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Toll-like Receptor 4 Mediates Fat, Sugar and Umami Taste Preference, and Food Intake and Body Weight Regulation

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Abstract

Objective—Immune and inflammatory pathways play important roles in the pathogenesis of metabolic disorders. In the present study we investigate the role of TLR4 in orosensory detection of dietary lipids and sugars.

Methods—Taste preferences of TLR4 knockout (KO) and wild type (WT) male mice under standard, and high fat and high sugar diets were assessed with 2-bottle tests. Gene expression of taste signaling molecules was analyzed in the tongue epithelium The role of TLR4 in food intake, and weigh gain was investigated in TLR4 KO and WT mice fed a high fat and high sugar diet for 12 weeks.

Results—Compared to WT mice TLR4 KO mice showed reduced preference for lipids, sugars, and umami in 2-bottle preference test. The altered taste perception was associated with decreased levels of key taste regulatory molecules in the tongue epithelium. TLR4 KO mice on a high fat and high sugar diet consumed less food and drink, resulting in diminished weight gain.

Conclusions—TLR4 signaling promotes ingestion of sugar and fat by a mechanism involving increased preference for such obesogenic foods.

Keywords

TLR4; taste; obesity; dietary sugar; high-fat diet; TRPM5

Introduction

The gustatory system allows animals to discriminate among foods in order to select nutritious diets and maintain energy balance. Although a broad range of economic, social and behavioral factors influences food choices in humans, the immediate pleasantness generated by taste is still for most individuals the driving force behind food consumption. The hedonic response to high caloric sugars and fats, and consequent over-consumption of

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such foods, likely plays a role in the increasing prevalence of obesity world wide (1). However, the cellular and molecular mechanisms underlying such eating behavior are largely unknown.

Toll-like receptors (TLRs) recognize structurally conserved molecular patterns expressed in pathogens triggering inflammatory and immune responses. Since the discovery that obese, type 2 diabetic, and metabolic syndrome subjects have increased levels of TLR4 expression in various tissues, many studies have been conducted to elucidate TLR4 functions in the metabolic consequences of diet-induced obesity (2). Interestingly, while most studies of TLR4 have focused on its role in macrophages and other immune system cells, recent findings have demonstrated that TLR4 is expressed in neurons where, in the absence of pathogens, it plays important roles in regulating the development and plasticity of neuronal circuits (3). Notably, hypothalamic activation of TLR4 signaling by fatty acids has been shown to influence anorexigenic signals (4). It has also been shown that multiple TLRs are expressed in the tongue gustatory papillae where they may initiate immune responses to pathogens (5). In this study, we investigated the possibility that TLR4 could be involved in taste perception and regulation of food preference and intake.

Methods

Animals and diets

Adult male TLR4−/− (TLR4 KO) and TLR4+/+ wild type (WT) mice (25–30 g) were kept in a 12 hour light/12 hour dark cycle on a standard diet (Harlan Teklad 2018). For the diet-induced obesity model, mice of each genotype were randomly assigned to a control diet (diet #101845; Dyets Inc., Bethlehem, PA) with water, or a high fat diet (diet #101842; Dyets Inc.) with water containing 12% sterile fructose. The macronutrient composition of the different diets utilized in the study is shown in Table 1. This research was approved by the National Institute on Aging Animal Care and Use Committee and was performed according to guidelines in the NIH Guide for the Care and Use of Laboratory Animals.

RNA extraction and real-time PCR

The tongue epithelium containing foliate and circumvallate papillae was dissociated from the underlying muscle, snap frozen and stored at −80°C. RNA was isolated using Trizol (Invitrogen) and purified with an RNA Micro Kit (Qiagen, Valencia, CA). Following treatment with DNase I, RNA was quantified and equal amounts were reverse-transcribed using the SuperScript First Strand Synthesis System (Invitrogen Life Technologies). Real-time PCR analysis was performed with a PTC 200 Pelthier Thermo Cycler and Chromo 4 Fluorescent Detector (BioRad, Hercules, CA), and Sybr® Green PCR Master Mix according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). The comparative Ct method was used to determine the normalized changes of the target gene relative to a calibrator reference (β-actin).

Two-bottle preference test

The test was performed in the home cage of individually housed mice given free access to food. Two sipper bottles (modified 15 mL polypropylene conical tubes with a sipper tube),

containing water were provided for the first 2 days to habituate the mice to the two bottles. After the mice were acclimatized to the experimental setting, they were presented with a bottle containing a tastant solution and a bottle containing water plus the proper vehicle for two days. The position of the bottles was switched daily to preclude potential position bias. A three day interval with plain water was given between tastant tests. The order of taste tests was: water, saccharin (2 mM), fructose (12%), quinine (0.1–1 mM), denatonium benzoate (3 mM), citric acid (10 mM), inosine-5′-monophosphate (10 mM), calcium chloride (75 mM), sodium chloride (300 mM), linoleic acid (0.1–2% versus 0.3% xanthan gum to minimize textural cues) and capsaicin (0.1%). To eliminate the potential effect of body weight on solution intake preference ratios, we calculated the ratio of 48 hours tastant intake over total liquid (water + tastant) consumption, were utilized.

Cell culture, lipid uptake and colocalization experiments

HEK 293 cells (ATCC) were cultured in Dulbecco's modified Eagle's medium containing 10% Hyclone Fetalclone III and 1% penicillin-streptomycin at 37°C in a humidified 5% CO_2 atmosphere. Based on the experimental end point cells plated on coverslips were transfected with either vector, pcDNA3-TLR4-YFP (gift from Doug Golenbock, Adgene plasmid #13018) or a mixture 1:1 of pcDNA3-TLR4-YFP and pcDNA3-CD36-Flag (gift from Nada Abumrad) using Fugene 6. After 24 hours cells were incubated with fresh complete media containing 1 μM 4,4-difluoro-5,7-diphenyl-4- bora-3a,4a-diazo-s-indacene-3-dodecanoid acid (Bodipy #3832) for lipid uptake experiments, or 100 μM linoleic acid (C18:2) for colocalization experiments. At different time points following treatment the cells were washed with phosphate buffered saline and fixed with 4% paraformaldehyde. CD36 expression was detected by immunocytochemistry using an anti Flag M2 antibody (Sigma). DNA was counterstained with DAPI. Z-stack photomicrographs were acquired on a Zeiss LSM 510 confocal using a 40x objective. Bodipy uptake was measured in 3D reconstructed images using Fiji. Data are expressed as total cell fluorescence (TCF).

Statistical analysis

Statistical analysis was performed by Student's *t*-test or analysis of variance (two-way ANOVA) as appropriate for the experimental design using Graph Pad Prism 6.0. The results are expressed as mean + SEM. Values of $p<0.05$ were considered statistically significant.

Results

TLR is expressed in the tongue epithelium

To study the impact of TLR4 signaling on taste perception we first analyzed the expression of several different TLRs in WT and TLR4 KO mice maintained on a standard diet by real time PCR. In agreement with previous reports (5) we found that cells in the tongue sensory epithelium of WT mice express TLRs 2, 3, 4 and 6 (Figure 1A), and two accessory proteins implicated in TLR4 signal transduction, cluster of differentiation 14 (CD14) and myeloid differentiation primary response gene 88 (MyD88). Compared to WT, TLR4 KO mice did not express TLR4 (Figure 1A, $P<0.001$, unpaired *t*-test) and had comparable levels of mRNAs encoding TLRs 2, 3 and 6, CD14 and MyD88 (Figure 1A, $P >0.05$, unpaired *t*-test).

Next we determined the expression levels of taste receptors and signaling molecules involved in taste transduction. The levels of mRNAs encoding taste receptor type 1 member 1 (T1R1), 2 (T1R2) and 3 (T1R3), that modulate the perception of sweet (T1R2+T1R3), and umami (T1R1+T1R3), as well as the capsaicin channel transient receptor potential cation channel subfamily V member 1 (TRPV1) were comparable between WT and TLR4 KO mice (Figure 1B, P >0.05, unpaired *t*-test). While the level of the mRNA encoding the fat taste receptor G-protein coupled receptor 120 (GRP120) was unchanged (Figure 1B, P=0.96, unpaired *t*-test), we surprisingly found a significant decrease in the expression of cluster of differentiation 36 (CD36) in TLR4 KO mice (Figure 1B, P <0.001, unpaired *t*-test). No changes were observed for the G-coupled proteins α-gustducin (αGus) and transducin (Gnat) (Figure 1B, P >0.05, unpaired *t*-test), but levels of mRNAs encoding the transient receptor potential cation subfamily member 5 (TRPM5) (Figure 1B, P<0.001, unpaired *t*-test), and the G protein-dependent phospholipase C β2 (PLCβ2) (Figure 1B, P <0.001, unpaired *t*-test) were also significantly decreased in TLR4 KO mice compared to WT mice. Overall, these results indicate that TLR4 deficiency is associated with altered taste signaling transducers expression.

TLR4 KO mice are less responsive to sweet, umami, spicy and fat tastants

To determine the taste preferences of TLR4 KO mice we performed a 48 hour two-bottle test. Naive animals were allowed to acclimate to single housing in cages with two sipper bottles for two days, and were then tested for the various tastants following the order stated in the Methods section. All mice had unrestricted access to standard diet (carbohydrate 44.2%; fat, 6.2%; proteins, 18.6%). Preference scores showed that TLR4 KO mice consumed less artificial (saccharine, P=0.01, unpaired *t*-test) and natural (fructose, P=0.044, unpaired *t*-test) sweet, as well as umami (IMP, P=0.04, unpaired *t*-test), and fat (LA, P=0.05, unpaired *t*-test) solutions (Figure 2). Notably TLR4 KO mice were also less sensitive to capsaicin (Cap, P=0.001, unpaired *t*-test). No difference between the genotypes was observed for calcium, bitter, salty, and sour tastes (data not shown).

TLR4-dependent altered orosensory perception influences diet-induced obesity

The reduced preference for sweet and fat molecules shown by the TLR4 KO mice suggested that TLR4 may impact the propensity of mice to consume high amounts of obesogenic diets rich in fat and simple sugars. We therefore tested high fat diet palatability in WT and TLR4 KO naive mice. Similarly to the two-bottle taste preference experiment, singly housed mice were given access to two hoppers containing a known amount of control diet (carbohydrate, 64.7%; fat, 7%; proteins, 20%) or high fat diet (carbohydrate, 47.6%; fat, 27.1%; proteins, 20%) and water. The position of the hoppers was changed after 24 hours to account for position bias. The amount of food consumed was measured after 48 hours and a preference score for the high fat diet was calculated as grams of high fat diet over total food consumed. Not surprisingly, we found that WT mice displayed a preference for the high fat diet (Figure 3A), with about 43% of the mice exclusively consuming the high fat food. TLR4 KO mice showed a reduced preference for the high fat diet with none of the animals exclusively partaking on it (Figure 3A, P=0.01, unpaired *t*-test). We next tested how the observed changes in orosensory perception influenced weigh gain in WT and TLR4 KO mice fed with a high fat high sugar diet. Mice of both genotypes were randomly assigned to either a

control diet supplemented with water or a high fat diet supplemented with 12% fructose (average sugar content in sodas) and their weight, food and liquid intake were monitored over a three month period. In mice on the control diet, there were no significant differences between the genotypes in the average food (Figure 3C) ($P>0.05$, two-way ANOVA) and water (Figure 3D) ($P>0.05$ two-way ANOVA) consumption, with identical caloric intake and weight gain at the end of the study (Figure 3B – E). In contrast, compared to WT mice TLR4 KO mice consumed less high fat diet ($P<0.05$, two-way ANOVA), and fructose solution ($P< 0.05$, two-way ANOVA) throughout the study (Figure 3C, D), resulting in an overall diminished caloric intake (Figure 3E, $P< 0.01$ two way ANOVA) and ultimately in weight gain (Figure 3B).

Influence of TLR4 on high fat diet-induced taste preferences

A growing body of evidence suggests that obesity modifies taste sensitivity (6). We tested if TLR4 signaling influences diet-induced taste changes subjecting WT and TLR4 KO mice to a two-bottle preference test in mice using the same control diet and HFD they had consumed for 12 weeks. Similarly to what we observed for naive mice on the standard diet, TLR4 KO mice on the control diet, showed decreased saccharin ($P=0.039$, two-way ANOVA), fructose ($P=0.037$, two-way ANOVA) and IMP ($P=0.005$, two-way ANOVA) preference scores compared to WT mice (Figure 4A). Consumption of HFD significantly decreased the preference scores for sweet (Saccharin, $P=0.039$; Fructose, $P=0.044$, two-way ANOVA), and umami ($P<0.0001$, two-way ANOVA) in WT mice (Figure 4A), but had no effect in TLR4 KO mice ($P>0.05$, two-way ANOVA). We also performed a dose-response experiment using increasing concentrations of linoleic acid (LA). Compared to mice kept on a standard diet all mice showed higher preferences scores for LA (Compare Figure 2 and Figure 4B). Multiple exposures to increasing concentrations of LA resulted in increased preference scores in WT mice on the control diet but not in those on the HFD (Figure 4B, $P=0.01$, two-way ANOVA). TLR4 KO mice showed lower preference for LA regardless of the diet regimen (Figure 4B). The analysis of the relative expression of taste receptors and transducers in the tongue epithelium revealed decreases in levels of mRNAs encoding CD36 ($P=0.041$, two-way ANOVA), TRMP5 ($P=0.027$, two-way ANOVA) and PLCβ2 ($P=0.002$, two-way ANOVA) in TLR4 KO mice on the control diet compared to WT mice, similar to the mice fed a standard NIH diet (Figure 4C). Surprisingly, a significant reduction of T1R2 ($p<0.001$, two-way ANOVA) expression was also found in TLR4 KO mice on the control diet compared to WT animals (Figure 4C). HFD consumption resulted in a decrease in levels of mRNAs encoding T1R2 ($p=0.005$, two-way ANOVA) and TRPM5 ($P=0.035$, two-way ANOVA) in WT but not in TLR4 KO mice (Figure 4C).

TLR4 mediates lipid uptake, and colocalizes with CD36 during linoleic acid cellular internalization

Fatty acids have been shown to activate the TLR4 signaling cascade trough the leucine rich repeat region in the extracellular domain which allows the association with hydrophobic ligands (7,8). CD36 and TLR4 cooperation is important for the recognition and internalization of bacteria (9), as well as oxidized lipoproteins (10). We tested the possibility that TLR4 could mediate lipid cellular uptake using the TLR- deficient cell line HEK 293. As shown in figure 5A the expression of TLR4-YFP increased about 3.4 fold the cellular

uptake of the fluorescent fatty acid analog (C12) compared to vector transfected cells ($P<0.001$ unpaired *t*-test). Qualitative differences in the localization patterns were also observed. In vector transfected cells, within 30 min of treatment the fluorescent C12 appeared to have a diffused distribution with few punctate perinuclear structures (Figure 5A). However, more defined lipid droplets colocalizing with TLR4 were observed in the TLR4-YFP transfected cells (Figure 5A). We next assessed the possibility that TLR4 and CD36 may cooperate in lipid internalization transfecting HEK 293 with both TLR4-YFP and CD36-Flag expressing plasmids, and incubating the cells with linoleic acid (C18:2). In untreated cells TLR4-YFP and CD36 had a diffuse distribution with minimal colocalization (Figure 5B). In cells expressing both proteins, within 15 min of incubation with linoleic acid, increased colocalization of both receptors in perinuclear areas was seen (Figure 5B). The absence of similar clustering in cells expressing only CD36 (Figure 5B) suggests that TLR4 expression provides the cells with additional mechanisms to mediate lipid uptake. Overall our results indicate that TLR4 can mediate the internalization of saturated fatty acids, and of unsaturated fatty acids in presence of CD36.

Discussion

Under conditions where a choice of food is possible, hedonic signals will override homeostatic physiological needs (11). Similarly, to drugs of abuse, palatable foods activate reward-learning regions and dopamine signaling (12), leading to the possibility that overeating and obesity could be the result of food addiction. Given the huge social and economic burden of obesity-related co-morbidities, it is crucial to improve our understanding of the mechanisms underlying pleasure and aversion to food. Since food ingestion represents a major route of pathogen entry, it is not surprising that many components of immune and inflammatory signaling pathways are expressed in the tongue epithelium (5, 13). Interestingly, in recent years functions for innate immune molecules unrelated with immuno-surveillance have begun to emerge. For example, the pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) (13), and the anti-inflammatory cytokine interleukin-10 (14) are secreted in gustatory buds, and TNFα knockout mice are less sensitive to bitter compounds (15). Conversely, elements of the taste transduction system are expressed in tissues of the body not traditionally associated with nutrient sensing. For example, the bitter receptor T2R5 and its entire taste transduction cascade are expressed in testis, where they regulate spermatogenesis (16). With regards to obesity, the role of TLR4 in the establishment of insulin resistance and in the metabolic consequences of diet-induced obesity is well characterized (17). To date, however, no study has analyzed the potential impact of TLR4 signaling in orosensory perception. Here we show that TLR4 KO mice display reduced preference for sweet, fat and savory tastants, and decreased intake of high fat and sugar diet and, ultimately, reduced weight gain.

The changes in tastant and food preferences of the TLR4 KO mice were associated with decreased expression of the key taste molecules CD36, PLC2β and TRPM5 in tongue epithelium. Our findings are consistent with previous reports showing that the production of the cytokine TNFα in the tongue epithelium is TLR4-dependent, and limited to a subset of gustatory cells that express T1R3 receptors (umami and sweet) and PLC2β (13). Although the functional consequences of a partial decrease in PLC2β and TRPM5 are unknown, their

complete ablation in mice results in loss of sweet, umami, fat and bitter taste responses (18–20). Substantiating the importance of palatability in feeding behavior, TRPM5 KO mice have attenuated weight gain when fed high caloric carbohydrates or fat diets as a result of reduced overeating (21, 22). In addition to changes in sweet, fat and savory perception we also found that TLR4 KO are less responsive to capsaicin. In sensory neurons TLR4 signaling enhances TRPV1 activity and calcium fluxes, thus pain sensitivity and itching (23, 24). It is possible that, at least partially, the changes in taste perception we observed in TLR4 KO mice are the result of diminished calcium responses via TRPV1 (i.e. capsaicin) or TRPM5 (i.e. sweet, umami, fat). CD36 can bind a variety of saturated and unsaturated long chain (C13-C21) fatty acids (25). It has been shown that both complete and partial ablation of CD36 abolishes fat preference in mice (26, 27), independently of post-ingestive mechanisms (28). A direct relationship between oral levels of CD36 and sensitivity to fat has also been observed in human subjects (29). In addition to taste modulation, CD36 heterodimerization with TLR4 is involved in lipopolysaccharide (LPS) recognition, as well as in the endocytosis of bacteria (4), amyloid beta and oxidized lipoproteins (10). Although it is known that fatty acids can act as TLR4 agonists, most studies have focus on their ability to elicit inflammatory related mediators such as cytokines (4, 8, 30). Our *in vivo* and *in vitro* findings suggest that, similar to what is observed for other endogenous substrates, CD36 and TLR4 can interact to mediate the cellular uptake of fatty acids. It has been previously shown that lauric acid (C12) enhances the homodimerization and the recruitment of TLR4 in lipid rafts (31). Our results showing that TLR4 expression enhances fluorescent C12 uptake, corroborate the previous findings, and further suggest the possibility that TLR4 may directly contribute to fatty acid endocytosis. Evidence for a direct physical binding of saturated fatty acids to TLR4 is still lacking. In the absence of CD36, the increased lipid uptake can be explained by the presence of soluble CD14 and lipopolysaccharide-binding protein in the serum added to the culture media. In fact, both proteins have been shown to act as mobile carriers of triacylated lipopeptides or lipoproteins to TLRs (32). Similar to CD36, CD14 is also required for phospholipase C2 and IP_3- intracellular calcium-dependent internalization of TLR4 complexes (33). *In vivo*, either CD14 or CD36 could facilitate the assembly of TLR4/lipid complexes and their consequent internalization via caveolae-dependent mechanisms (34). Such internalization could also provide a negative feedback mechanism to reduce palatability of fats, and thus their ingestion, during a meal (35). When we analyzed tastant preference following long-term consumption of the high fat and fructose diet, WT, but not TLR4 KO mice on the HFD showed decreased sweet, umami and fat preferences, as well as reduced expression of TRPM5 and T1R2 compared to animals on the control diet. The decrease in TRPM5 will lead to diminished calcium responses and reduced sensitivity to the various food components. Our results in WT mice substantiate previous findings showing that obese rodents and humans have reduced oral detection of sweet and umami solutions (36, 37). Over consumption in obese animals would then be needed to stimulate lingual receptors and release sufficient dopamine to signify reward (38). Interestingly, altered TLR4 signaling has been found in brain areas involved in the natural reward circuitry implicated in dependence (39). The fact that TLR4 KO mice preferences were not altered by diet composition, nor by weight status, strongly suggests that the TLR4 signaling pathway is involved in both orosensory and post-ingestive food driven adaptive changes, and likely contributes to overeating behavior.

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Answers to Study Important Questions

- This is the first report showing that TLR4 signaling plays a role in spontaneous preference for simple sugars and fat
- TLR4-dependent diet induced obesity is, in part, due to altered taste perception

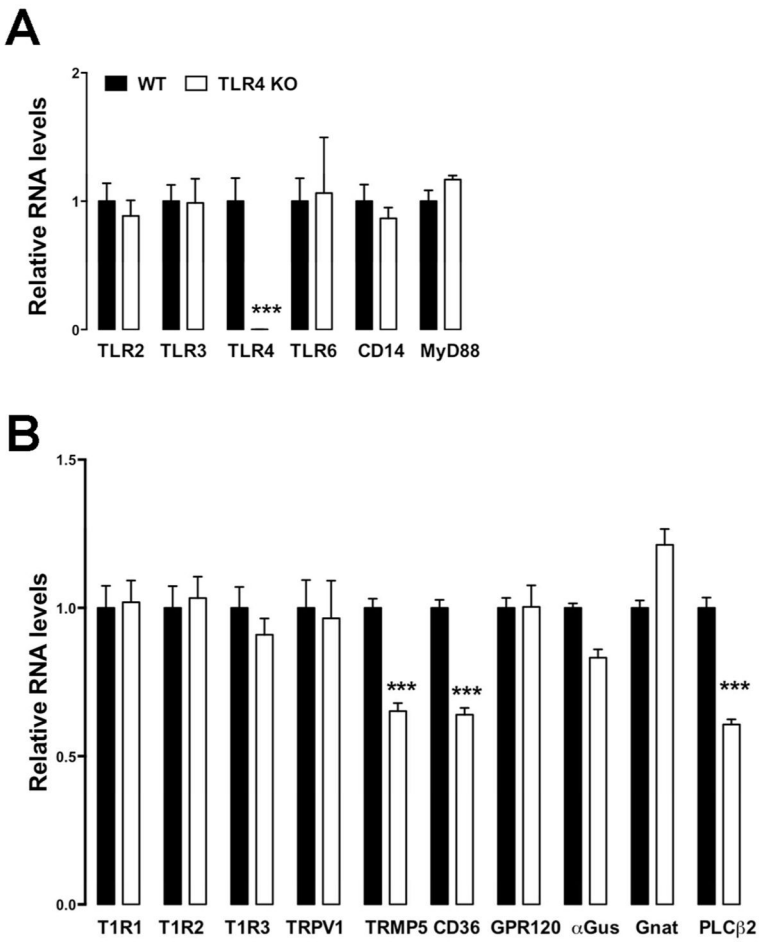

Figure 1.

Expression of Toll-like receptors and TLR4 downstream accessory proteins in the tongue gustatory epithelium. Real time PCR showing the relative levels of mRNAs encoding TLR-2, -3, -4, and -6, adaptor protein MyD88 and TLR4 lipopolysaccharide binding partner CD14 (**A**), and the indicated taste receptors and gustatory signaling transduction proteins (**B**) in WT and TLR4 KO mice. Data are mean and SEM. of 5–12 animals. ***$p \leq 0.001$ versus WT animals.

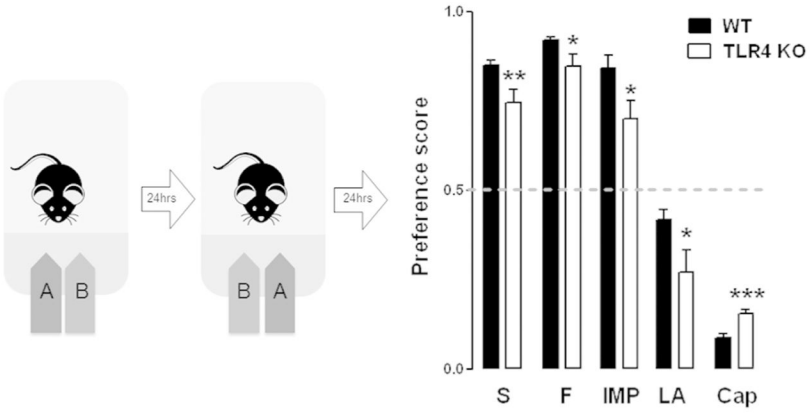

Figure 2.

TLR4 deficiency modifies sweet, umami, spicy and fat perception. Preference for various tastants was measured in WT and TLR4 KO mice during a 48 hour two-bottle preference test. The position of the bottles was changed after 24 hours to control for position bias. TLR4 KO mice displayed decreased avoidance of the spicy tastant capsaicin, and reduced preference for sweet, umami and fat tastants. S, saccharin 2 mM; F, fructose 12%; IMP, inosine -5′-monophosphate 10 mM; LA, linoleic acid 0.1%; Cap, capsaicin 0.1%. Data are mean and SEM. of 6–7 animals. *$p \leq 0.05$; **$p \leq 0.01$, ***$p \leq 0.01$ versus WT animals.

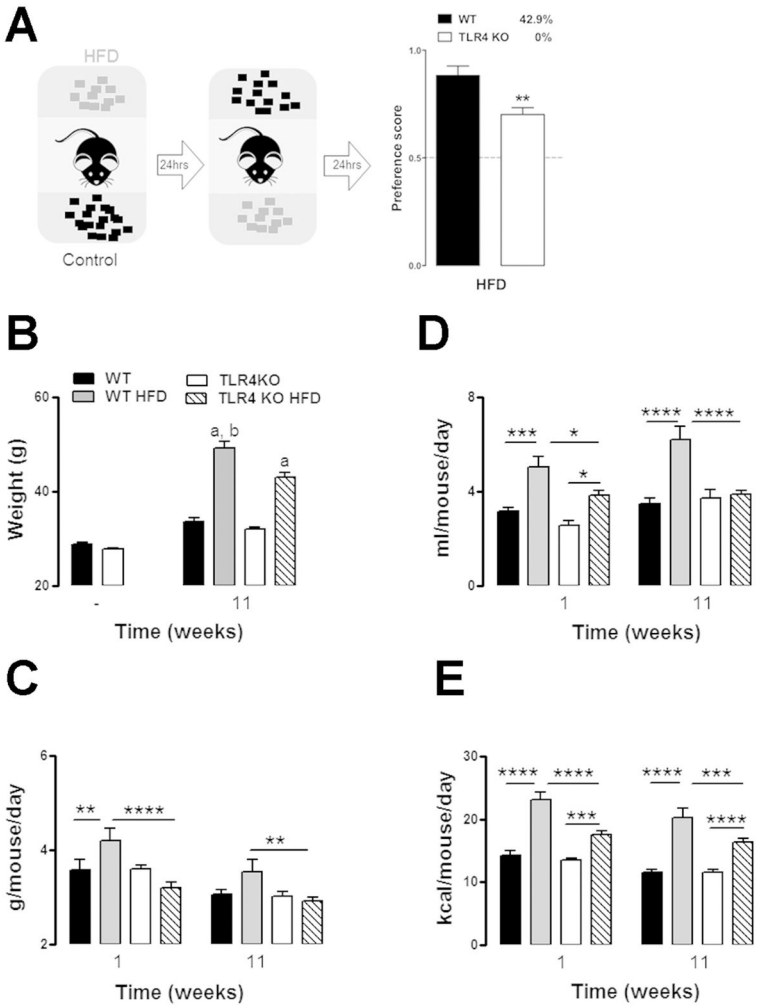

Figure 3.

TLR4 deficient mice display reduced preference for a high fat plus fructose diet, and are resistant to diet-induced obesity. **(A**) Naive WT and TLR4 KO mice were offered a choice between control and high fat diet (HFD). Compared to WT mice TLR4 KO mice showed decreased preference for the HFD. While 42.9% of the WT mice exclusively fed on the high fat diet, none (0%) of the TLR4 KO mice showed a similar behavior. Data are mean and SEM of 5–7 animals. ** $p \leq 0.01$ versus WT animals. WT and TLR4 KO mice were fed control or high fat diet supplemented with water or 12% fructose respectively, and their body weight (**B**), food intake (**C**), drink intake (**D),** and caloric intake (**E**) were monitored over a 12 weeks period. Values are mean and SEM of 17–19 animals per group. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001 versus TLRKO HFD; a, p≤0.001 versus control diet; b, p ≤0.001 versus TLR4 KO HFD.

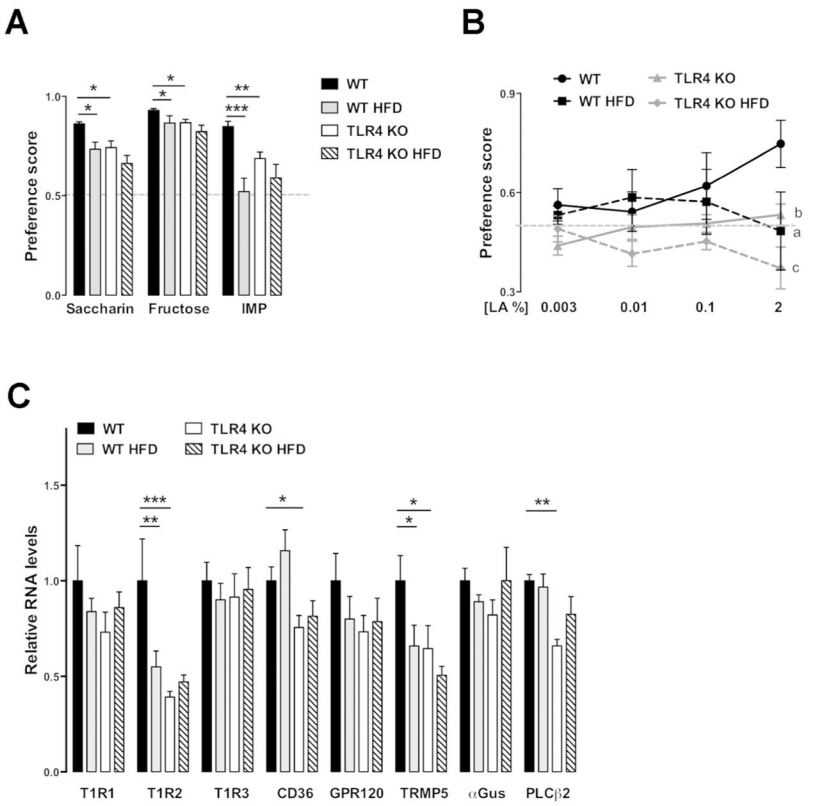

Figure 4.

Reduced preference for sweet and umami in obese mice is partially dependent on TLR4 signaling. Preference scores for the indicated tastants (**A**) and increasing concentrations of linoleic acid (LA) (**B**) in WT and TLR4 KO fed control or high fat plus fructose diet (HFD) for three months. Data are mean and SEM of 7–14 animals. (**C**) Relative levels of mRNAs encoding taste receptors and transducing molecules in the tongue epithelium. Values are the mean and SEM of 5–7 mice per group. *p≤0.05, **p≤0.01, ***p≤0.001 versus indicated group; a, p≤0.05 versus WT control diet; b, p≤0.05 versus WT control diet; c, p≤0.001 versus WT control diet.

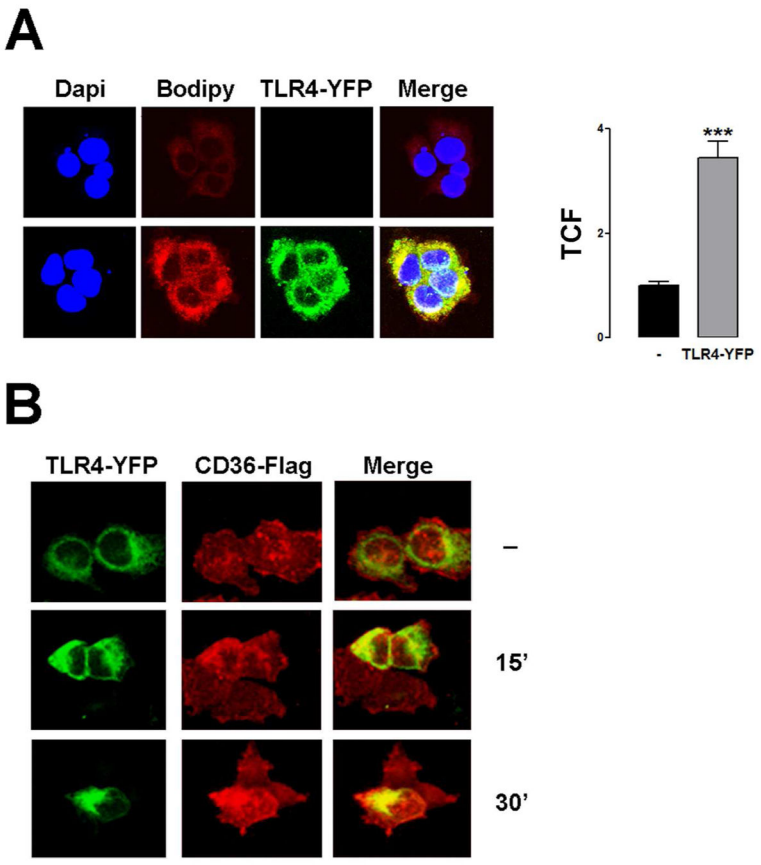

Figure 5.

TLR4 enhances cellular lipid uptake. (**A**) HEK 293 cells transfected with vector or TLR4-YFP expressing plasmids were incubated with fluorescent fatty acid analog C12 (Bodipy). The lipid uptake quantification expressed as total cell fluorescence (TCF), and representative images following 30 min incubation are shown. Data are mean and SEM of 3 independent experiments in triplicate. (n=46–48 cells). ***p≤0.001 versus vector transfected cells. (**B**) Representative images showing the distribution of TLR4-YFP and CD36-Flag in absence (−) and presence of 100 μM linoleic acid.

Table 1

Macronutrient composition of the diets utilized in the study. All diets were supplemented with minerals, amino acids and vitamins mixtures.

Macronutrients (%)	Standard diet (Harlan Teklad) 3.1 kcal/g
Crude protein	18.6
Fat (Soybean oil)	6.2
Saturated	0.9
Unsaturated	4.7
Carbohydrate	44.2
Fiber	18.2

Macronutrients (%)	Control diet (Dyets 101845) 3.8 kcal/g	High fat diet (Dyets 101842) 5.0 kcal/g
Crude protein	20.0	20.0
Fat	7.0	27.1
Soybean oil	7.0	7.0
Lard	-	2.0
Butter	-	18.1
Saturated	1.0	13.2
Unsaturated	5.3	11.8
Carbohydrate	64.7	47.6
Fiber	5.0	5.0