Title: A low-fat diet upregulates expression of fatty acid taste receptor gene *FFAR4* in fungiform papillae in humans: a co-twin randomised controlled trial

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Abstract

Fatty acid taste (FAT) perception is involved in the regulation of dietary fat intake, where impaired FAT is associated with increased fatty food intake. There are a number of FAT receptors identified on human taste cells that are potentially responsible for FAT perception. Manipulating dietary fat intake, and in turn FAT perception, would elucidate which receptors are associated with long-term regulation of FAT perception. This study aimed to assess associations between diet-mediated changes to FAT receptors and FAT perception in humans. A co-twin randomized controlled trial was conducted, where each matching twin within a pair were randomly allocated to either an 8-week low-fat (LF; <20% energy fat) or high-fat (HF; >35% energy fat) diet. At baseline and week 8, fungiform papillae were biopsied in the fasted state and FAT receptor gene expressions (*CD36*, *FFAR2*, *FFAR4*, *GPR84* and *KNCA2*) were measured using RT-PCR; and fatty acid taste threshold (FATT) was assessed using 3-alternate forced choice methodology. Linear mixed models were fitted, adjusting for correlation between co-twins. Intakes were compliant with the study design, with the LF and HF groups consuming 14.8% and 39.9% energy from fat, respectively. Expression of *FFAR4* increased by 38% in the LF group (P=0.023; time-diet interaction P=0.063). Δ*FFAR4* (Δ, week 8 – baseline) was associated with Δfat intake (g) ($\hat{\beta}$=-159.4; P<0.001) and ΔFATT ($\hat{\beta}$=-8.8; P=0.016). In summary, *FFAR4* is involved in long-term diet-mediated changes to FAT perception. Manipulating dietary fat intake, and therefore *FFAR4* expression, might aid in reducing taste-mediated passive overconsumption of fatty foods.

Introduction

The chemoreception of fatty acid (FA) in the oral cavity, also known as fatty acid taste (FAT), is involved in the regulation of dietary fat intake (1-3). Individuals with impaired FAT sensitivity are more likely to consume greater amounts of dietary fat due to compromised cephalic phase and post-ingestive satiety hormone responses following oral fat exposure (4-9). This is reflected throughout the alimentary canal, as individuals who are less sensitive to FAT in the oral cavity also have reduced hormonal response following FA stimulation in the gastrointestinal tract (8). FAT sensitivity is attenuated by oral fat exposure, and conversely can be increased by long-term reduced dietary fat intake (10-12) which may be due to regulation of some or all FAT receptors in the oral cavity.

Three types of lingual papillae – fungiform, foliate and circumvallate – house taste bud cells (TBCs) which express FAT receptors. A recent study of our group localized several candidate FAT receptor genes – *CD36*, *FFAR2*, *FFAR4*, *GPR84* and *KNCA2* – in TBCs of human fungiform papillae (13). Each of these receptors have specificities in the type of FA they bind (14), however the exact receptors that might be responsible for oral chemoreception of FA in humans remains unresolved. Previously, we showed that increased *CD36* was associated with short-term fat intake, particularly saturated fat, and acute dislike of fatty foods (13). However, no associations were found between any FAT receptors and FAT perception. Since FAT sensitivity can be modified by dietary fat intake (10-12), it may be possible to use a dietary fat intervention to elucidate which receptor might be responsible for long-term changes in FAT perception.

While no human studies have investigated changes in FAT receptors in response to changes in the dietary fat, animal studies have been conducted albeit with conflicting results. An 8-week high-fat (HF) (40% energy from fat) diet induced significant downregulation of *CD36* in rat circumvallate TBCs (3). However, this downregulation was not observed in fasted mice fed a HF (34.2%) diet for 4 weeks (15). Another study found that protein levels of CD36, rather than mRNA, decreased while FFAR4 protein increased in raft membranes of human and mouse fungiform TBCs following a two-month HF (40% energy from fat) diet (16). As for short-term, an acute HF (30% energy from fat) oral exposure triggered an immediate decrease in CD36 protein in mice circumvallate TBCs, which returned to pre-exposure levels following 11 hours of fasting, with no obvious effect was seen for FFAR4 protein (17). The discrepancies between the studies may be attributed to the fat content, the different area of the

tongue used for analysis, and/or fasting status. Diet-mediated regulation of FAT receptors has not been investigated in humans. Manipulating dietary fat intake, and in turn FAT taste perception, would elucidate which key receptors are associated with long-term regulation of oral chemoreception of FA.

This study aimed to determine which FAT receptors are responsible for diet-mediated changes to FAT perception in humans. The aim was accomplished by assessing the associations between changes to key FAT receptor genes expressed in fungiform papillae (as determined by Liu et al. (13)) and FAT perception following an 8-week dietary fat intervention. A secondary aim was to assess the associations between changes in macronutrient intakes and FAT receptor gene expressions.

Subjects and Methods

The study presented here is an 8-week follow-up analysis of a previous study where baseline data has been published (13).

Participants

A co-twin design was chosen as it controls for age, common environmental, and partial genetic factors shared by co-twins in each experimental group. Twins Research Australia (TRA) invited via mail 1881 twin pairs (3762 individuals) from the Melbourne metropolitan area to participate in a larger study on the effect of diet and genes on fatty acid taste (10). Twins were eligible to participate in the study if they were aged between 18-69 years, were able to attend three laboratory sessions in Burwood, VIC, and were willing to alter their diet for a period of 8 weeks. Both monozygotic (MZ) and dizygotic (DZ) twin pairs were invited to participate. Subjects were excluded from recruitment if they had any dairy allergies and intolerances, illnesses preventing them from eating foods included in the study, or if they were pregnant or lactating. Due to the nature of the twin study design, if one individual from a twin pair was excluded or withdrawn from the study, their co-twin was also excluded. In 66 pairs, both twins expressed interest in participating, and were then screened for eligibility. Forty-six twin pairs (92 individuals) aged between 18-68 years were recruited into the larger study, however only 13 pairs (26 individuals; 10 MZ pairs, 3 DZ pairs) consented to the additional testing described in this paper (Figure 1). Co-twins from each pair were

randomized into either a low-fat (LF) or HF diet, where one twin from each pair was allocated to the LF diet and the other twin allocated to the HF diet. Prior to recruitment, a block randomized sequence was generated with blocks of size two. TRA was responsible for recruitment and therefore characteristics of the participants were blinded to the researchers. Participants were allocated to the randomized sequence based on their TRA twin number; therefore, allocation of participants to diet group was concealed. Due to the nature of the intervention, blinding of participants was not feasible.

Ethics

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Deakin University Human Research Ethics Committee (ID: 2013-163). Written informed consent was obtained from all subjects. This study is registered as a clinical trial with the Australian New Zealand Clinical Trials Registry (ID: ACTRN12613000466741; http://www.anzctr.org.au/).

Study Outline

Participants attended two tasting sessions and two biopsy sessions at the Centre for Advanced Sensory Science (CASS) at Deakin University, Burwood, VIC. Recruitment and data collection occurred between July 2014 and May 2016. The first tasting session occurred on the day prior to beginning the dietary intervention, and the second tasting session occurred 8 weeks later on the last day of the intervention. Tasting sessions were conducted in a temperature and sound controlled environment with a 15 minute break in the middle of their session to prevent fatigue. Participants were asked to avoid eating or drinking anything but water and to avoid brushing their teeth or using mouthwash up to an hour prior to each tasting session. Tasting sessions measured fatty acid taste threshold (FATT) for oleic acid (C18:1) and anthropometric measurements. A 24-hour food recall was collected by a nutritionist during the first session. Between tasting sessions, participants recorded six 24-hour diet records, two weekdays and one weekend during the first 4 weeks, and two weekdays and one weekend during the final 4 weeks of the trial.

Collection of fungiform papillae (biopsy) occurred the day after each tasting session. Participants fasted for at least 10 hours prior to each fungiform papillae collection. Fungiform papillae tissue and serum blood samples were collected. After the biopsy session, participants were provided with a LF or HF breakfast snack depending on which diet they were allocated.

Dietary Intervention

The LF diet was defined as <20% of energy from fats and the HF diet was defined as >35% of energy from fats. These values were chosen as they fall outside the acceptable macronutrient distribution range for fat intake (20-35%) (18). Participants on the HF diet were encouraged to choose foods higher in monounsaturated and polyunsaturated fats rather than saturated fats in order to maintain a healthy diet. A diet booklet for each diet was created with the aid of an accredited practicing dietitian, which described the parameters of each diet; a list of foods which should and should not be eaten; and some example recipes that adhere to the diet. Participants were given the HF or LF booklet, their assigned diet was explained and they were taught how to interpret a nutrition information panel in order to identify which foods were acceptable for their diet.

They were requested to start the assigned diet the day after baseline measurement. As foods were not provided in this study, food choice was up to the participants. To maximize adherence to the diets, participants were contacted via phone fortnightly and questioned on their dietary habits. If the researcher felt that participants were not following the diet adequately, they were provided with suggestions and encouragement to aid in diet adherence. Participants were also asked a series of questions to ensure that they did not experience any negative effects from the diet. These questions included “Do you feel like you have less energy since starting the diet?”, “Do you feel like your weight has changed significantly since starting the diet?”, and “Is the diet affecting your day-to-day activities?” If the researcher felt that participants were suffering from major negative effects due to the diet (e.g. nausea, inability to work), they would be asked to stop the diet and were dropped out of the trial.

The first three completed diet diaries were inspected and reviewed at week 4 to assess compliance to the assigned diet.

Dietary Assessment

A single three-pass 24-hour dietary recall (19) of the day prior to the first tasting session was conducted by a trained nutritionist. For the ongoing diet records throughout the trial, participants were asked to avoid filling out diet records on a non-standard day (for example, if they attended a wedding reception). They were taught to quantify foods in standard serving sizes (cups, teaspoons, tablespoons, etc.) using a food model booklet, and asked to weigh their food and drinks wherever possible. Details such as brand, cooking method, and foods additives (e.g. sugar added to coffee) were included in the diet records.

Food recall and records were analysed for carbohydrate, protein, fat and fibre intakes (g and % of energy) using computer software FoodWorks (version 8, Xyris, Spring Hill, QLD, Australia).

Anthropometry

Body weight was measured after removal of shoes, heavy clothing, and any items in their pockets using electronic scales (OHAUS NV4101, Parsippany, NJ, USA), and height was measured using a free-standing stadiometer (SECA, Hamburg, Germany). BMI was calculated as weight (kg)/height $(m)^2$.

Fatty Acid Taste Receptor Expression

Fungiform papillae biopsy was conducted without anaesthetic by a registered doctor following the procedure described previously (20, 21). Fungiform papillae were chosen as they were the least invasive biopsy target and provide the best result compared to other oral sampling methods (22). For each participant, up to eight fungiform papillae were collected; four papillae chosen from the left side of the tongue and four from the right side of the tongue, chosen at random sites within the fungiform region of the tongue by the doctor. The eight papillae were pooled as an individual sample to reduce the impact of variation from different sites of collection. The gene expression of FAT receptors found within fungiform papillae tissue (13) including *CD36*, *FFAR2*, *FFAR4*, *GPR84* and *KCNA2* were analysed with real-time reverse transcription polymerase chain reaction (RT-PCR). RNA was extracted from the pooled fungiform papillae samples using TRIzol reagent (Life Technologies, USA) following the manufacture's protocol. The purified RNA pellet was dissolved in 20µl RNase-free H_2O, treated with RNase-free DNase set (Qiagen, Australia) and quantified with

NanoDrop ND-1000 spectrophotometer. The RNA integrity was measured with Bioanalyser 2100 (Agilent Technologies, USA) and the cut-off value was set at 5. For the RT-PCR, 1μg of total RNA was used to synthesize cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). Each cDNA sample was diluted 1:5 at first. Standards were then prepared with a serial dilution 1:5 from the top standard (an aliquot of the all the 1:5 dilution cDNA samples). For the standard curve, 1 gene copy was assigned to the lowest concentrated standard which had a Cp value. Gene concentration of other standards were set accordingly based on the dilution value. The notional concentration of each sample was calculated based on the standard curve (log concentration against Cp). The expression of the genes of interest were analysed with the Taqman gene expression assays (Life Technologies, USA) (Table 1). For each gene analysis, a negative control of the sample that had not been reversely transcribed was included. Housekeeping genes *GAPDH* and *RPLP0* were included for normalizing the transcript numbers.

Fatty Acid Taste Threshold (FATT)

Fat-free milk (Devondale, Southbank, VIC, Australia) solutions containing C18:1 (Sigma Aldrich, St. Louis, MO, USA) at varying concentrations (0.02, 0.06, 1.00, 1.40, 2.00, 2.80, 3.80, 5.00, 6.40, 8.00, 9.80, 12.00, and 20.00 mM) were prepared using established methods (38). Control samples were prepared in the same way, but without added C18:1.

FATT for C18:1 were determined using an ascending series 3-alternate forced choice methodology (10). To prevent confounding non-taste sensory inputs, participants wore nose clips and all tests were conducted under red light. FATT was transformed to an ordinal variable ranging from 0 to 12, with a higher score implying lower FAT sensitivity (2).

Statistical Analysis

Analyses were conducted using computer software SPSS (v24.0, IBM, NY, USA). Null hypotheses were rejected at $P < 0.05$. This study is a secondary analysis of participants from a larger study (10) who provided consent to additional testing, therefore sample size of this study was determined by feasibility of recruitment.

The effect of the diet on FAT receptor gene expressions were assessed using linear mixed models including diet (LF and HF), time (baseline and week 8) and the time-diet interaction

as fixed effects; with twin pair as a random effect and co-twin as the subject with repeated measures to account for the correlation between co-twins. A post-hoc Sidak's test was used to compare means between each fixed effect and to correct for multiple comparisons. Time-diet interaction, post-hoc Sidak's test and confidence intervals are reported. The above model was also used to assess differences between diet groups at baseline and to determine compliance to the prescribed diets.

To explore the strength of the association ($\hat{\beta}$) between change in FATT and change in FAT receptor gene expression over the 8 week period, the same model as above was conducted with Δ FATT (Δ, week 8 – baseline) as the outcome and Δ FAT gene expression as a fixed effect; and twin pair as a random effect. Pearson's correlations (*r*) for each association are also reported for descriptive purposes. The same analysis was repeated for the associations between Δ macronutrient intakes (total fat, saturated fat, monounsaturated fat, polyunsaturated fat, carbohydrate, protein, and dietary fibre) (g) and Δ FAT gene expression.

Results

Thirteen twin pairs were recruited and completed this study. However, four twin pairs were not included in the RT-PCR analysis of gene expression due to low concentration or poor integrity of the collected RNA samples according to the Nanodrop and Bioanalyser analyses. As a result, we present here the 9 pairs (7 MZ, 2 DZ; 8 female pairs, 1 male pair) with valid samples that underwent RT-PCR analysis (Figure 1). No harmful or unintended effects were observed in either group. None of the participants reported any significant changes to their weight, energy levels or ability to perform day-to-day tasks throughout the dietary intervention. Baseline characteristics of the participants are described in Table 2. No significant differences in baseline characteristics were observed between diet groups.

Dietary compliance

Intakes of energy from fat throughout the trial were within the aims of the study, with the LF group consuming 14.8 (95% CI: 10.0, 19.7) % energy from fat and the HF group consuming 39.9 (35.0, 44.7) % energy from fat (Supplementary Table 1). Intake of energy from fat at baseline was already considered high in the HF diet group at 36.4 (31.6, 41.3) %, so there was no significant change in fat intake across the trial in this group. There was a significant

decrease in intake of energy from fat in the LF diet group (-20.3 [-26.2, -14.4] %, $P < 0.001$). There was no significant change in total energy intake in either diet group, although a between group difference was observed at week 8 (2.3 [0.5, 4.2] MJ, $P = 0.021$).

Effect of dietary fat intake on FATT

No difference in FATT was observed between the LF and HF diet groups at baseline (Table 3). Over the 8 weeks, FATT decreased by 76 % in the LF diet group ($P < 0.001$) and increased by 23 % in the HF diet group ($P = 0.049$). At week 8, FATT in the HF diet group was 3.8 times higher than the LF diet group ($P < 0.001$), and there was a significant time-diet interaction for FATT ($P < 0.001$) (Table 3).

Effect of dietary fat intake on FAT receptor gene expressions

There was evidence for a time-diet interaction for *FFAR4* expression ($P = 0.063$), as expression increased by 38 % in the LF diet group ($P = 0.023$) (Table 3). No significant time-diet interactions were observed for *CD36*, *GPR84*, *FFAR2* and *KCNA2* expression, although *GPR84* expression was 61 % greater in the LF diet group than the HF diet group at baseline.

Associations between FATT and FAT receptor gene expressions

The relationship between FAT sensitivity and FAT receptor expression was assessed by comparing change (Δ) in FATT with change in receptor expression from baseline to week 8 (Table 4) (Figure 2). There was a significant negative association between Δ FATT and Δ *FFAR4*, indicating that as *FFAR4* expression increased, FATT also decreased concurrently (i.e. sensitivity to FAT increased). Conversely, the positive association between Δ FATT and Δ *GPR84* indicates that as *GPR84* expression increased, FATT also increased (i.e. sensitivity to FAT decreased). There were no significant associations between Δ FATT and Δ *CD36*, Δ *FFAR2* or Δ *KNCA2*.

Associations between dietary macronutrient intakes and FAT receptor gene expressions

There were significant negative associations between Δ *FFAR4* and Δ fat intake (g) ($\hat{\beta}$ = -159.4; r = -0.744; $P < 0.001$), Δ saturated fat intake (g) ($\hat{\beta}$ = -79.4; r = -0.759; $P < 0.001$), Δ monounsaturated fat intake (g) ($\hat{\beta}$ = -53.4; r = -0.711; $P = 0.001$), and Δ polyunsaturated fat intake (g) ($\hat{\beta}$ = -14.8; r = -0.533; $P = 0.023$). There was a statistical trend for a negative association between Δ *KCNA2* and Δ polyunsaturated fat intake (g) ($\hat{\beta}$ = -72.7; r = -0.459; $P = 0.056$). Finally, there was a significant positive association between Δ *FFAR2* and Δ dietary fibre intake (g) ($\hat{\beta}$ = 22.5; r = 0.560; $P = 0.016$). No significant associations were observed for Δ *CD36* or Δ *GPR84*, although there was a significant association between baseline *GPR84* expression and intake of energy from dietary fibre (%) ($\hat{\beta}$ = 8.8; r = 0.399; $P = 0.023$).

Discussion

This study assessed changes in fasting expression of FAT receptor genes following 8 weeks of LF or HF dietary intake, for the first time in humans. It is well established that FAT sensitivity is modulated by dietary fat intake (10-12). It was hypothesised that expression of FAT receptors would be similarly modulated due to dietary fat intake, as in rodent models (3, 17). However, only *FFAR4* was affected by the dietary intervention, with expression of *FFAR4* increasing by 38 % in the LF diet group from baseline to week 8. While the magnitude of reduction in *FFAR4* expression in the HF group (3 %) was much lower than the increase seen in the LF group, this is likely because in the HF group, fat (g) increased by only 34 % whereas in the LF group there was a 72 % reduction in fat consumed. Further, change in *FFAR4* expression was associated with change in FATT, or in essence, as *FFAR4* increased, FAT sensitivity also increased concurrently. This is supported by the associations between *FFAR4* and intakes of fat, saturated fat, monounsaturated fat and polyunsaturated fat, indicating that dietary fat with any level of FA saturation downregulates *FFAR4*. These results indicate that *FFAR4* expressed in fungiform papillae may be responsible for long-term changes in FAT perception. To that point, increasing expression of *FFAR4* might lead to an increased secretion of intestinal peptides following chemoreception of FA in the oral cavity or GIT (8), reducing subsequent desire to eat and therefore energy intake. Also, since systemic *FFAR4* is involved in the facilitation of energy homeostasis (23), brown fat activation (24) and inflammation (25), the ability to upregulate *FFAR4* via dietary intervention may have potential in the management of obesity and metabolic disease.

Interestingly, there was no effect of diet on *CD36* in fungiform papillae in the current study, which contradicts what is found in our previous research (13). In the current study, there was a small trend in the same direction as *FFAR4*, in that *CD36* increased by 16 % in the LF diet and decreased by 13 % in the LF diet after 8 weeks, however there was no significant time-diet interaction. This is likely because the baseline dietary data was based on a single 24-hour recall, whereas the intervention dietary data was based on six diet records over the course of 8 weeks. If participants consumed a HF meal on the night before testing, there might have been some residual effect on gene expression. Short-term oral exposure to dietary fat in mice decreased CD36 levels by two-fold within 1 hour of refeeding (17). However, 11 hours of fasting returned CD36 levels to pre-prandial levels. Therefore, it is possible that TBCs up- and downregulate *CD36* relatively quickly following acute oral exposure to fat compared to *FFAR4*. We speculate that the role of *CD36* may be to mediate short-term response to dietary fat, and is downregulated throughout an eating event, then upregulated to pre-meal expression following a prolonged fast; whereas *FFAR4* is involved in regulating long-term response to dietary fat between meals with greater gradual change in expression over longer periods of time. Analysis of *CD36* in human papillae immediately before and after consumption of dietary fat is necessary to confirm this. Some studies have also shown associations between *CD36* and hedonic preference for fatty foods in humans (13) and rodents (26-29), which strengthens the argument that the role of *CD36* is more specific to mediating fat intake within a given meal. There is also evidence of interaction between FFAR4 and CD36 in TBCs (30), although the significance of this interaction remains unclear.

While the dietary intervention was not designed to control for polyunsaturated fat intake as participants were able to choose to consume any food sources of fat, intake of polyunsaturated fat increased on the LF diet and decreased on the HF diet. Despite this, time-diet interaction for *KCNA2*, which is the receptor gene specific to polyunsaturated fatty acid (PUFA) chemoreception, was not significant. There was an 18 % increase in *KCNA2* observed in the LF diet group which was in the hypothesised direction, and essentially no change to *KCNA2* in the HF diet group likely due to the relatively minor increase in fat intake over the 8 weeks. There was a statistical trend for a negative association between Δ *KCNA2* and Δ polyunsaturated fat intake ($P = 0.056$), which indicates there may be some effect of polyunsaturated fat intake on *KCNA2*. Therefore, it could be suggested that KCNA2 might be involved in the regulation of PUFA intake, where an individual who is not meeting their PUFA intake requirement might express greater KCNA2 levels on TBCs to promote greater

subsequent PUFA intake. However, a dietary intervention that is well-controlled for PUFA intake is necessary to confirm this.

Another interesting outcome of this study was that there was no effect of the diet on *FFAR2*, despite there being strong evidence for an association between *FFAR2* and fat intake, particularly saturated fat, in the baseline data of this study (13). The previous finding was unexpected as FFAR2 is mainly responsible for short-chain fatty acid (SCFA) chemoreception (14). Instead, *FFAR2* might not be regulated by fat intake but rather by total energy, as high saturated fat intake (g and %) is highly correlated with increased energy and reduced dietary fibre intake in this sample (unreported). Further to this, changes in *FFAR2* was associated with changes to dietary fibre intake which is likely due to incidental changes to fibre intake as an artefact of the diets. This result is noteworthy, as dietary fibre intake leads to production of SCFA by biota in the gastrointestinal tract (GIT) (31). Since regulation of receptors is presumably analogous throughout the alimentary canal (8), it is possible that increased exposure to SCFA in the GIT may cause increased *FFAR2* in TBCs. Similarly, while there was no time-diet interaction for *GPR84* expression, there was an association between *GPR84* and energy from dietary fibre (%). As *GPR84* is mainly involved in the chemoreception of SCFA, we suspect this also due to increased exposure of SCFA in the GIT.

There was a strong time-diet interaction for FATT ($P < 0.001$), suggesting a physiological change to taste mechanisms was causing the change in FATT. While this is likely due to changes in FAT receptor gene expressions, namely *FFAR4*, there are many complexities to the taste system that should be discussed. First, small changes to gene expression may result in large physiological effects due to potent effects of a protein, differences in the amount of protein products, and the function of the half-life of the mRNA. To that point, we only measured gene expression in fungiform papillae as it was the least invasive tissue (21) whereas there may have been larger changes to gene expression in foliate and circumvallate papillae (32). Second, coordination and interaction of receptors may confound the results. For example, upregulation of either *FFAR4* or *CD36* independently may have an insignificant effect on FATT, but when upregulated together there may be a larger attenuation of FATT due to intracellular signal transduction (30). Finally, there may be salivary factors that were not measured in this study that are additionally causal to the FATT change (33, 34).

This randomised controlled trial has some limitations that should be noted when interpreting these results. First, the analysis of FAT receptor gene expressions was only conducted in fungiform papillae, as collection of foliate and circumvallate papillae in living humans is fairly invasive. The relative sensitivities and expressions of these genes within and between human taste papillae are not clear (35, 36), so the results from this study should be interpreted only as indicative of the entire oral cavity. Second, the sample size was small and there was only one male twin pair in the sample, so there may be limited power to detect small changes in gene expression. Third, while we explored associations between nutrient intakes and FAT receptor gene expressions, this study was designed as an intervention to dietary fat intake. Changes in other nutrient intakes were incidental, and therefore the observed associations need to be confirmed in trials that are designed around those nutrients specifically. Lastly, due to the small sample of twin pairs, quantitative genetic effects could not be evaluated in this study.

In summary, *FFAR4* is the only FAT receptor on fungiform papillae associated with FAT perception in the fasted state, and both are mediated by diet concurrently. The role of *FFAR4* appears to be to regulate long-term diet-mediated changes to FAT perception. Increasing expression of *FFAR4* in papillae, via diet or otherwise, could aid in reducing taste-mediated passive overconsumption of dietary fat. In addition, there is some evidence to suggest that *CD36* is involved in regulating short-term within-meal responses to oral FA exposure and *KCNA2* may also have some role in regulating PUFA intake, although future research should be conducted to confirm these.

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Conflict of Interest

None.

Authorship

CN, KD, NA, and RK designed the research; AC and DL conducted the research; AC, DL, and SB analysed the data; AC and DL wrote the manuscript; and RK had primary responsibility for final content.

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Table 1. Taqman gene expression assays used for the real time RT-PCR analysis.

Gene	Assay ID	Description
CD36	Hs01567185_m1	Probe spans exons
FFAR4	Hs00699184_m1	Probe spans exons
FFAR2	Hs00271142_s1	Probes are within single exon
GPR84	Hs01874713_s1	Probes are within single exon
KCNA2	Hs04187587_g1	Probe spans exons
RPLP0	Hs99999902_m1	Probe spans exons
GAPDH	Hs02758991_m1	Probe spans exons

RT-PCR, Reverse transcription polymerase chain reaction

Table 2. Baseline characteristics of participants.

	All Participants (n = 18)	LF Diet (n = 9)	HF Diet (n = 9)
Age (years)	41.6 (16.5)	-	-
Weight (kg)	72.5 (17.2)	71.9 (17.5)	73.0 (18.0)
BMI (kg/m^2)	26.9 (6.3)	26.7 (6.1)	27.2 (6.9)
Female (n)	16	8	8

Data presented as mean (SD). LF, low-fat; HF, high-fat

Table 3. Means and between-group differences in FATT and FAT receptor gene expression levels (relative gene copy number) in fungiform papillae TBCs over the 8-week trial.

	LF Diet	HF Diet	Between-Group Difference	Time-Diet Interaction (*P*)
FATT				< 0.001
Baseline	7.8 (5.8, 9.7)	7.4 (5.4, 9.3)	-0.4 (-3.1, 2.4)	
Week 8	1.9 (0.4, 3.4)***	9.1 (7.6, 10.6)*	7.2 (5.1, 9.3)†††	
CD36				0.248
Baseline	1.20 (0.65, 1.75)	1.26 (0.71, 1.81)	0.06 (-0.56, 0.68)	
Week 8	1.39 (0.87, 1.92)	1.10 (0.58, 1.63)	-0.29 (-0.88, 0.29)	
FFAR4				0.063
Baseline	0.84 (0.57, 1.12)	0.93 (0.66, 1.21)	0.09 (-0.30, 0.48)	
Week 8	1.16 (0.89, 1.43)*	0.90 (0.62, 1.17)	-0.26 (-0.65, -0.12)	
FFAR2				0.409
Baseline	0.95 (0.53, 1.36)	0.60 (0.18, 1.01)	-0.35 (-0.93, 0.23)	
Week 8	0.93 (0.48, 1.38)	0.75 (0.30, 1.20)	-0.18 (-0.86, 0.50)	
GPR84				0.214
Baseline	0.057 (0.020, 0.095)	0.022 (-0.015, 0.060)	-0.035 (-0.069, -0.001)†	
Week 8	0.041 (0.009, 0.074)	0.029 (-0.003, 0.062)	-0.012 (-0.025, 0.000)	
KNCA2				0.460
Baseline	0.17 (0.12, 0.22)	0.18 (0.14, 0.23)	0.01 (-0.04, 0.07)	

Week 8	0.20 (0.12, 0.28)	0.19 (0.11, 0.26)	-0.01 (-0.12, 0.09)

Data presented as mean and 95% CI. Between-group difference calculated as HF diet – LF diet; Estimated means, CIs and *P*-values obtained under a mixed model including twin pair as a random effect. Post-hoc Sidak's test, CI and time-diet interaction are reported.

FATT, fatty acid taste threshold; FAT, fatty acid taste; LF, low-fat; HF, high-fat

Significantly different from baseline: * $P < 0.05$, *** $P < 0.001$; significant between-group difference: † $P < 0.05$, ††† $P < 0.001$

Table 4. Associations between Δ FATT and Δ FAT receptor gene expressions.

	Δ FATT		
	r	$\hat{\beta}$	*P*
Δ *CD36*	-0.061	-0.8	0.822
Δ *FFAR4*	-0.590	-8.8	0.016
Δ *FFAR2*	0.270	3.4	0.311
Δ *GPR84*	0.517	68.7	0.040
Δ *KCNA2*	-0.302	-21.3	0.256

$\hat{\beta}$ and *P*-values obtained under a mixed model including twin pair as a random effect and time as a repeated effect; *r* obtained using Pearson's correlation for descriptive purposes only.

Δ, week 8 – baseline; FAT, fatty acid taste; FATT, fatty acid taste threshold

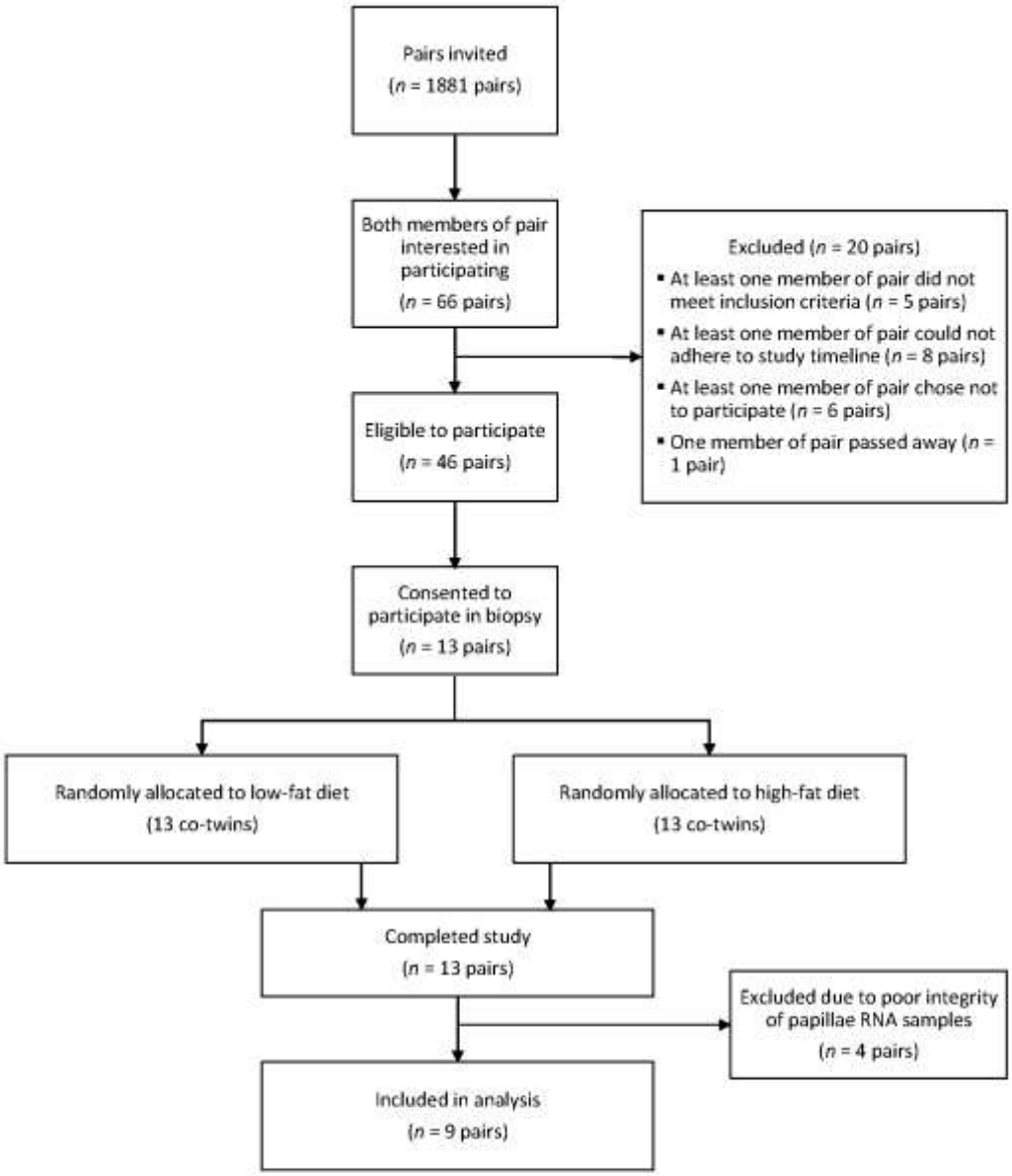

Figure 1. Study consort flow chart diagram.

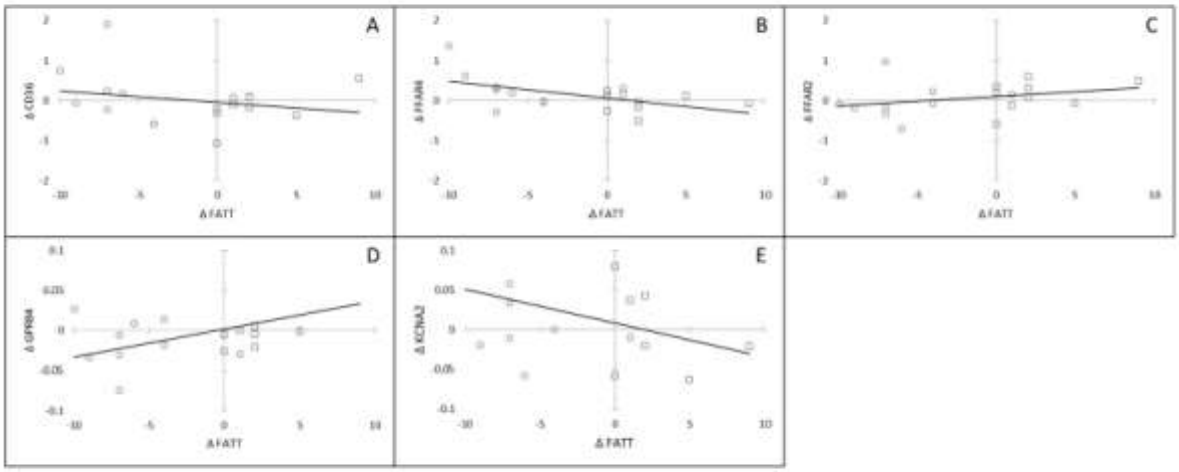

Figure 2. Scatterplots of Δ FATT and Δ FAT receptor gene expressions. Circle markers (○) indicate participants on the LF diet (n = 9); square markers (□) indicate participants on the HF group (n = 9). A: Δ FATT vs. Δ CD36; B: Δ FATT vs. Δ FFAR4; C: Δ FATT vs. Δ FFAR2; D: Δ FATT vs. Δ GPR84; E, Δ FATT vs. Δ KNCA2.

FATT, fatty acid taste threshold; FAT, fatty acid taste; LF, low-fat; HF, high-fat