Decreased expression of CD36 in circumvallate taste buds of high-fat diet induced obese rats

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\textbf{A B S T R A C T}

Mammals spontaneously prefer lipid rich foods. Overconsumption of high-fat diet leads to obesity and related diseases. Recent findings indicate that taste may participate in the orosensory perception of dietary lipids and the fatty taste may contribute to a preference for and excessive consumption of dietary fat. CD36, a trans-membrane glycoprotein, which is located in the taste buds of circumvallate papillae of rodents, appears to be a plausible receptor for this fatty taste. Obese subjects present a stronger preference for fatty foods, though the mechanisms involved are complex and not fully investigated. Our data from immunofluorescence and real-time RT-PCR showed that the expression levels of CD36 in circumvallate taste buds were significantly lower in high-fat diet induced obese rats as compared with that of control rats fed a normal diet. These results suggest that decreased expression of CD36 in circumvallate taste buds of high-fat diet induced obese rats may be associated with diminished fatty taste sensitivity and in order to compensate the preference for dietary fat, rats consume more fatty foods. Therapeutic strategies designed to alter or manipulate CD36 expression or function in taste buds may have important implications in treating obesity and related diseases.

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\textbf{Introduction}

The sense of taste informs the body about the quality of ingested food. Sweet, sour, salty, bitter, and umami are recognized as the basic taste stimuli. Recently, compelling evidence suggests the existence of a sixth primary taste modality, the “fatty taste”. This may contribute to a spontaneous preference for and overconsumption of fatty foods (for reviews see: Khan and Besnard, 2009; Passilly-Degrace et al., 2009) that leads to obesity (Takeda et al., 2001) and related diseases (Despres and Lemieux, 2006; Getz and Reardon, 2007).

CD36 is a trans-membrane glycoprotein that belongs to the class B scavenger. It is widely expressed in many cell types and has multiple functions (for reviews see: Febbraio et al., 2001; Silverstein and Febbraio, 2009). Recently, accumulating evidence has supported CD36 as a plausible taste receptor of dietary lipids in rodents (for reviews see: Abumrad, 2005; Calder and Deckelbaum, 2006). The lingual localization of CD36 in rats and mice was confirmed mostly in circumvallate taste buds, to a lesser extent in foliate papillae, and rarely in fungiform papillae (Laugerette et al., 2005). CD36 gene knock-out in mice abolished both fat preference and cephalic phase of digestion triggered by oral long-chain fatty acids (LCFAs) deposition and this loss of preference was thought to be lipid specific since wild-type and CD36-null mice exhibited a similar response for sucrose or quinine solutions (Laugerette et al., 2005). With addition to some molecular, behavioral and physiological studies (El-Yassimi et al., 2008; Gaillard et al., 2008; Saitou et al., 2009), it is easy to conclude in rodents that lingual CD36 acts like a lipid sensor, which binds LCFA in lipid rich foods and/or hydrolyzed by lingual lipase from dietary triglyceride, triggers specific taste signal transduction and contributes to fat preference.

Obese patients have a greater preference for fatty foods than lean ones (Drewnowski et al., 1985; Mela and Sacchetti, 1991). The mechanisms involved in this phenomenon are complex and are not fully demonstrated. Olfaction, taste and food texture may account for this phenomenon. Stewart et al. (2010) found that individuals who were defined as orally hyposensitive to C18:1 fatty acid with the gustatory cue, consumed more energy and fat and had higher body mass index values (BMIs) than those defined as hypersensitive ones. Diminished fatty taste sensitivity, in turn, may contribute to a faulty compensatory regulation resulting in increased preference for and intake of palatable high-fat meals, sustained overeating and body weight gain. Differences in oral taste receptors are known to influence individual taste sensitivity (Dinehart et al., 2006; Garcia-Bailo et al., 2009), thus alterations in fatty taste receptor may contribute to different levels of preference for fatty foods between obese and lean subjects. However, whether there is an expression

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change of CD36 (the plausible receptor of fatty taste) in circumvallate taste buds of high-fat diet induced obese rats is still unknown.

The current study is designed to compare the protein and mRNA expression levels of CD36 in circumvallate taste buds of obese rats induced by chronic high-fat diet with those of control rats fed normal diet and to try to explore the cause of this possible change in CD36 expression status and its significance in overconsumption of dietary fat and obesity.

### Materials and methods

#### Animals

Eight-week-old male Wistar rats, weighing 180–200 g, were obtained from the Center of Experimental Animals, Harbin Medical University (Harbin, China, Approval No. SCXX (HLJ) 2006-009) and randomly divided into two groups. One group \( n = 12 \) fed with high-fat diet \((4.36 \text{ kcal/g}) \) contained 15%, 40%, 45% calories as protein, fat and carbohydrate respectively, while the control group \( n = 12 \) fed with normal diet \((3.43 \text{ kcal/g}) \) contained 23%, 9% and 68% calories as protein, fat and carbohydrate respectively. The lard, which is mainly constituted of LCFAs, supplied the main fat component in the high-fat diet. The exact composition of the two diets is indicated in Table 1. Rats were housed individually in a vivarium maintained under controlled room temperature \((22 \pm 2 ^\circ \text{C}) \) humidity \((55 \pm 5\%) \) and with a 12:12 h light-dark cycle. Water and food were given ad libitum. Body weight was measured every week. All procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### Tissue preparation

After 8 weeks, animals were killed by an overdose of sodium pentobarbital given by intraperitoneal injection. The posterior parts of the tongue containing circumvallate papillae were immediately dissected. Samples used for molecular analysis were isolated using previously described methods \((Shen et al., 2005)\). In brief, by injecting 2 mg/ml dispase and 2 mg/ml elastase in mammalian physiological saline (in mM: 120 NaCl, 20 KCl, 2 BAPTA and 10 HEPES, pH 7.4) between the epithelium and the muscle layers of the tongue, lingual epithelium was separated from connective tissue and circumvallate taste buds were dissected under a microscope and rapidly used to isolate total RNA. Prior to sacrifice, final body weights were measured and levels of serum total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and glucose were analyzed by standard methods. Visceral fat (perinephric, epididymal and mesenteric fats) weights were also measured.

### Immunofluorescence

Excised circumvallate papillae were embedded in OCT medium (OCT, Jung, Nussloch, Germany) and quickly frozen in liquid nitrogen. The samples were cut into 6-μm-thick sections with a cryostat and fixed in 95% ethanol for 5 min then rehydrated in 0.01 M PBS (pH 7.4) for 10 min. Rehydrated sections were blocked in 5% goat serum (Boster Biotechnology Co., Wuhan, China) for 30 min at room temperature and then incubated overnight with 1:100 dilution of rabbit anti-rat CD36 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C. After several rinses in PBS, sections were incubated with FITC-conjugated goat anti-rabbit IgG (1:100, Santa Cruz Biotechnology) for 1 h at 37 °C. Finally, sections were covered-slipped using an anti-fading glycerol-based mounting medium. Immunostained sections were observed under a fluorescence microscope (BX51; Olympus, Tokyo, Japan). Staining specificity was assessed by treating slices in the absence of primary antibodies.

### Real-time reverse transcription polymerase chain reaction (real-time RT-PCR)

Total RNA from circumvallate taste buds was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. After assaying the RNA concentration using NanoDrop (Thermo Fisher Scientific, Wilmington, DE, USA), cDNA was synthesized by 150 ng total RNA with oligo-(dT)\(_{18}\) primers (KangChen Bio-tech Inc., Shanghai, China) and SuperScript™ III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). The sequences of primers specific for CD36 (sense: 5′-GCTGTCACAGCCTTATC-3′, anti-sense: 5′-TTATGGCAACCTGTGATAT-3′) \((product \text{ size } 214 bp)\) and GAPDH (sense: 5′-GGAAGCCTGTGCGCGTAT-3′, antisense: 5′-AAGGTGGAAGATGGGATT-3′) \((product \text{ size } 308 bp)\) were designed by Primer 5.0 and synthesized by Sangon biotech (Shanghai, China).

The PCR reaction mixture \((25 \mu l)\) contained of 0.25× Sybrgreen (Invitrogen, Carlsbad, CA, USA), 2.5 μl dNTPs \((2.5 \text{ mM each})\) (HyTest Ltd, Turku, Finland), 2.5 μl 10× PCR buffer (Promega Biotech Co, Madison, WI, USA), 1 unit Taq DNA polymerase (Promega), 1.5 μl MgCl\(_2\) \((25 \text{ mM})\) (Promega), 1 μl of forward and reverse primers \((10 \text{ μM})\), 1 μl of cDNA template and water. The reactions were run on Rotor-Gene 3000 (Corbett Research, Sydney, Australia) using the following program: 95 °C for 5 min, 35 cycles of 95 °C for 10 s, 59 °C for 15 s and 72 °C for 20 s. Data was analyzed using the two-standard curve method and GAPDH was used as an internal control to normalize the amount of input RNA. The mRNA expression levels are expressed as the \(n\)-fold difference relative to the mRNA expression in control rats.

α-Gustducin, which is considered as a specific molecular marker for taste bud cells and plays an important role in taste transduction of bitter, sweet and umami, but not fatty taste (Scalfani et al., 2007), was also assayed to assess the purity of papillae samples. The sequences of primers specific for α-Gustducin were: 5′-ATGAGGGTGAGATGTTGAT3′ (sense) and 5′-CTTAATTGAGGCGGATGAC-3′ (anti-sense) \((product \text{ size } 253 bp)\) and were also synthesized by Sangon Biotech (Shanghai, China). The PCR mixture and amplification program for α-Gustducin were the same as CD36 and GAPDH.

The amplified sequences were visualized by electrophoresis in 2% agarose gels.

### Statistical analyses

SPSS 13.0 software (SPSS Inc., IBM, Chicago, IL, USA) was used to conduct statistical analyses. Results were expressed as mean ± SEM. Body weight gain was analyzed by repeated measure
Table 2
Comparison of final body weight, visceral fat weight and plasma parameters of lipid metabolism and glucose in the two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Final body weight (g)</th>
<th>Visceral fat weight (g)</th>
<th>Total cholesterol (mmol/l)</th>
<th>Triglycerides (mmol/l)</th>
<th>HDL-C (mmol/l)</th>
<th>Serum glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>356.75 ± 6.65**</td>
<td>9.71 ± 0.71**</td>
<td>2.21 ± 0.31**</td>
<td>1.14 ± 0.07**</td>
<td>0.62 ± 0.04</td>
<td>5.26 ± 0.32</td>
</tr>
<tr>
<td>Control</td>
<td>261.58 ± 7.13</td>
<td>2.62 ± 0.23</td>
<td>1.13 ± 0.06</td>
<td>0.59 ± 0.06</td>
<td>0.63 ± 0.04</td>
<td>5.18 ± 0.31</td>
</tr>
</tbody>
</table>

HF, high-fat diet induced obese rats.

** P<0.05 vs. control group.

Results

High-fat diet induced obesity and hyperlipidemia in rats

After 8 weeks fed with high-fat diet, rats developed obesity that attained significantly higher final body weight (20%) relative to the control group (356.75 ± 6.65 g vs. 261.58 ± 7.13 g, P<0.01) (Table 2) and terms to be high-fat diet induced obese rats (HF). Moreover, the weights of visceral fat were significantly heavier (9.71 ± 0.71 g vs. 2.62 ± 0.23 g, P<0.01) (Table 2). The body weight gain in HF rats increased more rapidly than that of the control rats (F(1, 22) = 102.69, P<0.001) (Fig. 1). In addition, high levels of total plasma cholesterol and triglycerides were also detected in HF rats, while HDL-C and plasma glucose showed no significant difference between the two groups (Table 2).

CD36 immunopositive cells in circumvallate taste buds

In accordance with a previous study (Laugerette et al., 2005), immunoreactivity for CD36 was found with more intense labeling at the apical side of circumvallate taste cells in both groups (Fig. 2). By counting cells, we found that the number of CD36 immunopositive taste cells in HF rats decreased significantly as compared with that in the control rats (2.23 ± 0.07 vs. 3.66 ± 0.09 per taste bud, n = 6 each, P<0.01) (Fig. 2). Cell count was conducted according to the protocol described by Laugerette et al. (2005). In brief, 120 taste buds were randomly selected in circumvallate papillae of 6 rats and the CD36 immunopositive taste cells were counted in each taste bud. No immunoreactivity was observed in the absence of primary antibody.

mRNA expression of CD36 and α-Gustducin in circumvallate taste buds

In rat taste buds of circumvallate papillae, PCR products of expected sizes were observed by electrophoresis in 2% agarose gels (Fig. 3). In agreement with the immunofluorescence study, real-time RT-PCR detected mRNA expression levels of CD36 in circumvallate taste buds of obese rats induced by chronic high-fat diet was markedly lower (about 0.4 fold) as compared with that of the control rats (n = 6 each, P<0.01) (Fig. 4). No significant difference

Fig. 1. Body weight gain of the two groups. Body weight was measured every week and body weight gain was calculated. Rats fed with high-fat diet exhibited a significant increase in body weight gain (expressed as increase value to initial body weight). Symbols and error bars represent the mean ± SEM. n = 12 each. F(1, 22) = 102.69, P<0.001 vs. control group. HF, high-fat diet induced obese rats.

Fig. 2. CD36 immunolocalization in circumvallate taste buds of HF and control rats. CD36 immuno-positive taste cells were less expressed in circumvallate papillae of HF rat (A) than control rat (B). No immunoreactivity was observed in the negative control (C). Scale bar = 20 μm.

Fig. 3. Bands of expected sizes of PCR products visualized by electrophoresis in 2% agarose gels. The PCR products were visualized on a 2% agarose gel stained with ethidium bromide. Bands corresponding to the expected sizes of GAPDH (308 bp), CD36 (214 bp) and α-Gustducin (253 bp) were observed. M: 100 bp marker, 1: GAPDH, 2: CD36, 3: α-Gustducin.

Fig. 4. CD36 mRNA expression in circumvallate taste buds. The mRNA expression of CD36 was markedly lower in HF rats (A) than control rats (B), with about 0.4 fold as compared with that of the control rats (n = 6 each, P<0.01) (Fig. 4). No significant difference
in α-Gustducin mRNA expression was observed between the two groups (data not shown). Data were repeated twice and similar results were obtained.

Discussion

Both animals (Tsuruta et al., 1999; Takeda et al., 2001) and human beings (Drewnowski, 1997) are attracted to dietary fat. The palatability of fatty foods leads to excessive consumption of high-fat diet that contributes to obesity (Takeda et al., 2001) and related diseases (Despres and Lemieux, 2006; Getz and Reardon, 2007). Our study confirmed that long-term ingestion of a high-fat diet resulted in obesity in rats. Final body weight and visceral fat weight of HF rats were significantly higher than those of the controls and the body weight gain increased more rapidly. Moreover, these rats showed high levels of serum total cholesterol and triglycerides, though HDL-C and plasma glucose demonstrated no significant difference between HF rats and the control rats. Collectively, these data well demonstrated the phenotype of obesity induced by high-fat diet in rats.

Obese subjects appear to prefer lipid rich foods much more than lean ones (Drewnowski et al., 1985; Mela and Sacchetti, 1991) although the mechanisms involved are complex and are not fully investigated. Considering the possible existence of the fatty taste that may contribute to fat preference (for reviews see: Khan and Besnard, 2009; Passilly-Degrace et al., 2009), alterations in taste sensitivity to fatty acids may account for this phenomenon. Indeed, a recent study has defined 54 subjects as hypo- or hyper-sensitive to oral fatty acid with no additional orosensory cues such as olfaction, irritation nor texture but taste (Stewart et al., 2010). Individuals who were defined as orally hyposensitive to C18:1 fatty acid, consumed more energy and fat, were weaker at detecting small differences in custard containing varying amounts of fat and had higher BMIs than those defined as hypersensitive ones (Stewart et al., 2010). Additionally, animals that were hyposensitive to oral fatty acids consumed more dietary fat and developed obesity, while hypersensitive animals consumed less fat and were resisted to body weight gain when exposed to a high-fat diet (Gilbertson et al., 1998). Thus, diminished fatty acid taste sensitivity in obese subjects may be correlated with greater preference for and excessive consumption of fatty foods.

Differences in oral taste receptors are known to influence individual taste sensitivity, preference and intake of specific food and food component (Dinehart et al., 2006; Garcia-Bailo et al., 2009). The expression and function of taste receptors are influenced by both genetic contributions and environmental factors including exposure and consumption of specific nutrients (Garcia-Bailo et al., 2009; Huang and Stahler, 2009) and the expression level of taste receptors may have positive relationship with specific taste sensitivity (Lin et al., 1999). The present study first reported that both protein and mRNA expression levels of CD36 were down-regulated in circumvallate taste buds of high-fat diet induced obese rats compared with controls, which was somewhat in agreement with the previous study (Chen et al., 2010), that showed high-fat diet induced obese rats exhibited decreased mRNA expression of sweet taste receptor, diminished preference for saccharin and consumed less of this sweet component. However, as the T1R2 + T1R3 heterodimer responses to sweet component, only T1R3 mRNA was observed decreased in HF rats while T1R2 stayed the same in the study by Chen et al. (2010). It is hard to explain how this change in sweet taste receptor may affect the body weight or the nutritional status for the relations between sweet taste perception and body weight are contradictory (for reviews see: Bartoshuk et al., 2006; Donaldson et al., 2009), nevertheless, as far as we are aware, fat preference or fat taste perception has positive correlation with obesity. Avena et al. (2009) suggest that fat may be the macronutrient that results in excess body weight while sweet taste may be largely responsible for producing addictive-like behavior.

Down-regulation of CD36 in circumvallate taste buds of obese rats may be associated with decreased fatty taste sensitivity, raising the possibility that more fat needs to be consumed to evoke a comparable oral taste response. Takeda et al. (2001) found that obese rats induced by chronic high-fat diet did have significant and constant larger amount of fat intake during the whole diet weeks. However, further researches are needed to investigate why this down-regulation of CD36 occurs. In general, we may make an explanation using the phenomenon known as habituation, which has been reported for numerous orally detected compounds (Zhao and Henness, 2009). For example, increased dietary salt reduced oral Na"+ sensitivity and augmented the preference level of Na"+ in common foods, while acute and chronic Na"+ restriction and deplere result in the reverse phenomenon (Bertino et al., 1986; Beauchamp et al., 1990). We hypothesize that a similar relationship may exist within fat intake and oral fatty taste sensitivity, whereby oral exposure to a diet rich in lipids would be associated with diminished oral fatty acid sensitivity. This is greatly in accordance with our present results that showed decreased expression of CD36 in circumvallate taste buds of obese rats induced by long-term high-fat diet while the specific molecular marker of taste cells α-Gustducin, which also plays an important role in taste transduction of sweet, bitter and umami, but not fatty taste (Sclafani et al., 2007) stayed the same. Indeed, a relationship between habitual fat consumption and the preference for fatty foods has recently been demonstrated in human beings (Ledikwe et al., 2007).

In conclusion, high-fat diet induced obese rats represented decreased expression levels of CD36 in circumvallate taste buds that may be associated with diminished fatty taste sensitivity and in order to compensate the high fat preference, rats consumed more fatty foods that lead to sustained body weight gain. Therapeutic strategies designed to alter or manipulate CD36 expression or function in taste buds may have important implications in treating obesity and related diseases.

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