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High Fat Diet Differentially Regulates the Expression of Olfactory Receptors in the Duodenum of Obesity-Prone and Obesity-Resistant Rats

Stefany D. Primeaux,

Joint Diabetes, Endocrinology and Metabolism Program, Louisiana State University System, Louisiana State University Health Science Center-New Orleans (LSUHSC-NO), New Orleans, LA 70112, USA. Internal Medicine-Endocrinology, Diabetes and Metabolism, Louisiana State University System, Louisiana State University Health Science Center-New Orleans (LSUHSC-NO), 1542 Tulane Ave, Box T4 M-2, New Orleans, LA 70112, USA

H. Douglas Braymer, and

Pennington Biomedical Research Center, Baton Rouge LA 70808, USA

George A. Bray

Pennington Biomedical Research Center, Baton Rouge LA 70808, USA

Stefany D. Primeaux: sprime@lsuhsc.edu

Abstract

Background—The gastrointestinal tract is important in the regulation of food intake, nutrient sensing and nutrient absorption. Obesity-prone Osborne-Mendel (OM) rats are less sensitive to the satiating effects of a duodenal infusion of fatty acids than obesity-resistant S5B/Pl (S5B) rats, suggesting that the gastrointestinal tract differentially senses the presence of fat in these two strains. A microarray analysis was conducted to identify genes that were differentially expressed in the duodenal enterocytes of OM and S5B rats.

Aims—The present experiment evaluated the expression of olfactory receptors in the duodenal enterocytes of OM and S5B rats. It was hypothesized that olfactory receptors present in the duodenum of OM and S5B rats would be differentially regulated by the intake of a high fat diet.

Methods—The mRNA levels of four olfactory receptors (Olr1744, Olr50, Olr124, Olr1507) were assessed from the duodenal enterocytes of OM and S5B rats consuming a high fat diet for 14 days.

Results—The duodenal mRNA levels of Olr1744, Olr124 and Olr1507 were significantly elevated in OM rats fed the high fat diet, but not S5B rats. No differences in the expression of Olr50 receptor mRNA were detected.

Conclusions—These data suggest that several olfactory receptors present in the duodenum are selectively regulated by high fat diet intake in obesity-prone OM rats. Therefore, these receptors

may play a role in the sensing and regulation of dietary fat, and may be important for the individual susceptibility to obesity in these two strains.

Keywords

Obesity-prone; Obesity-resistant; Olfactory receptors; Duodenum; High fat diet

The role of the gastrointestinal tract (GI) in the detection and absorption of nutrients is an increasingly important component for understanding the intake of foods high in fat and the subsequent prevalence of obesity. Following ingestion of nutrients, the release of hormones and the activation of receptors alter the physiology of the GI tract and affect further ingestion of nutrients. Evidence from animal models of diet-induced obesity suggests that the detection of dietary fat by the GI tract is subject to individual differences in susceptibility to obesity. Multiple studies have investigated differential sensing of dietary fat by the GI tract of obesity-prone Osborne-Mendel (OM) and obesity-resistant S5B/Pl (S5B) rats [1–4]. Duodenal infusions of Intralipid (a highly emulsified triglyceride mixture) and sodium linoleate on sham-fed animals suppressed food intake more completely and for a longer time in S5B rats compared to OM rats [1]. Recently, we reported a diminished ability of the GLP-1 agonist, Exendin 4, to reduce high fat diet (HFD) intake in OM rats, compared to S5B rats [4]. These data demonstrated strain differences in the ability of the GI tract to respond to the presence of dietary fat and suggested that the S5B rats were more sensitive to dietary fat than OM rats.

To further investigate the differences in the GI tract of OM and S5B rats, a microarray analysis was conducted from the duodenal enterocytes of OM and S5B rats fed a HFD or a low fat diet (LFD). The results from this microarray analysis established strain differences in response to HFD in genes related to immunity, lipid and fatty acid metabolism, signal transduction and olfaction (see Fig. 1; Table 1) [5]. Though numerous genes were altered in these two rat strains by HFD intake, one gene, *Olr1744*, was particularly intriguing. The expression of a large number of olfactory receptors contributes to the complexity of the olfactory system's ability to detect and discriminate odorous compounds [6]. Recently, these receptors have been detected in the GI tract [7, 8].

Several studies have reported differential protein expression in adipose tissue, liver, and skeletal muscle of obesity-prone and -resistant rats and suggest that these proteins play a role the susceptibility to obesity [9–11]. The purpose of the present experiment was to assess the expression of olfactory receptors in the duodenum of OM and S5B rats consuming a HFD or a LFD. Based on our microarray analysis, it was hypothesized that *Olr1744* would be increased by the consumption of HFD in OM rats, but not S5B rats. It was further hypothesized that the expression of several additional olfactory receptors in the duodenum would be differentially affected by the intake of a HFD in OM and S5B rats.

Male OM and S5B rats (8–9 weeks old) were bred in the AAALAC approved Pennington Biomedical Research Center vivarium and housed on a 12/12 h light/dark cycle with food and water available ad libitum. All procedures were approved by the Pennington Center Institutional Animal Care and Use Committee. Animals were given access to a pelleted high fat diet (55 % kcal from fat) or pelleted low fat diet (10 % kcal from fat) for 2 weeks prior to

sacrifice [4]. Food intake was measured daily and body weight was measured weekly. For real-time PCR, a 2.5-cm section of the duodenum was removed, cleaned and the enterocytes were removed by gentle scraping with a clean metal spatula. Excised duodenal enterocytes were immediately frozen on dry ice and stored at -80°C until further processing.

RNA was isolated from duodenal enterocytes using Tri-Reagent (Molecular Research Ctr, Cincinnati, OH) and RNeasy Minikit procedures (Qiagen, Valencia, CA) and based on previous experiments [12]. A microarray analysis on these samples was conducted by the Pennington Center Genomics Core (see Fig. 1; Table 1). Rigorous requirements for the microarray analysis (p value of less than 0.05 and fold-change of greater than 4) led to the discovery of a significant alteration in the olfactory receptor, Olf1744, in these strains. The application of slightly less stringent criteria revealed potential diet differences in Olf1507, Olf50, and Olf124. Biological function for these genes was based on the Panther Classification System (www.pantherdb.org).

Reverse transcription was conducted using the High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA) and based on previous studies [4]. Primers were designed using Primer Express (Applied Biosystems) to assess and verify differences in these four olfactory receptors—Olf1744: For-5'-TCATCTTCTCCTTGCCCTTCTG-3', Rev-5'-AGCACCGGCTGGATCTCA-3'; Olf50: For-5'-TTCTATCCTCCTCCACACAACCTAA-3', Rev-5'-AGGCAGGCATTGTATTCAATCTC-3'; Olf1507: For-5'-TCATCTTCTCCTTGCCCTTCTG-3', Rev-5'-AGCACCGGCTGGATCTCA-3'; Olf124: For-5'-GCCTTGGGCACTTGTGTTG-3', Rev-5'-GCGATGAGTTAGGAACTGAACAAT-3'; Cyclophilin: For-5'-CCCACCGTGTTCTTCGACAT-3', Rev-5'-CTGTCTTTGGAACCTTGTCTGCAA-3'. For real-time PCR, SYBR Green 2× Master Mix (Applied Biosystems), primers and cDNA were added to 384-well plates. The quantity of Olf1744, Olf1507, Olf50, and Olf124 was based on a standard curve and normalized to cyclophilin levels (ABI Prism 7900 Sequence Detection System, Applied Biosystems). A significance level of $p < 0.05$ was used for all tests.

Following 14 days access to the HFD or LFD, a significant strain $\times$ diet interaction was detected for body weight gain ($F = 20.28$, $p < 0.05$, data not shown). As expected, obesity-prone OM rats gained more weight than S5B rats, and OM rats consuming HFD gained more than OM rats consuming LFD. A significant diet $\times$ strain interaction was detected for average daily food intake of HFD and LFD ($F = 17.36$, $p < 0.05$, data not shown). Overall, the rats consumed more HFD than LFD, and OM rats consumed more HFD than the S5B rats. A significant diet $\times$ strain interaction was detected for Olf1744 ($F = 5.63$, $p < 0.05$), Olf1507 ($F = 5.52$, $p < 0.05$) and Olf124 ($F = 15.47$, $p < 0.05$) (see Fig. 2a–c). Differences were not detected for Olf50 (see Fig. 2d). Post hoc analyses revealed a significant increase in the duodenal expression of Olf1744, Olf1507 and Olf124 in the duodenal enterocytes of OM rats consuming a HFD, compared to OM rats consuming a LFD and S5B consuming either a HFD or a LFD ($p < 0.05$).

OM and S5B rats are models used to assess the mechanisms underlying feeding behavior and obesity [1–4, 13, 14]. OM rats are susceptible to diet-induced obesity and gain more

weight and body fat when eating a HFD than S5B rats, which are resistant to diet-induced obesity. The purpose of the present experiment was to examine HFD-induced differences in the expression of several olfactory receptors in the duodenal enterocytes of OM and S5B rats. Based on a microarray analysis, it was hypothesized that Olf1744 would be increased by the consumption of HFD in OM rats, but not S5B rats. Several additional olfactory receptors were investigated.

The criteria to detect gene expression differences in this microarray analysis were rigorous and led to the detection of six genes down-regulated by HFD in OM rats, ten genes down-regulated by HFD in S5B rats, ten genes up-regulated by HFD in OM rats and five genes up-regulated by HFD in S5B rats (see Table 1; Fig. 1). An effect of diet was detected in the olfactory receptor, Olf1744 [5]. The present analysis was expanded to include olfactory receptors which showed differential expression but did not reach statistical significance ($p > 0.05$). Expression of Olf1744, Olf1507, and Olf124 mRNA in the duodenal enterocytes was significantly elevated in the obesity-prone OM rats consuming a HFD (See Fig. 2). However, expression of Olf50 mRNA in the duodenal enterocytes was not altered by strain or diet, suggesting that only specific olfactory receptors are modulated by the intake of a HFD.

Previous investigations examining the potential function of olfactory receptors in the GI tract revealed a role for these receptors in the release of serotonin from the GI tract [7, 8]. The expression of olfactory receptors in the human GI tract has been evaluated and activation of olfactory receptors in these cells has been linked to increased influx and increased intracellular levels of Ca^{++} and increased release of serotonin [7, 8]. Serotonin controls gut motility and secretion, enteric nervous activity and has been implicated in pathologic conditions such as irritable bowel syndrome, eating disorders, vomiting and diarrhea [7, 8, 15–22]. Serotonin receptor type 3 appears to play a prominent role in GI-related pathology. In people, variants in the serotonin receptor types 3A and 3B have been associated with anorexia nervosa and bulimia nervosa [19, 22]. Additionally, serotonin receptor type 3 antagonists are used to treat chemotherapy-induced nausea and vomiting and irritable bowel syndrome [21, 22].

In the OM and S5B rats, the differential expression of olfactory receptors in the duodenal enterocytes by HFD may affect the release of serotonin in the GI tract. Earlier studies from this laboratory have investigated the role of serotonin on the intake of HFD in OM and S5B rats [23, 24] and demonstrated strain differences in the response to serotonergic drugs. Overall these data report a significant increase in mRNA levels of several olfactory receptors in the duodenum of obesity-prone OM rats fed a HFD. Olfactory receptors are chemoreceptors and though their exact role in the GI tract is not understood, it is apparent from these data that these receptors are susceptible to diet manipulations. More studies are needed to examine the function of these olfactory receptors in the detection of fat/fatty acids and their potential modulation of serotonin and GI motility in these animal models.

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Fig. 1.

A microarray analysis was conducted on duodenal enterocytes Osborne-Mendel (OM) and S5B/Pl (S5B) rats fed a high fat diet (HFD) or low fat diet (LFD) for 14 days. Biological function for these genes was based on the Panther Classification System. **a** Genes down-regulated by HFD in obesity-prone OM rats. **b** Genes up-regulated by HFD in obesity-prone OM rats. **c** Genes down-regulated by HFD in obesity-resistant S5B rats. **d** Genes up-regulated by HFD in obesity-resistant rats. $p < 0.05$ and fold-change greater than 4

Fig. 2.

Olfactory receptor mRNA levels were measured in the duodenal enterocytes of Osborne-Mendel (OM) and S5B/Pl (S5B) rats consuming a high fat diet (HFD) or a low fat diet (LFD) for 14 days. **a** The olfactory receptor, OLR1744, mRNA was increased in the duodenal enterocytes following the consumption of the HFD in obesity-prone OM rats. **b** The olfactory receptor, OLR1507, mRNA was increased in the duodenal enterocytes of OM rats consuming the HFD. **c** The olfactory receptor, OLR124, mRNA was increased in the enterocytes of obesity-prone OM rats consuming a high fat diet. **d** The olfactory receptor, OLR50, mRNA was not altered by the consumption of a high fat diet in OM or S5B rats. Data are shown as mean $\pm$ SEM, $*p < 0.05$, ($n = 6-9$ rats/group)

Table 1

Genes altered by the consumption of a high fat diet in obesity-prone and obesity-resistant rats

Gene	Gene ID ^a	Fold change	p Value
<i>Down-regulated by high fat diet in OM rats (LFD/HFD)</i>			
Lipid, fatty acid metabolism			
Cytochrome P450, subfamily 1A	Cyp1a1	10.52	<i>p</i> = 0.048
Nitrogen metabolism			
Nitric oxide synthase	Nos	4.53	<i>p</i> = 0.001
Nucleotide metabolism			
Homeobox protein distal-less DLX	Dlx3	4.09	<i>p</i> = 0.026
<i>Up-regulated by high fat diet in OM rats (HFD/LFD)</i>			
DNA metabolism			
A Helicase Reco5	Recq15	4.0	<i>p</i> = 0.004
Immunity and defense			
Fibrinogen alpha/alpha E chain	Fga	4.98	<i>p</i> = 0.002
Lipid, fatty acid metabolism			
Apolipoprotein C-II	Apoc2	5.59	<i>p</i> = 0.034
Olfactory receptors			
Olfactory receptor MOR263	Olr1744	5.10	<i>p</i> = 0.013
Signal transduction			
Angiopoietine-1	Angpt1	4.01	<i>p</i> = 0.019
<i>Down-regulated by high fat diet in S5B rats (LFD/HFD)</i>			
Calcium mediated signaling			
Calcium binding protein	L-CaBP2	4.89	<i>p</i> = 0.001
Carbohydrate metabolism			
Solute carrier family 2	Slc2a5	8.21	<i>p</i> = 0.0004
Receptors			
Acetylcholine receptor, nicotinic β 1	Chrb1	6.49	<i>p</i> = 0.012
P2Y purinoreceptor 12	P2y12	5.24	<i>p</i> = 0.019
Latrophilin 3	Lphn3	4.82	<i>p</i> = 0.044
Endocytosis			
Vacuolar membrane protein	Vps11	4.02	<i>p</i> = 0.036
Signaling molecule			
DAPP1/BAM32	Dapp1	4.49	<i>p</i> = 0.035
<i>Up-regulated by high fat diet in S5B rats (HFD/LFD)</i>			
Lipid, fatty acid metabolism			
2,4 dienoyl-CoA reductase	Decr1	6.29	<i>p</i> = 0.35
Signal transduction			
RAS homolog gene family, member V	Rhov	4.21	<i>p</i> = 0.036

^a Gene IDs and classifications from the Panther Classification System (www.pantherdb.org)