Oral administration of uridylic acid increases plasma leptin, but suppresses glucose and non-esterified fatty acid concentrations in rats

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Abstract

Nucleic acids have been known to have biological effects on the digestive and immune systems, although less attention has been paid to the action on metabolism. In the present study, in order to investigate the effects of oral ingestion of uridylic acid (5′-uridine monophosphate, 5′-UMP) on hormonal and metabolic levels, we measured changes in the plasma concentrations of leptin, insulin, glucose, non-esterified fatty acids (NEFA), weights of the liver and abdominal fat and fat accumulation in the liver and M. gastrocnemius in male rats. Intragastric administration of 5′-UMP via a stomach tube at a dose of 44mg/day for 7 days slightly (P=0.098) blunted the body weight gain without causing a significant change in food intake. The administration significantly reduced the plasma concentrations of glucose (P=0.004) and NEFA (P=0.004), whereas it significantly increased (P=0.03) plasma leptin concentration. The weights of perirenal (but not epididymal) fat (P=0.083) and the liver (P=0.061) were slightly increased. The triacylglyceride concentration in M. gastrocnemius was slightly increased (P=0.097), although the muscle weight was not significantly changed (P=0.197). In summary, acute oral administration of 5′-UMP was effective in the rat in reducing plasma concentrations of glucose and NEFA, an effect that was accompanied by an elevated plasma leptin concentration.

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Introduction

Ruminant colostrum contains a large amount of uridine and its derivatives such as uridine monophosphate (5′-uridylic acid, 5′-UMP) and uridine diphosphate (UDP). The concentrations of 5′-UMP and its derivatives can be as high as 1000, 2000 and 9000 µmol/l for cattle, goats and sheep, respectively (Gil, 1981). On the other hand, human colostrum contains several kinds of nucleotides (CMP, UMP, GMP and AMP) at relatively low levels between 1.0 and 23.0 µmol/l (Duchen and Thorell, 1999). These nucleic acids and their derivatives are reported to induce a large number of biological actions in the digestive system (Nunez et al., 1990; Uauy et al., 1990; Brunser et al., 1994; Bueno et al., 1994; Ortega et al., 1994), the immune system (Carver et al., 1990; Van Buren et al., 1983, 1985, 1994; Kulkarni et al., 1989; Matsumoto et al., 1995; Nagafuchi et al., 1997; Rudolph et al., 1986) and the microbial flora in the gut (Gil et al., 1986).

Recently, we fed neonatal calves milk containing 5′-UMP at 2g/day for 1 week, and demonstrated that 5′-UMP feeding suppressed perirenal fat deposition and the postprandial increase in plasma insulin (Katoh et al., 2005). However, to our knowledge, the effect of 5′-UMP on metabolic levels has not yet been reported in non-ruminant animal species. It is important to determine the biological significance of nucleic acids and their derivatives, because a variety of commonly eaten foods, such as formula, cookies and cakes, are made from cow’s-milk-derived materials.

Therefore, our objective in this study was to investigate the effects of oral 5′-UMP intake on metabolic parameters in young male rats. The findings clearly demonstrate that 5′-UMP feeding significantly affects the hormonal and metabolite levels.
Materials and methods

Experimental animals

The animals were treated according to the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences” (The Physiological Society of Japan), and the present experiment was approved by The Animal Care Committee of Tohoku University.

Twenty Wistar rats (10 weeks old, male, mean body mass 250 g, CLEA, Tokyo, Japan) were housed in a controlled environment (2 rats in a cage), exposed to a 12-h light, 12-h dark cycle, and provided with Purina standard rodent chow and water ad libitum for at least 1 week before the onset of the experiment.

Intragastric injection of uridylic acid

The rats were administered with 1 ml of saline alone (control) or 1 ml of saline containing 5′-UMP (44 mg/head = 124 mg/kg metabolic body size/head) through a stomach tube (9.5 cm length) attached to a plastic syringe once a day at 1300 h for 7 successive days. The dose of 5′-UMP per metabolic body size (44 mg/day) was equivalent to that given to young calves, and it was shown to change the metabolic parameters efficiently (Katoh et al., 2005).

Blood and tissue sampling

At 1000 h on day 8, the animals were decapitated so that blood could be collected and tissue samples taken. The heparinized (10 U/ml) blood samples were centrifuged at 8000 g for 20 min, and then plasma samples in some portions were stored at −30°C until the hormones and metabolic parameters were assayed. The perirenal and epididymal fat, liver and M. gastrocnemius were sampled, weighed, soaked in liquid nitrogen and stored at −80°C until the assay.

Analyses

The glucose and NEFA concentrations in plasma were determined using commercial kits (Glucose CII-Test and NEFA C-Test, respectively; Wako Pure Chem., Osaka, Japan). Protein concentrations were determined by the method described previously (Lowry and Passonneau, 1972). Leptin and insulin concentrations were assayed using commercial RIA kits (Linco, MO, USA).

Statistics

Results are represented as means ± S.E. The unpaired Student’s t-test was employed to compare the two mean values (Zar, 1984). In the comparison of daily food intake (Fig. 1), Bonferroni’s multiple range test following one-way ANOVA was also employed (Wallenstein et al., 1980). Statistical significance was set at P < 0.05. When the P value was 0.05 < P < 0.10, the term “slightly” was used.

Results

Fig. 1A depicts the mean daily food intake (g) for each group over 7 days. The cumulative food intake over a 7-day treatment period was 1094 and 991 g for the control and the 5′-UMP

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Plasma concentrations of hormones and metabolites in control and UMP-fed rats</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>4.7±0.1</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>4.4±0.7</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>126.2±2.7</td>
</tr>
<tr>
<td>NEFA (mEq/l)</td>
<td>0.597±0.042</td>
</tr>
</tbody>
</table>

In UMP-fed (n = 10) and control (n = 10) rats, physiological saline (1 ml) with or without 44 mg UMP was injected into the stomach through stomach tubing at 1300 h every day for 7 days. Blood samples were then taken after decapitation. The unpaired Student’s t-test was employed.
group respectively. No significant difference was found between the two groups on any day nor among the values over the 7 days for each group.

Fig. 1B demonstrates the difference in body weight before and after the 7 days. The body weight for the control group was slightly increased ($P=0.071$) from 266.0±1.5 to 272.1±2.8g, while that for the 5′-UMP group was not significantly changed from 266.4±6.6 to 266.6±8.5g ($P=0.954$). Eventually, the body weight increase for the 5′-UMP group was slightly smaller than that for the control group ($P=0.098$).

Table 1 demonstrates the plasma concentrations of leptin, insulin, glucose and NEFA. The plasma concentrations of leptin were significantly increased, while those of glucose and NEFA were significantly decreased by the UMP ingestion. However, plasma insulin concentration was not significantly changed.

Table 2 demonstrates tissue weight of the liver, *M. gastrocnemius*, and epididymal and perirenal fat in the rats that ingested UMP. The weight of the liver and perirenal fat was slightly ($P=0.061$ and 0.083, respectively) increased in the rats that ingested UMP.

Fig. 2 shows the triacylglyceride concentrations in the liver and *M. gastrocnemius*. The triacylglyceride concentration in the liver was significantly ($P=0.046$) reduced, but that in *M. gastrocnemius* was slightly ($P=0.097$) increased in the rats that ingested UMP.

Discussion

The present study has, for the first time, demonstrated that the ingestion of UMP at a dose of 44mg/day (124mg/kg metabolic body size/day) over 7 days significantly reduced plasma glucose and NEFA levels, a reduction that was accompanied by a significant increase in the plasma leptin level and a slight reduction in body weight gain. The negative-feedback control of the peripheral and CNS administration of leptin is well known to suppress feeding behavior and white adipose mass (Friedman and Halaas, 1998; Halaas et al., 1995; Schwartz et al., 1996). However, the mechanism by which the UMP ingestion increases plasma leptin levels remains to be clarified.

In our previous paper with young calves (Katoh et al., 2005), the reduced postprandial plasma glucose level and increased triacylglyceride accumulation in the Longissimus muscle were demonstrated by the UMP feeding. In the present study, a slightly increased triacylglyceride accumulation was also demonstrated in the *M. gastrocnemius* of the rat (Fig. 2). This finding may suggest an enhanced insulin-sensitive glucose uptake by the skeletal muscles, as it has been reported that the major mechanism of insulin-sensitive glucose uptake by the skeletal muscles, as it has been reported that the major mechanism of insulin-sensitive glucose uptake by the skeletal muscles, as it has been reported that the major mechanism of insulin-sensitive glucose uptake by GLUT-4 (Baron et al., 1988; Rose et al., 1997) positively correlates with fat accumulation, but negatively correlates with glycogen content (Derave et al., 2000). However, the detailed mechanism of the reduced triacylglyceride accumulation in the rat liver remains to be clarified. The reduction in the liver was not found in milk-fed calves (Katoh et al., 2005).

There apparently exist some differences, which may result from a species difference, in the effects of UMP ingestion on metabolic parameters. In calves fed with milk containing UMP (2g/day, 126mg/kg metabolic body size) (Katoh et al., 2005), the plasma NEFA concentration was not changed, although the postprandial increase in the plasma concentrations of glucose and insulin after milk ingestion were significantly smaller than in the control calves. In the present study with rats, however, the UMP ingestion significantly reduced the plasma levels of glucose and NEFA without changing the insulin level (Table 1).
In addition, the feeding of UMP caused a reduction in the perirenal fat mass in young calves (Katoh et al., 2005), although the opposite result, if any, was suggested in the present study (Table 2). One possibility for the discrepancy between rats and calves may be the difference in their ages, as we used suckling calves in our previous paper (Katoh et al., 2005) but young weaned rats in the present study.

It is increasingly known that UMP can interact with P2Y purine receptors and exert biological actions as reported in many tissues (Konduri et al., 2004; Anderson and Parkinson, 1997). At least four different types of receptors are known for uridine nucleotides (Anderson and Parkinson, 1997). In pulmonary circulation, uridine nucleotides were shown to cause comparative vasodilatation as adenine nucleotides did (Konduri et al., 2004). Activation of P2Y receptors (one of G-protein coupled receptors) by ATP caused intracellular calcium metabolism in mammary epithelial cells (Katoh et al., 2001).

In summary, we, for the first time, have demonstrated that 5'-UMP, a common nucleic acid which is contained in cow’s colostrum at a higher concentration than other nucleic acids, has the effect in the rat of suppressing plasma glucose and NEFA levels, an effect that is accompanied by an increased leptin level.

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