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Research Report

Nutritional status alters saccharin intake and sweet receptor mRNA expression in rat taste buds

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ARTICLE INFO

Article history:
Accepted 5 February 2010
Available online 12 February 2010

Keywords:
Sweet preference
Dietary-induced obesity
Saccharin
OB-Rb
Sweet taste receptors

ABSTRACT

Sweet taste usually signifies the presence of caloric food. It is commonly accepted that a close association exists among sweet taste perception, preference, and nutritional status. However, the mechanisms involved remain unknown. To investigate whether nutritional status affects the preference for palatable solutions and alters sweet taste receptor gene expression in rats, we measured saccharin intake and preference using a two-bottle preference test, and changes in body weight, plasma leptin levels, and gene expression for the sweet taste receptor in taste buds in high-fat diet-induced obese rats and chronically diet-restricted rats. We found that the consumption and preference ratios for 0.01 and 0.04 M saccharin were significantly lower in the high-fat diet-induced obese rats than in the normal diet rats, while the serum leptin levels were markedly increased in obese rats. Consistent with the changes in saccharin intake, the gene expression level of the sweet taste receptor T1R3 was significantly decreased in the high-fat diet-induced obese rats compared with the control rats. By contrast, the chronically diet-restricted rats showed remarkably enhanced consumption and preference for 0.04 M saccharin. The serum leptin concentration was decreased, and the gene expression of the leptin receptor was markedly increased in the taste buds. In conclusion, our results suggest that nutritional status alters saccharin preference and the expression of T1R3 in taste buds. These processes may be involved in the mechanisms underlying the modulation of peripheral sweet taste sensitivity, in which leptin plays a role.

1. Introduction

The gustatory system plays a critical role in the life and nutritional status of an organism. Sweet taste is usually described as a predictor of the caloric properties of food. It induces a hedonic response and promotes food ingestion (Anderson, 1995). Previous studies have shown that the changes in homeostatic status alter the intake of sweeteners

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Abbreviations: CNS, central nervous system; CR, chronically restricted diet rats; CV, circumvallate papilla; GPCRs, G-protein-coupled receptors; CT, chorda tympani; HF, high-fat diet induced obese rats; OB-Rb, full-length "b" isoform of the leptin receptor; PLC-β2, phospholipase C-β2; TC, total cholesterol; TG, triglyceride; TRPM5, a cation channel member of the transient receptor potential super family, subfamily M, member 5

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doi:10.1016/j.brainres.2010.02.026

(Berridge, 1991; Campbell, 1958; Glockner et al., 1986; Smith and Duffy, 1957; Zverev, 2004). These alterations also modulate the responses of the taste neurons, the transmission and integration of gustatory information in the central nervous system (CNS) (Giza and Scott, 1987; Hajnal et al., 2005; Kaplan et al., 2000; Kovacs and Hajnal, 2008), indicating a close relationship among nutritional status, ingestive behavior, and the gustatory system. While it is still not clarified that whether the altered sweet taste perception induced by metabolic state of the animal actually changes its taste sensitivity or whether it merely modulates the animals' affective response to the taste stimulus.

Results from two groups indicate that adaptations to changes in nutritional status may occur in the periphery. For example, the responses of the chorda tympani (CT) nerve to sweeteners are significantly enhanced in diabetic rats and in high-fat diet-induced obese rats (HF rats) (Kawai et al., 2000; Shimizu et al., 2003). It was suggested that the marked decrease in the gustatory threshold in acute fasted rats may be related to the effects of hunger on the taste receptors (Glockner et al., 1986; Zverev, 2004). The taste bud may serve as an important target for gustatory sensitivity modulation (Shin et al., 2008). Neural coding of gustatory information begins in the oral cavity with the interaction between taste stimuli and the taste receptors. This is a complicated physiological process in which numerous chemical modulators are involved (Herness et al., 2005). The sweet taste receptor is a heterodimer that consists of two proteins (taste receptor, type 1, member 2 T1R2/member 3 T1R3) belonging to the G-protein-coupled receptor (GPCR) superfamily (Nelson et al., 2001). Both T1R2 and T1R3 subunits are capable of binding sugars (Nie et al., 2005) and play distinct roles in the detection of sugars and/or the activation of T1R2/T1R3 (Xu et al., 2004). Several intracellular signaling elements, including α-gustducin, phospholipase C-β2 (PLC-β2), and TRPM5 (a cation channel member of the transient receptor potential superfamily, subfamily M, member 5), may act downstream of the receptors and play important roles in this process. It has been shown that the mRNA expression of α-gustducin is significantly increased in taste buds of diabetic rats (Zhou et al., 2008). Leptin, a hormone normally released from adipocytes, plays an important role in maintaining energy homeostasis (Ahima et al., 1996; Schwartz et al., 2000). Leptin was recently found to act on the full-length "b" isoform of the leptin receptor, OB-Rb, in taste cells to suppress sweet sensation (Shigemura et al., 2004). However, little is currently known about the cellular and molecular mechanisms that link nutritional status with changes in sweet taste preference and sensitivity in the periphery.

In this study, we examined the preference for saccharin solutions in rats under different nutritional conditions by using two-bottle preference tests. We measured the mRNA expression of the sweet taste receptor and the related downstream elements in taste buds by quantitative real-time RT-PCR. We also monitored changes in body weight and plasma leptin levels to assess whether the preference is correlated with the plasma metabolic profiles and gene expression of the sweet taste receptor in HF rats and chronically diet-restricted rats (CR rats).

2. Results

2.1. *Effects of nutritional status on metabolic profiles of rats*

Overall, the results of the one-way ANOVA revealed significant effects of diet on body weight gain from week 1 ($F_{(2,21)}$ = 34.228, P<0.001) to the end of week 6 ($F_{(2,21)}$ = 32.239, P<0.001) of the study. As shown in Fig. 1, rats fed with the high-fat diet for 6 weeks became significantly obese and their final body weight was 371.15±4.12 g (n=8), which was approximately 20% greater than that of control rats (299.25±4.90 g; n=8). Fisher's LSD comparisons revealed that the body weight gain in the obese rats was significantly greater than that of control rats at weeks 4, 5, and 6 (P=0.003, P=0.004, and P=0.001, respectively). However, rats exposed to chronic diet restriction were much leaner, weighing only 234.88±4.20 g (n=8) at week 6. From week 1, the magnitude of weight gain in the CR rats was markedly lower compared with that of the control group rats (P<0.001).

Table 1 shows the plasma TC, TG, and leptin concentrations in the three groups in different specific caloric need states. The HF rats were hyperleptinemic, with plasma leptin levels that were 20% higher than those in control rats (P=0.028). The serum total cholesterol (TC) and triglyceride (TG) levels were 2.2-fold (P=0.003) and 1.1-fold (P=0.049) higher, respectively, in HF rats than in control rats. However, in CR rats, there were no significant changes in plasma TC, TG, or leptin as compared with control rats. Further analyses showed that the plasma TC (P=0.049), TG (P=0.000), and leptin (P=0.005) levels were significantly lower in CR rats than in HF rats (Table 1).

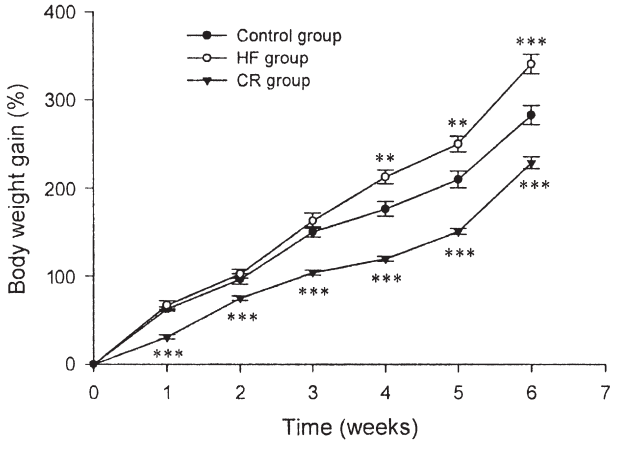

Fig. 1 – Effects of ingestion of HF and CR diets for 6 weeks on body weight gain. HF rats exhibited a significant increase in body weight (expressed as the percentage of initial body weight) at 4–6 weeks, while CR rats showed a markedly decreased weight gain at 1–6 weeks compared with control rats. **P<0.01 and ***P<0.001 compared with control group. Abbreviations: CR, chronically restricted diet rats; HF, high-fat diet-induced obese rats.

Table 1 – Average plasma concentration in male rats in different nutritional states.

	HF group ($n=8$)	CR group ($n=8$)	Control group ($n=8$)
Cholesterol (mmol/l)	1.73±0.10*	1.39±0.04##	1.51±0.06
Triglyceride(mmol/l)	1.35±0.25**	0.41±0.32###	0.61±0.09
Leptin (ng/ml)	7.45±0.30*	5.79±0.36##	6.18±0.46

Values are mean±SEM; *$P<0.05$, **$P<0.01$, and ***$P<0.001$ compared with control values. #$P<0.05$, ##$P<0.01$, and ###$P<0.001$ compared with HF rats values.

2.2. *Effects of nutritional status on intake and preference ratios for saccharin solutions*

As illustrated in Fig. 2A, the HF rats consumed a lower absolute volume of sweet solutions as compared with the control rats, at saccharin concentrations of 0.01 M (16.33±1.06 vs. 22.25±1.63 ml, $P=0.042$) and 0.04 M (14.51±1.03 vs. 18.64±0.68 ml, $P=0.045$). The CR rats showed a significant increase in the intake of 0.04 M saccharin compared with the control rats and in the intake of 0.01, 0.02, and 0.04 M saccharin compared with the HF rats (Fig. 2A). Fig. 2B clearly shows that the saccharin preference ratios were different between the groups. The HF rats showed a lower preference for 0.01, 0.02, and 0.04 M saccharin compared with the control rats (0.01 M: 51.75±3.08% vs. 64.38±2.75%, $P=0.028$; 0.02 M: 49.17±3.06% vs. 63.75±4.55%, $P=0.025$; 0.04 M: 49.20±2.69% vs. 60.45±1.49%, $P=0.023$) in the two-bottle choice test. By contrast, the CR rats exhibited significantly greater preference for 0.04 M saccharin only, as compared with the control rats, and for 0.01, 0.02, 0.04 M saccharin compared with HF rats (76.08±4.66% vs. 60.45±1.49%, $P=0.002$) (Fig. 2B).

2.3. *mRNA expressions of sweet taste receptors and related genes in taste buds*

In rat taste buds, PCR products of the expected size were observed (Fig. 3). Real-time PCR results revealed that the mRNA expression of the sweet taste receptor subunit T1R2 was not altered in HF rats or in CR rats, showing no significant effect of the different diets ($P>0.05$) (Fig. 4A). However, the mRNA expression of T1R3 in the taste buds was significantly lower (67%) in HF rats compared with that in the control rats ($P=0.013$), whereas chronic diet restriction had no effect compared with the control group ($P=0.546$). Nevertheless, the difference between the HF group and the CR group was statistically significant ($P=0.003$) (Fig. 4B). Although there were no significant changes in the mRNA expression of the downstream proteins α-gustducin, PLC-β2, and TRPM5 in the taste buds in any of the three groups, there was an apparent tendency for the expression of these genes to decrease in HF rats and increase in CR rats compared with the controls. Relative to the controls, the HF rats showed a 32% reduction in α-gustducin gene expression while CR rats showed a 23% and 28% increase in the expression of PLC-β2 and TRPM5, respectively. The mRNA expression of α-gustducin was significantly different between the HF rats and CR rats ($P=0.006$) (Fig. 4C–E). The CR rats showed an increase in OB-Rb mRNA by 70% ($P=0.024$) and 60% ($P=0.043$) versus control rats and HF rats, respectively (Fig. 4F).

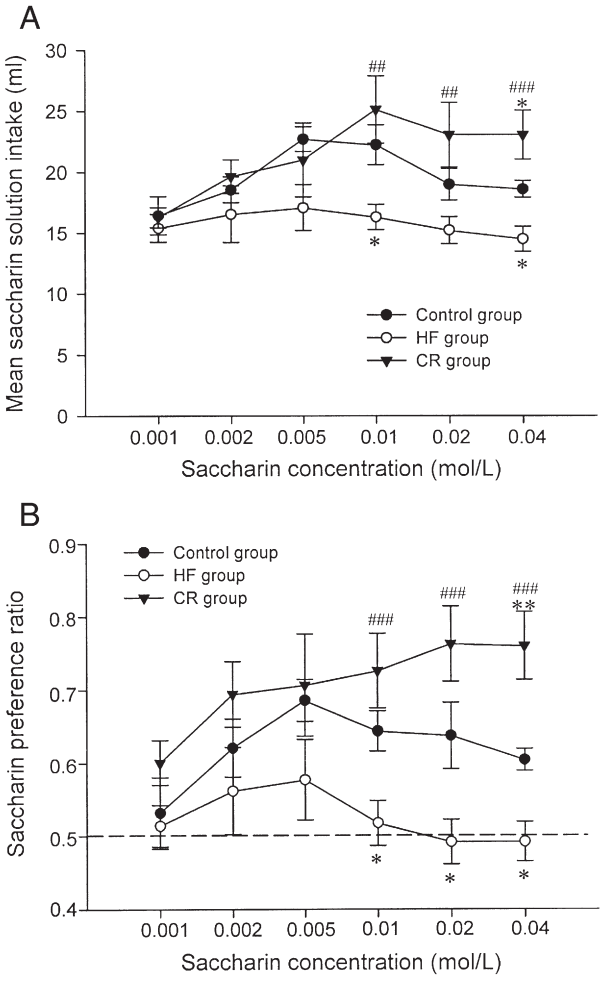

Fig. 2 – Results of two-bottle preference test in the three groups of rats upon a change of nutritional status. (A) Mean saccharin intake in the three groups of rats. HF rats showed significantly decreased intake of 0.01 and 0.04 M saccharin relative to the control rats, while CR rats consumed a markedly lower absolute volume of 0.04 M saccharin solution. (B) Mean saccharin preference ratios. HF rats showed lower preference for 0.01, 0.02, and 0.04 M saccharin solutions, whereas CR rats showed greater preference for 0.04 M saccharin alone. *$P<0.05$, **$P<0.01$, compared with control rats. #$P<0.05$, ##$P<0.01$, and ###$P<0.001$ compared with HF rats. Abbreviations: CR, chronically restricted diet rats; HF, high-fat diet-induced obese rats.

3. Discussion

Many investigators have used two-bottle preference tests to investigate rodent taste preferences for taste solutions (Bachmanov et al., 2001b; Harriman, 1967; Scalera, 1992), while some researchers considered that the behavioral outcome can be significantly altered by postingestive effects of a substance (Ackroff and Sclafani, 1994; Sclafani and

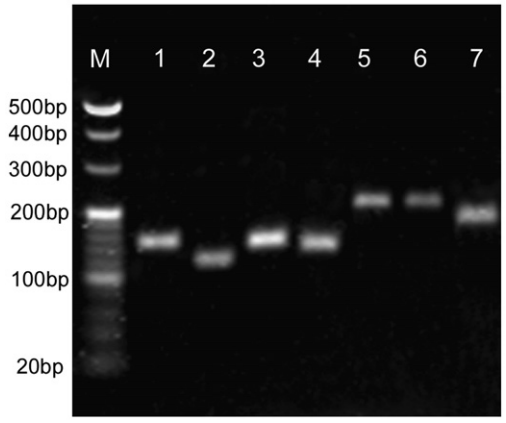

Fig. 3 – RT-PCR analysis of mRNA expression of rat sweet taste receptors and related components in taste buds. The PCR products were visualized on a 3% agarose gel stained with ethidium bromide. Bands corresponding to the expected sizes of T1R2, T1R3, α-gustducin, PLC-β2, TRPM5 and β-actin mRNA were observed. Molecular markers (M) using a 20-bp DNA ladder are shown. The predicted sizes of the PCR products are as follows: T1R2 (204 bp), T1R3 (203 bp), α-gustducin (129 bp), PLC-β2 (158 bp), TRPM5 (155 bp), OB-Rb (177 bp), and β-actin (156 bp). PCR products indicative of genomic contamination were not observed. M: 20 bp marker; 1: β-actin; 2: α-gustducin; 3: PLC-β2; 4: TRPM5; 5: T1R2; 6: T1R3; 7: OB-Rb. Abbreviations: CR, chronically restricted diet rats; HF, high-fat diet-induced obese rats; OB-Rb, full-length "b" isoform of the leptin receptor; PLC-β2, phospholipase C-β2; TRPM5, a cation channel member of the transient receptor potential super family, subfamily M, member 5.

Nissenbaum, 1987; Spector, 2000). It has been known that T1R2/T1R3 sweet taste receptor is expressed in the gut and stimulates the release of gut peptides glucagon like peptide-1 and glucose-dependent insulinotrophic peptide (Margolskee et al., 2007). In the present study, saccharin was used to be a sweet stimulus, which is implied that except for initiating sugar ingestion in the mouth as a taste stimulus, saccharin also binds its receptor in the gut and triggers some related physiological responses. However, as a non-caloric sweetener, saccharin has less postingestive effects compared with caloric postingestive effects and would thus act as a gustatory stimulus used in our experiment (Agmo and Marroquin, 1997; Kushner and Mook, 1984).

In 1967, Collier and Novell first investigated the rat's preference for saccharin and reported the inverted U-function for saccharin intake: saccharin intake and preference increased with concentration and then declined at higher concentrations (Collier and Novell, 1967). In this study, we assessed the sweet taste capacity of rats exposed to different nutritional conditions using two-bottle preference tests (saccharin vs. water). The similar inverted U curves were also shown (Fig. 2). Our results further demonstrated that HF rats exhibited suppressed intake and preference for 0.01 and 0.04 M saccharin solutions compared with control rats, while the CR rats showed markedly enhanced saccharin intake and preference for 0.04 M saccharin (Fig. 2). A previous study obtained a different result from the present investigation, in which the diet had little effect on saccharin taste preference (Tordoff, 2007). This discrepancy may partially be attributed to the following: first, the diet selection did not differ significantly from one another in that experiment: one kind of diet is a cereal-based chow diet (4.40% fat, 46.60% carbohydrate, and 24.50% protein; 3.1 kcal/g), another diet contained 7.0% fat, 62.90% carbohydrate, and 20.00% protein and had an energy content of 3.8 kcal/g, while the diet composition used in the present study is different markedly (Table 2). Second, the C57BL/6J and 129X1/SvJ mice were used after feeding with two different diet mentioned above, whereas we employed the male SD rats fed by high-fat diet for 6 weeks.

The intake and preference for sweeteners may be affected by changes in sweet taste perception and sensitivity (Shin et al., 2008; Tepper and Seldner, 1999), which refer to complex physiological and behavioral mechanisms, including sensory functions, taste hedonics, and affective response to the taste stimulus.

Many previous studies have proved that the physiological state of the animal actually changes its sensitivity to the gustatory stimulus. The alterations may happen at different levels of the taste transmission pathway: taste receptor cells (Huang and Staehler, 2009), taste afferent nerves (Inoue and Tordoff, 1998; Jacobs et al., 1988), nucleus of the solitary tract (Jacobs et al., 1988; McCaughey and Scott, 2000; Nakamura and Norgren, 1995) and parabrachial nucleus (Huang and Yan, 2008; Shimura et al., 1997). Other investigations pointed out that the changes in metabolic state also modulated the sensory pleasure of sweetness (Cabanac, 1971, 1979; Laeng et al., 1993) and hedonic reactions of rats to sweeteners (Berridge, 1991; Grill et al., 1996). Our results showed that the intake and preference of sweetness in HF rats were decreased, and the real-time quantitative PCR assay, the depressed expression of T1R3 in taste buds, was consistent with the behavioral data, implying a relation of behavioral changes of the rat with T1R3 subunit in taste cells, which is associated with the peripheral sweet sensitivity.

The taste perception is dynamically modulated by efferent signals from the CNS, hormonal action, or peripheral adaptation. Energy excess or deprivation may affect the sensitivity of some nuclei in the CNS that are involved in taste perception (Sudakov, 1993), which in turn act on the gustatory receptors (Plata-Salaman, 1991). Chronic alterations in plasma leptin levels are also likely to underlie the changes in sweet taste threshold and sensitivity (Nakamura et al., 2008; Ninomiya et al., 2002; Shimizu et al., 2003).

Leptin, the product of the obese (*ob*) gene, is a hormone predominantly released from adipose tissue. It plays an important role in regulating food intake and body weight by binding to Ob-Rb in the hypothalamus (Friedman and Halaas, 1998; Tartaglia et al., 1995; Zhang et al., 1994). Consistent with previous investigations (Ahima et al., 1996; Ahren et al., 1997; Guerre-Millo, 1997; Lin et al., 1998; Madiehe et al., 2000), the results from the present study also showed that HF rats gained more body weight and had higher plasma leptin levels and that CR rats had lower serum leptin levels compared with the control rats. One recent study in humans has revealed that an increase in sweet recognition threshold is closely related to the enhancement of circulating leptin (Nakamura et al., 2008).

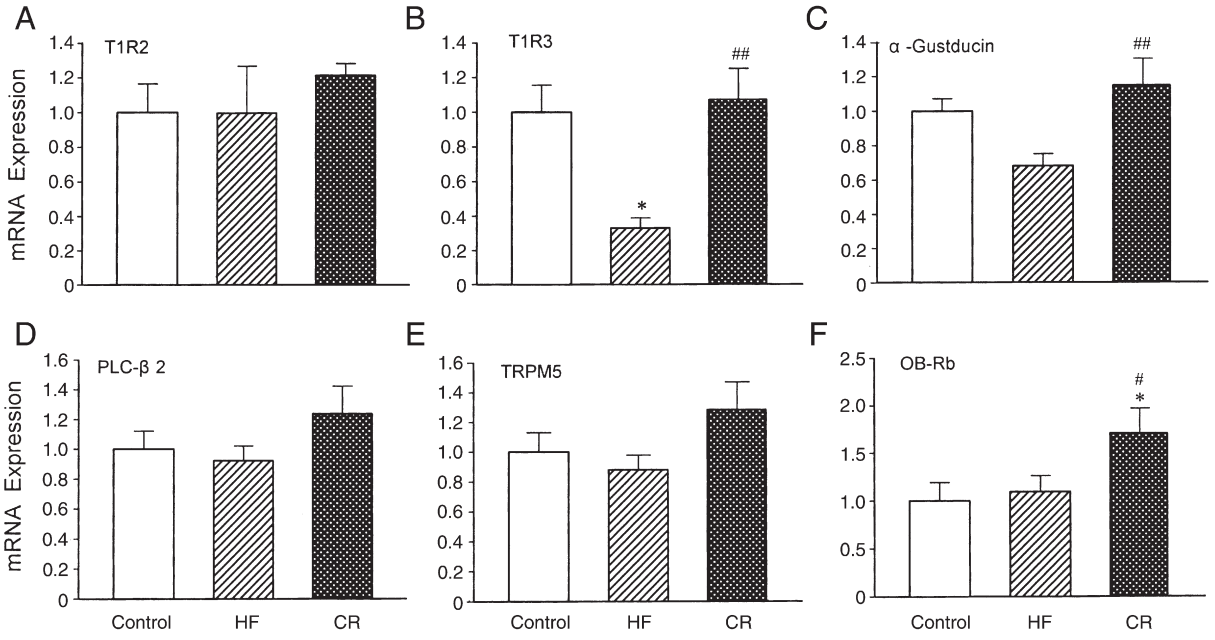

Fig. 4 – Gene expression of sweet taste receptors and related downstream elements in taste buds of rats under different nutritional states. Gene expression of T1R2, α-gustducin, PLC-β2, and TRPM5 was not significantly altered in HF or CR rats. *T1R3* gene expression was decreased by HF diet. *OB-Rb* gene expression was markedly increased by chronic diet restriction. $^{*}P<0.05$, compared with control rats. $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared with HF rats. Abbreviations: CR, chronically restricted diet rats; HF, high-fat diet-induced obese rats; OB-Rb, full-length "b" isoform of the leptin receptor; PLC-β2, phospholipase C-β2; TRPM5, a cation channel member of the transient receptor potential super family, subfamily M, member 5.

Another investigation indicated that the increased serum leptin affects the gustatory mechanisms in the diabetic and obese conditions (Ninomiya et al., 2002). It is suggested that the altered circulating leptin level caused by the different diets in the present study may be related to the altered sweet sensation of rats under different nutritional conditions.

Table 2 – Composition of the standard chow diet and high-fat diet.

Nutritive ingredient	High fat, g (%)	Standard chow diet, g (%)
Dried skim milk	1.5	2.0
Soybean meal	30.0	20.0
Lard	15.0	0
Yolk powder	10.0	0
Wheat bran	10.0	19.0
Corn starch	11.5	38.0
Fish meal	7.5	10.0
Lucerne powder	3.2	3.0
Yeast powder	2.0	1.0
Zymoprotein	4.2	3.0
Salt	0.5	0.5
Calcium phosphate	2.0	1.6
Calcium Carbonate	0.5	0.6
Choline Cl-70	0.1	0.1
Mineral Premix	1.0	0.6
Vitamin Premix	1.0	0.6
Fat energy, kcal/100 g (%)	892.0 (50.52)	161.4 (12.98)
Carbohydrate, kcal/100 g (%)	556.1 (31.50)	729.0 (58.62)
Protein, kcal/100 g (%)	317.7 (17.98)	353.2 (28.40)
Total energy, kcal/100 g	1765.8 (100)	1243.6 (100)

It is generally believed that Ob-Rb, the signal-transducing long form of the Ob-R, is not only expressed in the CNS (Couce et al., 1997; Hakansson et al., 1998) but also in taste cells (Kawai et al., 2000). In addition, the taste afferent nerve responses to saccharin were markedly suppressed and the electrophysiological responses of the taste cells were affected by the administration of leptin, suggesting that the taste organ is also a target for leptin (Kawai et al., 2000). Shigemura et al. found that leptin inhibited the behavioral responses to sweeteners, partially through its binding to OB-Rb on the taste cells (Shigemura et al., 2004). It was also supported by previous results that the Ob-Rb-deficient *db/ db* mouse displayed enhanced neural responses and elevated preference ratio to sweet tastants (Ninomiya et al., 1995, 1998).

A series of reports have indicated that the expression of Ob-Rb mRNA in the CNS is sensitive to changes in the circulating levels of leptin and fasting (Baskin et al., 1998; Bennett et al., 1998; Lin and Huang, 1997). Some late investigations also reported that the chronic ingestion of HF-diet affects the expression of Ob-Rb in white adipose tissue, liver, and hypothalamus in rats (Liu et al., 2007; Priego et al., 2009; Wrann et al., 2010). In the present study, we found that the mRNA expression of OB-Rb in taste receptor cells was increased, but the serum leptin level was decreased in CR rats. These findings might result in the reduced binding of leptin to OB-Rb in the taste buds. Therefore, it seems reasonable that the increased intake and preference for

saccharin solutions in CR rats may, at least in part, be attributed to the attenuation of the depressive effect of leptin on sweetener intake.

It is known that the transduction of sweet taste signaling begins by the binding of the specific structure of sweeteners to heterodimeric T1R2/T1R3 (Damak et al., 2003; Lindemann, 2001; Nelson et al., 2001; Zhao et al., 2003), which activates, at least in part, second messenger cascades, PLC-β2 via the coupled taste-specific G-protein, gustducin, formed by α-gustducin (Glendinning et al., 2005; Ruiz-Avila et al., 2001; Wong et al., 1996), Gβ1 and Gγ13 (Huang et al., 1999), and stimulates calcium release (Bernhardt et al., 1996) . In the GPCR–gustducin–PLC-β2 pathway, TRPM5 depolarizes the taste receptor cells and leads to neurotransmitter release (Perez et al., 2002; Zhang et al., 2003). Accordingly, α-gustducin, PLC-β2, and TRPM5 are key proteins involved in the sweet taste transduction pathway.

Except for coexpressing with T1R2 (T1R2+3, a sweet receptor), T1R3 also combines with T1R1 to function as a broadly tuned L-amino acid receptor (Nelson et al., 2002). Sweet and amino acid taste, which trigger the behavioral attraction, share a common receptor pathway and evolutional origin. T1Rs superfamily and the specific downstream elements are not only expressed in taste buds but also in the gastrointestinal tract (Bezencon et al., 2007; Hofer et al., 1996; Mace et al., 2007) and forebrain regions, which are responsible for nutrient sensing (Ren et al., 2009), where those molecules play an important role as a sugar sensor in regulating the energy homeostasis of the organism.

Interestingly, Ren and colleagues revealed that the expression of the sweet taste receptor and α-gustducin in the hypothalamus was regulated by the nutritional status of the organism based on in vivo studies in mice (Ren et al., 2009). The expression of T1R2 in the hypothalamus increased significantly after 24-h food deprivation and decreased significantly in obese hyperglycemic *ob/ob* mice. Interestingly, in the present investigation, we obtained similar results in taste buds.

Many studies in vitro and in vivo have proven that heteromerization of T1R2 and T1R3 is essential to create a fully functional sweet taste receptor (Li et al., 2002; Nelson et al., 2001; Nie et al., 2005; Zhao et al., 2003), and it is more efficacious for T1R3 to interact with sweeteners than T1R2 (Nie et al., 2005). Damak et al. also reported that the ability to sense sugars was closely related to the sweet taste receptor T1R3 (Damak et al., 2003). T1R3, the product of *Sac* gene (Bachmanov et al., 2001a; Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Nelson et al., 2001), accounts for up to 80% of variation in sweet taste preference of mice (Reed et al., 2004). One polymorphism in *Sac* gene significantly associated with saccharin sensitivity (Reed et al., 2004), and it perturbs the sweet receptor function by affecting the binding of T1R3 to sweet ligands or eliminating receptor function by preventing receptor dimerization (Max et al., 2001; Reed et al., 2004). Homomeric T1R3 receptors might be weakly activated by sweeteners and function as a low-affinity sweet receptor (Zhao et al., 2003). The reduced affinity of T1R3 for sugars is significantly correlated with reduced sweet taste sensitivity in mice (Inoue et al., 2004; Reed et al., 2004). Consistent with these results, the present study revealed reduced gene expression of T1R3 in taste buds and decreased saccharin intake and preference ratios in HF rats. It is likely that the decreased behavioral responses are partially mediated by the reduced expression of T1R3.

Because of the important role of T1R3 in detecting not only sweet but also umami taste, it is reasonable to infer that the altered expression of T1R3 in taste buds induced by high-fat diet may cause the change of umami taste response. The behavioral data and analysis of the nerve recording obtained from *t1r3* KO mice indicated that T1R3 is the primary or even only taste receptor in the detecting artificial sweeteners (Damak et al., 2003), while more than one subtype of receptor involves in the umami response (Delay et al., 2009; Maruyama et al., 2006). It seems that T1R3 is more important in detecting saccharin than umami.

α-Gustducin is a heterotrimeric guanine nucleotide-binding protein (G protein) expressed in taste receptor cells and is coupled with GPCRs (McLaughlin et al., 1992). Previous studies showed that α-gustducin-knockout mice were less responsive to sweet compounds, suggesting that α-gustducin plays an important role in sweet taste transduction. The significant differences in the gene expression of α-gustducin in taste buds between the HF rats and CR rats might be one reason for the differences in saccharin intake in these rats. The signal transduction components PLC-β2 and the TRPM5 ion channel are essential for sweet taste detection. PLC-β2- or TRPM5-knockout mice were unresponsive to sugars (Zhang et al., 2003). TRPM5-knockout mice showed greatly reduced responses in two-bottle preference tests and greatly diminished the CT nerve responses to sweetener (Damak et al., 2006; Liman, 2007). Our data showed a tendency for decreased gene expression of PLC-β2 and TRPM5 in HF rats and increased expression in CR rats, although these differences were not statistically significant.

Sweet taste preferences, beginning with gustatory receptors in the mouth, involve the integration of gustatory, olfactory, and vision information and modulation by hormonal and metabolic state. It is essential for the organism to alter the taste preference according to the nutritional status. One interesting topic for future investigation is the possibility that the nutritional status alters the sensitivities and detect threshold of other sweet tastants and the CT nerve response induced by different sweeteners. It is also an intriguing question how ingestion of sweeteners affects the co-expression of T1R2/T1R3 and Fos protein in hypothalamus of rats in different nutritional status.

The relationship between sweet taste preference and metabolic status (HF and CR) is far from being fully understood because of the complex interactions among genetic, biological, and psychological factors. Possible changes in the periphery that account for the behavioral alterations were analyzed in this study. Nutritional status has pronounced effects on sweet taste perception and may modulate the intake and preference for saccharin solutions by altering taste signaling at the initial processing, the receptor level. It seems that the changes in sweet tastant consumption in relation to nutritional status may be caused not only by changes in the expression of T1R3 but also by changes in serum leptin levels and Ob-Rb expression levels in taste cells. The present study indicates that chronic changes in nutritional status may affect the

intake and preference for saccharin solutions through complicated mechanisms that involve changes in the mRNA expression in taste buds.

4. Experimental procedures

The entire experiment was conducted in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC), for the use and care of animals in research. All efforts were made to minimize the number of animals used and limit the distress of the animals.

4.1. *Animals*

Male Sprague–Dawley rats (provided by the Medical Experimental Animal Center of Shaanxi Province, China) with an initial weight of 60 g were caged individually and had ad libitum access to standard laboratory chow and tap water, unless otherwise stated. Room temperature was maintained at 21±2 °C and a 12:12-h light–dark cycle (lights on at 07:00 h and off at 19:00 h) was used.

After 1 week of habituation to the animal facility, animals (n=48) were randomly divided into three groups: (1) HF rats (n=16) given a high-fat/high-calorie diet (4.14 kcal/g); (2) CR rats (n=16) given a food ratio limited to 50% of the baseline daily food intake level (average standard chow diet intake for the previous 3 days); and (3) control rats (n=16) given ad libitum access to standard chow diet (2.86 kcal/g) and water. About 50.52% and 12.98% of the available energy were due to lipids in the HF and the control diets, respectively. The two diets were formulated in the laboratory, and their exact composition is indicated in Table 2. The dietary regimens were maintained for 6 weeks. Food intake and body weight were recorded daily at 9:00 AM throughout the study.

4.2. *Two-bottle preference test*

Half of the rats in each group were used in the two-bottle choice (saccharin vs. water) test after 6 weeks of feeding. Two 25-ml graduated bottles were presented on the front of the cages, 3 cm apart, and were fitted with metal drinking spouts. For the first 7 days, the rats were exposed to two bottles, which both contained water, for 1 h in the morning (9:30–10:30 h) and 1 h in the afternoon (16:00–17:00 h). After the rats were acclimatized to the experimental situation, one bottle was filled with saccharin (Sigma Chemical Co., St. Louis, MO) solutions and the other with tap water. The saccharin solutions were prepared at concentrations of 0.001, 0.002, 0.005, 0.01, 0.02, and 0.04 M using tap water. The tastant solutions were presented at ascending concentrations, and each saccharin concentration was presented for 1 day. All rats maintained standard chow diet throughout the behavioral test.

The position of the two bottles was switched between tests to prevent position preference. Saccharin and water consumption was measured every afternoon by weighing the bottles before and after each test. The preference ratio was calculated as the ratio of tastant consumed to the total liquid consumed.

4.3. *Serum total cholesterol, triglyceride, and leptin assays*

After 6 weeks of dietary feeding, the remaining animals were anesthetized with urethane (1.2 g/kg, ip) (10: 00–12: 00 AM). Five milliliters of blood was collected from the abdominal aorta, and serum was separated by centrifugation (1500 rpm for 10 min) and stored at –80 °C until analysis. Serum TC and TG were measured by the CHOD-PAP and GPO-PAP methods, respectively (cholesterol and triglyceride kit, Beijing Puerweiye BioTech Co. Ltd., China).

The serum leptin concentration was measured by radioimmunoassay (RIA) system (rat leptin RIA kit, Linco Research Inc., St Charles, Missouri). The sensitivity of the leptin RIA kits was 2 μIU/ml. The intra- and the interassay coefficients of variation are <5% and <10% for the leptin kit, respectively. All samples were within the liner detection range and were analyzed in duplicate.

4.4. *Isolation of taste cells*

After the tongue was excised under deep anesthesia, the rats were then killed by cervical dislocation. The entire taste buds were isolated from the circumvallate papilla (CV) by injecting 2 mg/ml elastase and 2 mg/ml dispase in mammalian physiological saline (in mM: 120 NaCl, 20 KCl, 10 HEPES, and 2 BAPTA, pH 7.4) between the epithelium and the muscle layers of the tongue. The taste buds were collected using a suction pipette and immediately placed into a 1.5-ml microcentrifuge tube containing 100 μl of TRIzol reagent (Invitrogen, CA) for reverse transcriptase (RT)-PCR experiments performed in accordance with previously reported methods (Shen et al., 2005).

4.5. *Real-time quantitative PCR*

RT-PCR experiments were performed on total RNA isolated from taste buds using the PureLink Micro-to-Midi Total RNA Purification System (Invitrogen, San Diego, CA, USA). After assaying the RNA concentration using NanoDrop (Thermo Fisher Scientific, Wilmington, DE, USA), total RNA was reverse transcribed into cDNA using a commercial RT-PCR Kit according to the manufacturer's instructions (TaKaRa, Cat. No. DRR037A, Japan). We added 1 μg of total RNA, 0.5 μl of random 6-mers (50 μM), 0.5 μl of PrimerScript RT Enzyme Mix I, 0.5 μl of Oligo dT Primer (50 μM) in a total volume of 10 μl of RNase-free water to a sterile RNase-free microcentrifuge tube . We heated the tube to 37 °C for 15 min, followed by 85 °C for 5 s. Reactions were also set up in which the reverse transcriptase enzyme was omitted as a control to detect genomic DNA contamination.

After first-strand synthesis, 2 μl of cDNA was added to 25 μl of the PCR mixture comprising 2 μl of the RT reaction product, 12.5 μl of 2× SYBR premix Ex Taq, 0.5 μl of each forward and reverse primer, and 9.5 μl dH_2O. The primers and accession numbers of the genes studied are listed in Table 3. β-Actin mRNA was used as an internal control to normalize the amount of input RNA. The thermal cycling parameters were 1 cycle at 95 °C for 10 s followed by 40–45 cycles at 95 °C for 5 s, and 58–60 °C for 30 s and was performed on a thermal cycler (BIO-RAD PCR thermal cycler, iQ5, American).

Table 3 – Nucleotide sequences for the primers used in the real-time PCR assays.

Target gene	GenBank Accession No.	Primer sequence (5′–3′)	Orientation	Product size (bp)
β-actin	NM_031004	5′-CTATCGGCAATGAGCGGTTCC -3′	Forward	156
		5′-TGTGTTGGCATAGAGGTCTTTACG -3′	Reverse	
T1R2	XM_001074791	5′-TCCGCCATTACCGTGTCCAAC-3′	Forward	204
		5′-ACCAGCACCACAATCCAGTTCC-3′	Reverse	
T1R3	NM_130818	5′-GTGGCAGAGCCCTACACCTGTACT-3′	Forward	203
		5′-TGCCTTGCAGTCCACACAGTC-3′	Reverse	
α-gustducin	NM_173139	5′-TGTGCCAAATGAACAAGACGTTCT-3′	Forward	129
		5′-CTGATCTCTGGCCACCTACATCAA-3′	Reverse	
PLCβ-2	NM_053478	5′-GCGGATCCACCAAGACATGA-3′	Forward	158
		5′-CAACGGCAGCCAGATAGCAG-3′	Reverse	
TRPM5	XM_344979	5′-AGTTTCAACGCTACCACCTCATC-3′	Forward	155
		5′-GCTGTTTGTGCTGGGCTTC-3′	Reverse	
OB-Rb	NM_012596	5′- AGCAGTCCAGCCTACACTCTTG -3′	Forward	177
		5′- ACCACATACCTCCTCACACTACAC -3′	Reverse	

All quantitative PCR assays were performed in triplicate, and a minimum of three independent experiments were conducted. The amplified sequences were visualized by electrophoresis in 3% agarose gels. The mRNA expression levels are expressed as the *n*-fold difference relative to the mRNA expression in control rats.

4.6. *Statistical analysis*

All data are presented as means ± standard error of the mean. The formula used to calculate the normalized expression of quantitative PCR target genes was as follows:

$$\text{Normalized expression} = (E_{\text{target}})^{\Delta\text{Ct target (control}-\text{sample)}} \div (E_{\text{ref}})^{\Delta\text{Ct ref (control}-\text{sample)}},$$

in which E_{target} represents the expression of the target gene; E_{ref} represents the expression of the reference gene; ΔCt, the difference between the experimental group and the control group; and Ct, the cross threshold. Significant differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by post hoc multiple comparisons. $P<0.05$ was considered to be statistically significant. Statistical analyses were performed using Statistical Program for Social Sciences statistical software (SPSS 13.0; SPSS Inc., Chicago, IL, USA).

Acknowledgments

The authors wish to thank Dr. J.S. Tang for valuable comments on this manuscript and Professor Scott Herness and Zhao FL for technical advice. This work was supported by the National Natural Science Foundation of China (No. 30970973).

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