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Anti-obese action of raspberry ketone

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Abstract

Raspberry ketone (4-(4-hydroxyphenyl) butan-2-one; RK) is a major aromatic compound of red raspberry (Rubus idaeus). The structure of RK is similar to the structures of capsaicin and synephrine, compounds known to exert anti-obese actions and alter the lipid metabolism. The present study was performed to clarify whether RK helps prevent obesity and activate lipid metabolism in rodents. To test the effect on obesity, our group designed the following in vivo experiments: 1) mice were fed a high-fat diet including 0.5, 1, or 2% of RK for 10 weeks; 2) mice were given a high-fat diet for 6 weeks and subsequently fed the same high-fat diet containing1% RK for the next 5 weeks. RK prevented the high-fat-diet-induced elevations in body weight and the weights of the liver and visceral adipose tissues (epididymal, retroperitoneal, and mesenteric). RK also decreased these weights and hepatic triacylglycerol content after they had been increased by a high-fat diet. RK significantly increased norepinephrine-induced lipolysis associated with the translocation of hormone-sensitive lipase from the cytosol to lipid droplets in rat epididymal fat cells. In conclusion, RK prevents and improves obesity and fatty liver. These effects appear to

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stem from the action of RK in altering the lipid metabolism, or more specifically, in increasing norepinephrine-induced lipolysis in white adipocytes.
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Keywords: Raspberry ketone; Anti-obesity; Lipolysis; White adipocyte; High-fat diet

Introduction

Raspberry (European red raspberry, Rubus idaeus) is one of the oldest fruits known to people and has been used throughout the centuries for nutritional and medicinal purposes. Like its popular relatives the strawberry and blueberry, raspberry contains an abundance of sugars, vitamins, minerals, and polyphenols. Studies on the biological effects of raspberry components have yielded many results. In one study, for example, the ellagic acid in raspberries was confirmed to inhibit tumor inductions in the liver, lungs and esophagus (Ravai, 1996). Other work has been conducted to explore the makeup of the unique fragrance and flavor of raspberry by isolating various of its aromatic compounds. Raspberry ketone (4-(4-hydroxyphenyl) butan-2-one; RK), one of the major aromatic compounds of raspberry (Gallois, 1982), is widely used as a fragrance in cosmetics and as a flavoring agent in foodstuffs (Guichard, 1982). In one study investigating the intragastric administration of RK (1 mmol/kg), about 90% of the dose was excreted as metabolites via the urine within 24 h in rats, guinea pigs and rabbits (Sporstol and Scheline, 1982). Before now, however, there have been no reports on the biological effects of RK.

RK has a structure similar to the structures of capsaicin and synephrine (Fig. 1). Capsaicin (N-[(4-hydroxy-3-methoxyphenyl)-methyl]-8-methyl-6-nonamide), a pungent principle of hot red pepper, has been reported to decreased the adipose tissue weight and serum triacylglycerol content by enhancing energy metabolism (Kawada et al., 1986a, b). Synephrine (1-(4-hydroxy-phenyl)-2-methylaminoetha-

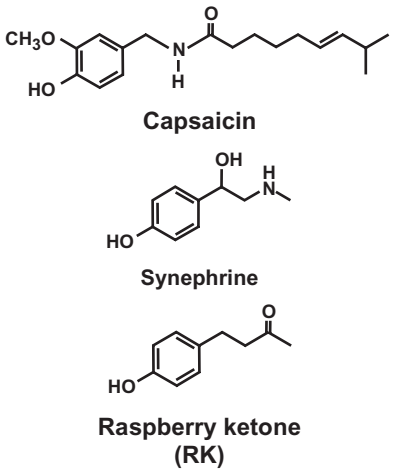

Fig. 1. Structural formulas of Capsaicin, Synephrine, and Raspberry ketone.

nol), a compound found in Citrus plants, exerts a lipolytic activity in fat cells (Carpene et al., 1999). Noting the structural similarities in these three compounds, we hypothesized that RK might influence the lipid metabolism in ways similar to capsaicin and synephrine. In this study we examined the effects of RK on obesity and lipid metabolism.

Materials and methods

Estimation of body, visceral adipose, and liver weights: Effects of RK in preventing obesity

Male ICR mice (4-weeks old) were obtained from CLEA Japan (Osaka, Japan) and housed in a temperature- and humidity-controlled room set to a 12/12 h light/dark cycle. After the animals were given a standard laboratory diet (Oriental Yeast Co., Ltd.) and water ad libitum for 1 week, they were divided into five groups matched for body weight. One group, the normal diet group, was fed a standard laboratory diet. RK with 99.9% purity is purchased from Takasago International Corporation (Tokyo, Japan). The other four, the experimental groups, were fed either of two experimental diets, the high-fat diet and the high-fat diet plus RK. The experimental diets shared the following basic composition: beef tallow 40%, casein 34–36%, corn starch 10%, sugar 9%, vitamin mixture (AIN-93G) 1% and mineral mixture (AIN-93G) 4% (w/w per 100 g diet) with water ad libitum. The compositions for the respective experimental groups were as follows (each n = 6): high-fat diet group, casein 36% and basic components; High-fat diet plus RK group, different amounts of casein (34%, 35% and 35.5%) and RK (2%, 1% and 0.5%). In a previous study, a high-fat diet formulated with different amounts of casein, from 22% to 36%, resulted in similar degrees of obesity in mice, with no significant variability in body weights or visceral adipose tissue weights (Han et al., 1999a, b). Based on these results, we added RK to the high-fat diet instead of a portion of the casein. The total food intake of each group was recorded at least three times weekly, and the body weight of each mouse was recorded once weekly. After 10 weeks of feeding on the indicated experimental diet, various visceral adipose tissues and the liver were quickly removed under anesthesia with diethyl ether and weighed.

Estimation of body, visceral adipose, and liver weights: Effect of RK in improving obesity

After giving male ICR mice (5-weeks old) the basic high-fat diet described above with water ad libitum for 6 weeks, the animals were divided into two groups matched for body weight (each n = 6). One group was continued on the same high-fat diet and the other was given a high-fat diet plus RK (casein 35% and RK 1%). The total food intake of each group was recorded at least three times weekly, and the body weight of each mouse was recorded once weekly. After 5 weeks of feeding on the high-fat diet or the high-fat diet plus RK, the liver and various visceral adipose tissues were quickly removed under anesthesia with diethyl ether and weighed.

Histology

The liver samples were fixed in 4% paraformaldehyde-phosphate buffered saline. Fixed samples were dehydrated by sequentially increased ethanol concentrations, cleared in xylene, and then embedded in paraffin. The embedded samples were sectioned and stained with hematoxylin and eosin (HE).

Measurement of lipolysis

Young male Crj:Wistar rats weighing between 150 to 200 g were given a standard laboratory diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. The animals were sacrificed by cervical dislocation to minimize endogenous catecholamine secretion, and their epididymal adipose tissues were quickly removed. Isolated fat cells were obtained from the removed epididymal adipose tissue by the method of Rodbell (Rodbell, 1964). The fat cells (200 μl packed volume) were incubated at 37 °C for 1 h in 500 μl of Hanks' buffer (pH 7.4) supplemented with 2.5%(w/v) bovine serum albumin and the indicated concentrations of RK and norepinephrine. After incubation, the reaction mixture was centrifuged at 100 × g at room temperature for 30 s to separate the medium and fat cells. The glycerol content of the medium was estimated by the method of Warnick (Warnick, 1986).

Localization of HSL in fat cells

Fat cells (200 μl packed volume) were separated from the reaction mixture by the method described above, added to 450 μl of homogenization buffer (25 mM Tris-HCl, pH 7.4, containing 254 mM sucrose, 1 mM EDTA, 100 μM benzamidine, 20 μM leupeptin, 2 mg/ml soybean trypsin inhibitor, and 1 μM okadaic acid) in a plastic tube, and agitated 20 times using a hand-held plastic pestle. After centrifugation at 5,500 × g for 10 min at 4 °C, the supernatant and fat layer were suspended in Laemmli sample buffer (Laemmli, 1970) containing 1 and 20% (w/v) SDS, respectively (Egan et al., 1992). An aliquot of each suspension (10 to 15 μl) corresponding to 200 μl of packed fat cells was subjected to SDS-PAGE using gels containing 8% acrylamide (Laemmli, 1970) and subsequent Western blotting (Morimoto et al., 2001) with an anti-HSL antiserum (Holm et al., 1988).

Statistical analysis of data

All values are expressed as mean ± SE. Data were analyzed by one-way ANOVA and differences among means of groups were analyzed using Fisher's Protected LSD and Dunnett's test. Differences were considered significant at $p < 0.05$.

Results

Effects of RK on body, visceral adipose, and liver weights: Effect in preventing obesity

The mice fed on the high-fat diet containing 40% beef tallow for 10 weeks had a significantly higher body weight and significantly heavier visceral adipose tissues (e.g., epididymal, retroperitoneal and mesenteric adipose tissues) than the mice fed on the normal diet (Fig. 2). In the mice fed the high-fat diet plus RK (2%), the body weight elevation that took place over the initial 6 weeks on the high-fat diet was significantly reduced (Fig. 2A) and the final weights of the visceral adipose tissues were significantly lower than those in the mice fed on the high-fat diet alone (Fig. 2B). The body weight reduction was proportionally equal to the reduction in the visceral adipose tissue weight after 10 weeks. The mean energy intake per week per mouse (kilo-joule (kJ)/week/mouse) during the whole experimental period

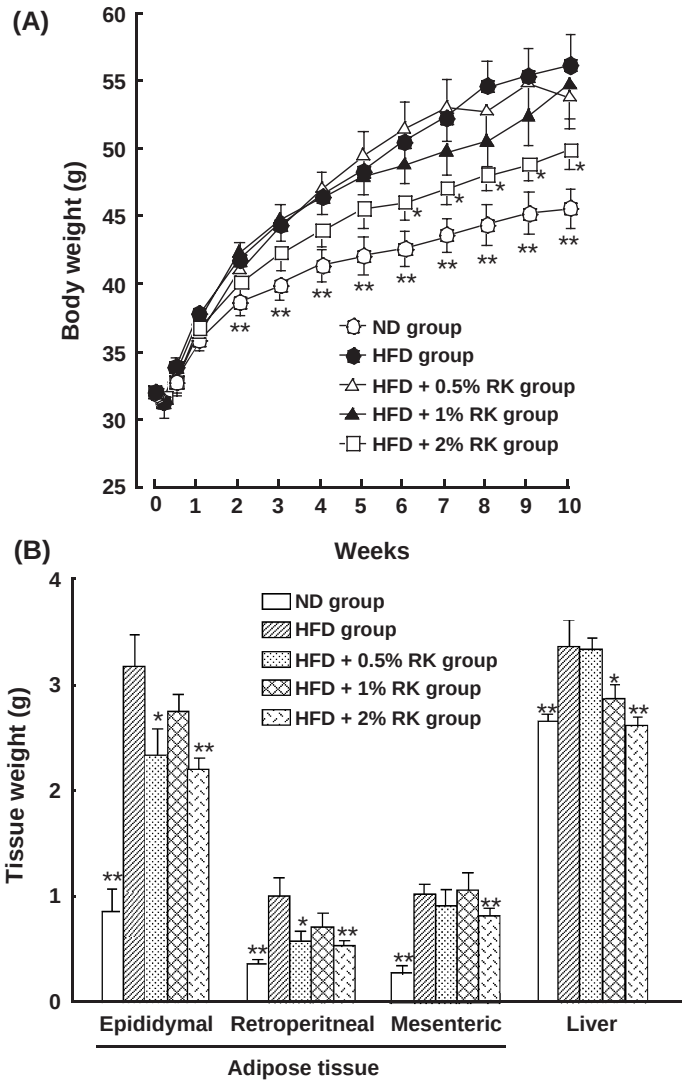

Fig. 2. Effect of raspberry ketone on body weight (A) and the weights of the liver and various visceral adipose tissues (B) in mice fed a high-fat diet for 10 weeks. The meaning of each symbol is indicated in the figure. The experimental procedures are described in the Materials and Methods section. Each value represents the mean ± SE of 6 mice; $*p < 0.05$ and $**p < 0.01$ vs. values in high-fat diet group. ND, normal diet; HFD, high-fat diet; RK, raspberry ketone.

differed significantly between the normal diet group and high-fat diet group (500.3 ± 9.1 kJ/week/mouse in the normal diet versus 760.7 ± 38.4 kJ/week/mouse in the high-fat diet group), but it did not significantly differ between the high-fat and high-fat plus 0.5%, 1% or 2% RK diet groups (760.7 ± 38.4 kJ/week/mouse (high-fat diet) versus 807.8 ± 14.7 kJ/week/mouse (0.5% RK diet), 805.0 ± 14.6 kJ/week/mouse (1% RK diet), and 799.4 ± 14.5 kJ/week/mouse (2% RK diet)). The high-fat diet containing 1% or 2% RK also significantly suppressed the increase in liver weight induced by the high-fat diet alone (Fig. 2B).

Effects of RK on body, visceral adipose and liver weights: Effects in improving obesity

Fig. 3A shows the changes in body weights of the groups fed on the high-fat diet or the high-fat RK (1%) diet for 5 weeks, which have been fed on the high-fat diet for 6 weeks. The body weights of the mice fed the high-fat diet alone and the high-fat RK (1%) diet both fell over the first week of RK feeding. The body weights of both these groups increased thereafter, but the weight gain in the animals on the high-fat RK (1%) diet tended to slow over the next 2 weeks. The final visceral adipose tissues and liver weights of the groups are shown in Fig. 3B. The high-fat diet led to significant increases in the

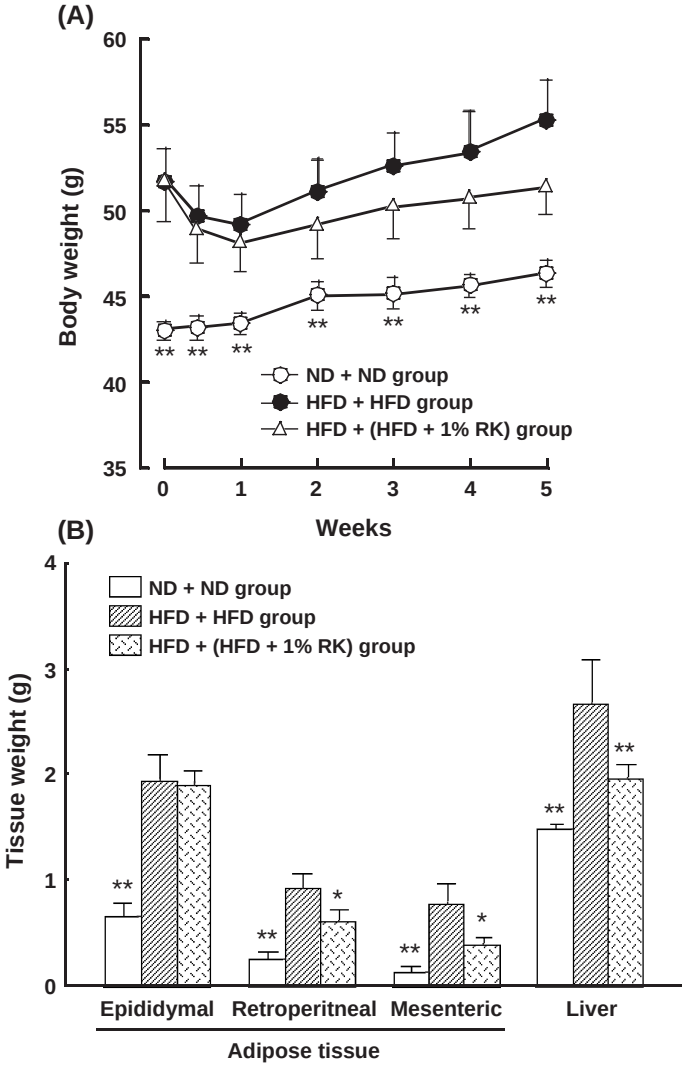

Fig. 3. Effect of raspberry ketone on body weight (A) and the weights of the liver and various visceral adipose tissues (B) in mice fed a high-fat diet for 5 weeks after feeding on a high-fat diet for 6 weeks. The meaning of each symbol is indicated in the figure. The experimental procedures are described in the Materials and Methods section. Each value represents the mean ± SE of 6 mice; $^{**}p < 0.01$ vs. values in high-fat diet group. ND, normal diet; HFD, high-fat diet; RK, raspberry ketone.

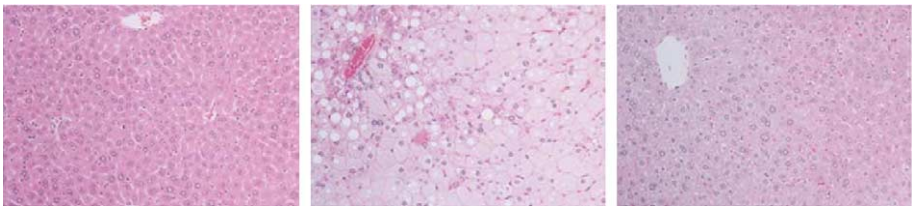

Fig. 4. Hepatic histology. The experimental procedures are described in the Materials and Methods section. ND, normal diet; HFD, high-fat diet; RK, raspberry ketone.

weights of the visceral adipose tissues and liver compared to the weights measured in the normal diet group. RK (1% in the high-fat diet) significantly reduced the final weights of the liver and retroperitoneal and mesenteric adipose tissues compared to the levels measured in the animals on the high-fat diet alone. The hepatic triacylglycerol levels in the normal group, high-fat diet group, and high-fat RK (1%) diet group were 10.7 ± 1.6, 35.6 ± 6.8, and 17.9 ± 1.9 mg/g liver, respectively. Concomitantly, the high-fat diet led to an enlargement of the hepatocytes and an increase in the number of lipid droplets in the liver compared with the levels observed in the normal diet group, while RK (1% in the high-fat diet) returned the hepatocyte size to normal and decreased the number lipid droplets (Fig. 4). In addition, plasma triacylglycerol levels tended to be reduced in the high-fat diet group because of a decrease in triacylglycerol secretion from the liver. However, RK tended to increase plasma triacylglycerol levels (data not shown).

Effects of RK on norepinephrine-induced lipolysis and localization of HSL in white adipocytes

By incubating fat cells with various concentrations of RK in the presence of norepinephrine, measurements of the amounts of glycerol released into the medium allowed us to estimate the lipolysis. As shown in Fig. 5A, norepinephrine at a concentration of 3×10^{-8} M increased the amount of glycerol released by the fat cells. RK enhanced glycerol release by fat cells in a concentration-dependent manner. Fig. 5B shows representative immunoblots of the HSL in the supernatant and fat layer in norepinephrine- and RK-stimulated cells. RK at the concentration of 10^{-3} M significantly increased the amount of HSL protein in the fat layer and concomitantly reduced that in the supernatant (Fig. 5C). RK in the absence of norepinephrine had no affect whatsoever on the lipolysis or localization of HSL in the fat cells (data not shown).

Discussion

We previously induced obesity in normal-weight mice by feeding them for 10 weeks on a high-fat diet containing 40% beef tallow. Increases in body weight, fat storage, hepatic triacylglycerol content, and the frequency of fatty liver were all noted (Han et al., 1999b). In the present study increases in the body weight and weights of visceral adipose tissues were successfully prevented in mice fed a high-fat diet plus RK without changing the energy intake (except the normal diet group) (Fig. 2). At the end point of

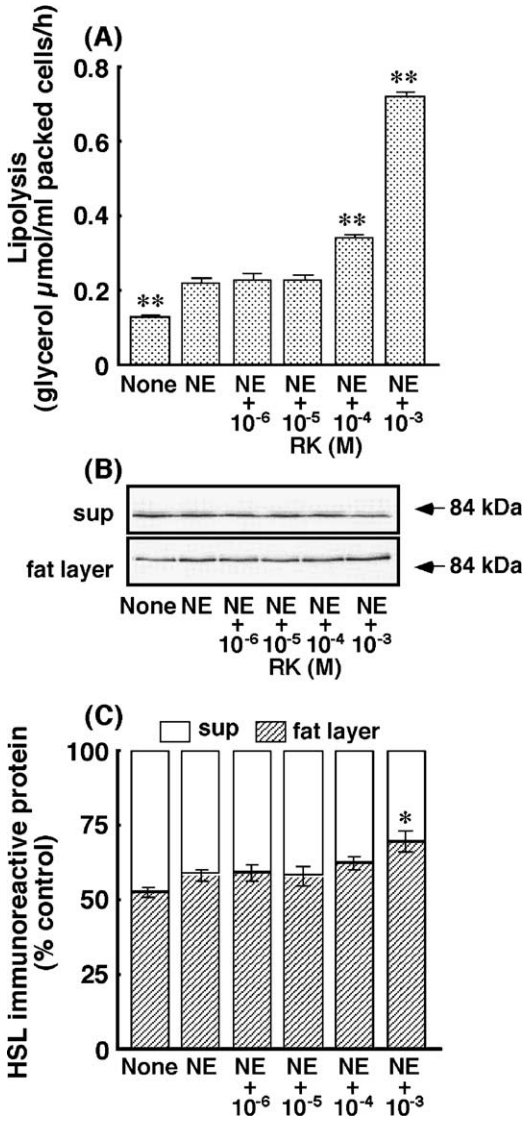

Fig. 5. Effect of various concentrations of raspberry ketone on lipolysis (A) and localization of HSL (B, C) in rat epididymal fat cells. The experimental procedures are described in the Materials and Methods section. (A): The glycerol content of the medium was estimated by the method of Warnick (Warnick, 1986). Lipolysis was expressed as μmol glycerol released per ml packed fat cells per h. (B): A representative immunoblot showing the HSL protein levels of norepinephrine- and raspberry ketone-treated cells is presented. (C): HSL immunoreactive protein levels of the supernatant and fat layer tabulated as the percentage of the signal density detected by enhanced chemifluorescence determined using a FluorImager, Fluorescence Imaging Analyzer (Amersham Pharmacia Biotech UK Ltd. Bucks, UK). Each column represents the mean ± SE of four separate experiments; *$p <$ 0.05 and **$p < 0.01$ vs. values of norepinephrine-treated cells. NE, norepinephrine; RK, raspberry ketone.

the experiment, the RK-induced inhibition of the elevations in the weights of the body and visceral adipose tissues did not appear to be dose dependent. Earlier, however, from weeks 6 to 9, the suppression of body weights by 1 and 2% RK was clearly dose dependent (Fig. 2A). In addition, RK reduced the final liver weight at doses of both 1% and 2% (Fig. 2B). On the basis of these findings, we decided that a dose of 1% RK was sufficient to prevent high-fat-diet-induced increases in body and tissue weights. It was also shown that 1% RK remedially affected the increases in body weight, visceral adipose tissues weights (Fig. 3), and hepatic triacylglycerol content (Fig. 4) in mice fed a high-fat diet. These results indicate that RK prevents the obesity and the fatty liver induced by feeding a high-fat diet.

Dietary fat is digested by pancreatic lipase and absorbed from the small intestine (Verger, 1984; Hernell et al., 1990). In strategies to prevent obesity, one of the key steps is to inhibit the digestion and absorption of the dietary fat. To explore this strategy further, we studied the effects of RK on fat absorption. RK at a concentration of 5% reduced the elevation of plasma triacylglycerol after oral administration of a lipid emulsion containing corn oil in rats, whereas a lower concentration of RK 1% elicited no such effect (data not shown). Moreover, RK at concentrations of 1–20 mg/ml inhibited rat pancreatic lipase activity in an assay system using trioleoylglycerol emulsified with lecithin (Tsujita et al., 1996), whereas the same concentrations exhibited no such inhibitory effect when the trioleoylglycerol was emulsified with gum arabic instead of lecithin in the same system (data not shown). This means that the site of the inhibitory action of RK on trioleoylglycerol hydrolysis may be the substrate rather than the enzyme. In any case, these results suggest that RK suppresses the dietary fat absorption by inhibiting the trioleoylglycerol hydrolysis. Significantly, however, the inhibitory effect of RK on fat absorption is not the main anti-obese mechanism. We know this because the minimum RK dose required to exert these effects is much higher than that required to exert anti-obese effects.

Dietary sugars such as glucose and fructose are also known to increase hepatic lipogenesis and promote fat accumulation (Herzberg and Rogerson, 1988). RK (1%) suppressed weight elevation in visceral and subcutaneous adipose tissues induced by the over-intake of fructose (Y. Morimoto, personal communication). Given that RK reduces both fat- and sugar-induced fat accumulation, we postulated that its anti-obese action is conferred mainly during the stages of fat decomposition.

Based on these experiments, we examined the effect of RK on lipolysis of white adipocytes. The catecholamines norepinephrine and epinephrine are known to stimulate lipolysis via beta-adrenergic receptor. Similarly, synephrine can activate in vitro lipolysis in rat adipocytes via activation of the same receptor (Carpene et al., 1999). This prompted us to examine the effect of RK on lipolysis in rat epididymal fat cells and the ability of RK to bind various subtypes of beta-adrenergic receptors. While RK failed to stimulate lipolysis in the absence of norepinephrine and failed to bind to beta 1, 2 and 3-adrenergic receptors (data not shown), it was successful in increasing norepinephrine-induced lipolysis at the concentration of 10^{-3} M (Fig. 5A). We find it noteworthy that RK has a lipolytic activity that takes place via a mechanism unrelated to that of synephrine. Noting that the rate-limiting step in lipolysis in fat cells, the hydrolysis of triacylglycerol, is catalyzed by HSL (Khoo et al., 1976; Belfrage et al., 1984), we decided to examine the effect of RK on HSL activity in rat fat cells. We found that RK did not enhance the HSL activity at the concentration of 10^{-3} M (data not shown). In another paper we reported that the conversion of the HSL translocation to its substrate on the surfaces of lipid droplets is a crucial step for triacylglycerol hydrolysis (Morimoto et al., 1999, 2001). When we examined the localization of HSL in fat cells in the present study, RK at a concentration of 10^{-3} M significantly increased the amount of HSL protein in the fat layer and concomitantly reduced that in the supernatant (Fig. 5B, C). These results

suggest that RK enhances norepinephrine-induced lipolysis not via the HSL activation, but via an increase in the translocation of HSL from the cytosol to the lipid droplets in the fat cells.

Capsaicin was reported to exert anti-obese activity by enhancing the energy metabolism (Kawada et al., 1986a, b). This effect might be due to an increase of thermogenesis in brown adipose tissues (BAT) through the stimulation of the sympathetic nervous system (Watanabe et al., 1994). If this is so, we can speculate that RK also stimulates the energy metabolism via a mechanism similar to that capsaicin. Capsaicin administration increases the oxygen consumption in rats (Kawada et al., 1986b), while RK supplementation increases the oxygen consumption and reduces the respiratory quotient (R.Q.) in rats (T. Shimazu, personal communication). In another study, the effect of RK on energy metabolism was examined by measuring cytochrome c oxidase activity in mouse BAT. Both the specific activity and total activity of cytochrome c oxidase activity were significantly increased by RK (Y. Morimoto, personal communication). These results indicate that RK activates the BAT thermogenesis and enhances the energy metabolism. In any case, more detailed studies in the future should help clarify the mechanisms by which RK enhances energy metabolism.

In conclusion, the present investigation demonstrated that RK has an anti-obese function. RK stimulated the metabolism of white and brown adipose tissues and inhibited small intestinal absorption of dietary fat by suppressing pancreatic lipase activity. As an agent effective in preventing both fat- and sugar-induced obesity, RK might exert its anti-obesity effect via an increase of norepinephrine-induced lipolysis in white adipocytes and an enhancement of thermogenesis in BAT.

References

Belfrage, P., Fredrikson, G., Strålfors, P., Torquist, H., 1984. Adipose tissue lipase. In: Borgström, B., Brockman, H.L. (Eds.), Lipase. Elsevier, Amsterdam, pp. 365–416.

Carpene, C., Galitzky, J., Fontana, E., Atgie, C., Lafontan, M., Berlan, M., 1999. Selective activation of β3-adrenoceptors by octopamine: comparative studies in mammalian fat cells. Naunyn-Schmiedeberg's Archives of Pharmacology 359, 310–321.

Egan, J.J., Greenberg, A.S., Chang, M.K., Wek, S.A., Moos Jr, M.C., Londos, C., 1992. Mechanism of hormone-stimulated lipolysis in adipocytes: translocation of hormone-sensitive lipase to the lipid storage droplet. Proceedings of the National Academy of Sciences of the United States of America 89, 8537–8541.

Gallois, A., 1982. Quantitative evaluation of raspberry ketone using thin-layer chromatography. Sciences des Aliments 2, 99–106.

Guichard, E., 1982. Identification of the flavoured volatile components of the raspberry cultivar lloyd george. Sciences des Aliments 2, 173–185.

Han, L.-K., Kimura, Y., Okuda, H., 1999a. Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet. International Journal of Obesity 23, 174–179.

Han, L.-K., Takaku, T., Kimura, Y., Okuda, H., 1999b. Anti-obesity action of oolong tea. International Journal of Obesity 23, 98–105.

Hernell, O., Straggers, J.E., Carey, M.C., 1990. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. Biochemistry 29, 2041–2056.

Herzberg, G.R., Rogerson, M., 1988. Interaction of dietary carbohydrate and fat in the regulation of hepatic and extrahepatic lipogenesis in the rat. British Journal of Nutrition 59, 233–241.

Holm, C., Kirchgessner, T.G., Svenson, K.L., Fredrikson, G., Nilsson, S., Miller, C.G., Shively, J.E., Heinzmann, C., Sparkes, R.S., Mohandas, T., Lusis, A.J., Belfrage, P., Schotz, M.C., 1988. Hormone-sensitive lipase: sequence, expression, and chromosomal localization to 19 cent-p13.3. Science 241, 1503–1506.

Kawada, T., Hagihara, K.I., Iwai, K., 1986a. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. The Journal of Nutrition 116, 1272–1278.

Kawada, T., Watanabe, T., Takaishi, T., Tanaka, T., Iwai, K., 1986b. Capsaicin-induced β-adrenergic action on energy metabolism in rats: influence of capsaicin on oxygen consumption, the respiratory quotient, and substrate utilization. Proceedings of the Society for Experimental Biology and Medicine 183, 250–256.

Khoo, J.C., Steinberg, D., Huang, J.J., Vagelos, P.R., 1976. Triglyceride, diglyceride, monoglyceride, and cholesterol ester hydrolysis in chicken adipose tissue activated by adenosine 3′ 5′-monophosphate-dependent protein kinase. The Journal of Biological Chemistry 251, 2882–2890.

Laemmli, U.K., 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Morimoto, C., Sumiyoshi, M., Kameda, K., Tsujita, T., Okuda, H., 1999. Relationship between hormone-sensitive lipolysis and lipase activity in rat fat cells. Journal of Biochemistry 125, 976–981.

Morimoto, C., Kameda, K., Tsujita, T., Okuda, H., 2001. Relationships between lipolysis induced by various lipolytic agents and hormone-sensitive lipase in rat fat cells. Journal of Lipid Research 42, 120–127.

Ravai, M., 1996. Quality characteristics of raspberries and blackberries. Cereal Foods World 41, 773–775.

Rodbell, M., 1964. Metabolism of isolated fat cells. The Journal of Biological Chemistry 239, 375–380.

Sporstol, S., Scheline, R.R., 1982. The metabolism of 4-(4-hydroxyphenyl)butan-2-one (raspberry ketone) in rats, guinea-pigs and rabbits. Xenobiotica 12, 249–257.

Tsujita, T., Matsuura, Y., Okuda, H., 1996. Studies on the inhibition of pancreatic and carboxylester lipases by protamine. Journal of Lipid Research 37, 1481–1487.

Verger, R., 1984. Pancreatic lipase. In: Borgström, B., Brockman, H.L. (Eds.), Lipase. Elsevier, Amsterdam, pp. 83–150.

Warnick, G.R., 1986. Enzymatic methods for quantification of lipoprotein lipids. Methods in Enzymology 129, 101–123.

Watanabe, T., Kawada, T., Kato, T., Harada, T., Iwai, K., 1994. Effects of capsaicin analogs on adrenal catecholamine secretion in rats. Life Sciences 54, 369–374.