) | Free (mg/g liver) | Ester (%) | (mg/g liver) | (mg/g liver) |
| I      | Control | 52 ± 4.2* | 3.7 ± 0.3 | 93 ± 0.2 | 40 ± 5.6 | 48 ± 11.4 |
| II     | Wax     | 41 ± 5.7a | 3.2 ± 0.5 | 92 ± 1.0 | 36 ± 2.3 | 57 ± 12.5 |
| III    | Fatty alcohol | 49 ± 5.6 | 3.4 ± 0.3 | 93 ± 0.7 | 40 ± 4.0 | 51 ± 11.7 |

* Mean ± SD. a Significantly different from the control diet (p<0.05).

Table 5. Gas chromatographic analysis of neutral sterol in feces.

| Intake | Excretion |
|--------|-----------|
|        | Intake    | Excretion |
|        | Cholesterol | Unknown** | Feces*** | Cholesterol | Coprostanol + unknown** |
|        | (mg/day) | (g/day) | (mg/day) | (mg/day) | (mg/day) |
| I      | Control  | 173 ± 17* | 0 ± 0 | 1.6 ± 0.3 | 131 ± 18 | 5 ± 2 |
| II     | Wax      | 179 ± 22 | 5.5 ± 0.7ab | 1.6 ± 0.2 | 123 ± 18 | 5 ± 2b |
| III    | Fatty alcohol | 174 ± 24 | 51.0 ± 6.7a | 1.6 ± 0.3 | 113 ± 18 | 59 ± 12a |

* Mean ± SD. ** The figures under 'unknown' are for peaks which occurred at the chromatogram position (Rt) corresponding to that of the excretion of coprostanol in the control group. *** Dry weight. a Significantly different from the control diet (p<0.005). b Significantly different from group III (p<0.005).

total and free Chol levels in the rats fed 0.5% of cane wax supplementation (group II) were significantly low as compared to those of the rats fed on control diet (group I). No significant changes in total or free Chol level were found in the rats given fatty alcohol (group III).

The serum TG and PL levels in all the groups of animals were almost the same.

Liver lipid concentration

The concentrations of Chol, PL and TG in the liver are shown in Table 4. The total Chol level of group II was significantly lower than that of group I, but free-Chol levels of all experimental groups were almost identical. There were no significant differences observed in PL and TG levels among these experimental groups.

Fecal excretion of neutral sterols

The fecal excretion of neutral sterols determined by gas chromatographic

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Fig. 1. Gas chromatograms of sterols of the control diet and feces (group I). (A) Diet 1, 5-α-cholestane; 2, Chol. (B) Feces 1, 5-α-cholestane; 2, unknown + coprostanol; 3, Chol.

Fig. 2. Gas chromatograms of sterols of the wax diet and feces (group II). (A) Diet 1, 5-α-cholestane; 2, unknown; 3, Chol. (B) Feces 1, 5-α-cholestane; 2, unknown + coprostanol; 3, Chol.

Analysis is shown in Table 5. The Chol intakes from the diet in each group were almost the same because there were no significant differences in the amounts of food intake. The amounts of feces excreted by all experimental group animals were wax on rat serum and liver lipid concentrations by supplying diets containing levels among those groups.

In addition to the figures for Chol, Table 5 shows chromatographic data for the peaks labeled ‘unknown.’ These peaks have retention time identical to that of the coprostanol peak as shown in Figs. 1–3. The source of the peak was not determined in this experiment. The differences between the unknown peak values of intake and excretion were insignificant.

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DISCUSSION

The main components of the sugar cane rind lipids, wax and fatty alcohol, were partially purified and fed to Wistar strain male rats at the 0.5% level. There were no significant differences found in the food intake, body weight gain and liver weight among the control and experimental diet groups. However, the addition of 0.5% cane wax to the control diet resulted in a significant lowering of the serum and liver Chol levels in the rats, suggesting a suppressive effect of cane wax on serum and liver lipid levels. It is worth mentioning that the sugar cane rind wax had a hypocholesterolemic action in rats even when they were fed on a diet containing high Chol in combination with sodium cholate.

It is necessary to determine coprostanol for the estimation of Chol excretion, especially when Chol-free diet is given. However, Sugao et al. (13) suggested that the conversion of Chol into coprostanol was depressed when the Chol and sodium cholate were combined in the diet as in the present experiment. Therefore, it would be safe to say that the absorption of Chol can be estimated by the amounts of intake and fecal excretion. Since there were no significant differences in the fecal excretion

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of Chol among all the experimental animal groups, the hypocholesterolemic effect of cane wax that was found in this study was assumed to be due to neither the acceleration of excretion nor the inhibition of absorption of Chol.

Thus, we are now investigating the mechanism of this lowering effect of cane wax on rat serum and liver lipid concentrations by supplying diets containing levels of fat different to those found in the diet of the present experimental animals. Furthermore, the fecal excretion of bile acids is also being determined and the results will be reported later.

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