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ARTICLE

Genetics and Epigenetics

Obesity is associated with altered gene expression in human tastebuds

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Abstract

Background The role of taste perception in the development and persistence of obesity is currently unclear due to conflicting results from psychophysical and other studies. No study to date has assessed whether there is an underlying fundamental difference in the physiology of taste tissue between lean and obese individuals.

Method/subjects We analysed the transcriptomic profile (RNA-seq) of human fungiform taste papillae biopsied from lean ($n = 23$) and obese ($n = 13$) Caucasian females (age range 18–55) to identify differences in gene expression.

Results Obesity status was the major contributor to variance in global gene expression between individuals. A total of 62 genes had significantly different gene expression levels between lean and obese ($P < 0.0002$), with the specific taste associated genes phospholipase C beta 2 (*PLCβ2*) and sonic hedge-hog (*SHH*) having significantly reduced expression in obese group. Genes associated with inflammation and immune response were the top enriched biological pathways differing between the lean and the obese groups. Analysis of a broader gene set having a twofold change in expression (2619 genes) identified three enriched theme groups (sensory perception, cell and synaptic signalling, and immune response). Further, analysis of taste associated genes identified a consistent reduction in the expression of taste-related genes (in particular reduced type II taste cell genes) in the obese compared to the lean group.

Conclusion The findings show obesity is associated with altered gene expression in tastebuds. Furthermore, the results suggest the tastebud microenvironment is distinctly different between lean and obese persons and, that changes in sensory gene expression contribute to this altered microenvironment. This research provides new evidence of a link between obesity and altered taste and in the future may help design strategies to combat obesity.

Introduction

Obesity is partly characterised as a condition of excess intake of macronutrients above the physiological requirements of the body. In particular, increased sugar and fat intake have both been associated with weight gain and obesity [1, 2]. Taste and orosensory signalling are important in the cephalic phase response which impacts on nutrient intake, satiety, and termination of food consumption [3–5]. Therefore, it is plausible that obese individuals have an altered taste/orosensory system, which may result in a delay in fullness and increased nutrient intake thus contributing to obesity. However, the role of taste perception in the development and persistence of obesity is currently unclear.

The current evidence of a link between taste and obesity is conflicting. In psychophysical studies, which generally assess differences in taste acuity (in one or several tastes) between lean and obese, the evidence is mixed with findings of increased [6] and decreased [7–12] taste acuity in obese, or, no association [13–18]. Similarly, studies assessing bariatric surgery patients before and after surgery or individuals before and after weight loss show a trend for increased taste acuity following surgery or weight loss; however, the findings are inconsistent [19–22].

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To uncover new insights into a link between taste perception and obesity we undertook a different approach to assess if there are underlying fundamental differences in the physiology of taste tissue between lean and obese individuals. To complete this, a global transcriptomic analysis (RNA-seq) of human fungiform papillae containing taste-buds was undertaken to identify differences in gene expression between lean and obese groups. The relationship between obesity status and the global gene expression profile of human taste tissue has not previously been explored.

Materials and methods

Study details

The research complies with the Declaration of Helsinki for Medical Research, all procedures were approved by the CSIRO Human Research Ethics Committee (HREC13/06), and informed written consent was obtained from all participants. The work presented is part of a larger research study registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613000639729).

Study participants

The study recruited lean (BMI 18–25) and obese (BMI > 30) females (18–55 years) of European descent living in the Sydney metropolitan area with adequate written and spoken English language to ensure they could provide informed consent and follow testing instructions. Further, individuals were excluded from the study if they were currently or recently dieting, if their weight had fluctuated more than 3 kg in the past 3 months, were smokers, suffered head trauma/surgery, oral cancers, tongue lesions (including tongue piercings), chronic diseases or infections (self-reported e.g. diabetes, autoimmune disease, or chronic ear infections), who were allergic to or did not wish to consume dairy products or peanuts, or were on medications that may impair taste sensitivity. A homogenous study population was selected for analysis in the current study to reduce any possible confounding factors that might be introduced which may also result in gene expression changes. Participants were remunerated to cover their time and travel costs for participating in the study. As the global fungiform papillae gene expression has not been previously reported, it was difficult to determine appropriate sample size of the study. Post hoc analysis of the study sample size utilising RNA-seq sample size calculator identified that the study could adequately detect a twofold change in gene expression ($\alpha = 0.05$, $\beta = 0.8$ and $\sigma = 0.6$) [23].

Fungiform papillae count

Fungiform taste papillae were quantified by counting the density of papillae on tongue. Blue food colouring (Queen, Woolworth's Australia, diluted 1 in 20 parts water) was placed on the anterior surface of the tongue. A filter paper card with a 6 mm diameter hole was placed on the tongue with the circle located 1 cm back from the tip and to the left of the centre of the tongue, a location with density measures that correlates with the total number of fungiform papillae on a tongue [24]. Photographs were captured using a Nikon DSLR D90 camera with a Sigma 105 mm DG Macro Lens and EM-140 DG Macro flash. Papillae density was determined independently by two investigators by manual counting of papillae in the circle using Adobe Photoshop CS6. Differences in count were resolved by the two investigators and a multiplication factor of 3.54 was applied to convert to papillae/cm^2.

Fungiform papillae biopsy

Fungiform papillae biopsy were collected as previously described (6 papillae per participant) [25]. Fungiform papillae were biopsied from study participants after a 2 h morning fast (water excepted) between the hours of 10–11 am [25, 26]. Briefly, fungiform papillae were cut from the anterior tongue (1–2 cm from tongue apex and to the left) by a trained medical professional, rinsed in ice-cold phosphate buffered saline (PBS) and transferred immediately into RNAlater (Life Technologies, USA) where they were stored at −80 °C. RNA was extracted from all samples on consecutive days in three batches (see sample batching). To extract RNA, papillae were thawed on ice, transferred to 150 µl TRIzol and homogenised with Kimble chase cordless motor pellet pestle (Sigma-Aldrich, USA) for 3–4 min. Homogenised samples were passed through a 19 gauge needle 15 times and RNA was extracted following the manufacturers protocol. Samples were further purified using sodium acetate and stored at −80 °C [25]. Sample quality was assessed on the Agilent Bio-analyser using the Agilent RNA 6000 Nano Kit.

Batching of fungiform papillae samples

Batching of samples was undertaken according to a balanced experimental design to reduce the potential for batch effects due to unequal distribution of biological groups or other experimental/confounding factors that could be introduced during sample extraction and sequencing. Samples were separated into three equal groups using the R package OSAT v.1.10.0 with optimal block [27], taking into account the: (i) obesity status, (ii) age group (stratified into four groups 18–25, 26–35, 36–45 and 45–55 years),

and, (iii) the doctor performing fungiform papillae biopsy. Chi-squared analysis and visualisation of the frequency of the three factors between the groups confirmed an even distribution and a well-balanced experimental design (data not shown).

RNA-seq library preparation and sequencing

RNA extracted from fungiform papillae were converted into sequencing libraries using an input of 700 ng RNA per sample with the Illumina TruSeq Stranded mRNA following the manufacturer's protocol. The RNA quality of samples was good (average RIN value 7.4) with two samples excluded from library preparation and sequencing due to low quality RNA (RIN > 6.0). The 36 prepared TruSeq libraries were separated into 4 sequencing pools of 9 libraries (3 samples from each OSAT batch) and each pool was sequenced in two lanes of a whole flow cell using Illumina HiSeq 2000 (2 × 125 bp Paired-End Dual index sequencing reads and v4 Illumina SBS chemistry). Sequencing of fungiform papillae from the 36 participants generated a total of 2.9 billion reads (average 44 million paired reads/sample and 98.8% of reads/sample were unique sequences).

Sequence alignment and differential gene expression

Sequencing quality of the raw reads was assessed using FastQC v0.11.3 [28]. The raw reads were not trimmed as the per base sequence quality was high across the length of the sequencing reads. K-mer, GC content and duplicate reads were acceptable and homogenous between samples and sequencing lanes [29]. Sequence alignment and differential gene expression was completed following the Tuxedo workflow [30], which included Tophat v2.1.0 [31] and Bowtie v2.2.5 [32] alignment of raw reads to the USCS hg19 reference sequence and transcriptome annotation (accessed from Illumina iGenomes website, https://support.illumina.com/sequencing/sequencing_software/igenome.html), and Cufflinks v2.2.1 for transcript assembly and to determine differential gene expression [33, 34]. The average read alignment to the reference sequence was 75% and no evidence of systematic changes across experimental conditions observed. Sequence alignments were indexed using Samtools v1.2 [35] for visualisation with IGV v2.3.55 [36, 37]. Differential gene expression was determined using CuffDiff (component of Cufflinks) which uses a negative binomial model estimated from data to obtain variance estimates from which p-values are determined and significance was achieved in genes with p-value <0.0002 and false discovery rate (FDR) < 0.05. Additionally, further analysis was conducted on all genes having a twofold change in expression between lean and obese.

Data analysis

Data analysis was completed in R [38] using the R package CummeRbund v2.16.0 [30]. Principal components analysis (PCA) was completed using prcomp and the package factoextra [39] to generate and visualise PCA plots. Additional plots were generated using the R package ggplot2 [40]. Specifically, box and whisker plots represent the distribution of data (minimum, first quartile, median, third quartile, and maximum) and dots represent outliers (1.5 times the interquartile range from the first and third quartiles). Enriched biological themes in gene lists were determined using the functional annotation tool in DAVID [41, 42]. This analysis was completed on two gene lists from differentially expressed genes identified through CuffDiff and by all genes having twofold change in gene expression (Table S2). Additional comparisons of taste associated genes were completed by analysis of the expression levels of 71 genes associated with taste (taste receptors and signalling molecules—Table S3). This list was reduced to 44 after a screening criteria was applied (50% of all samples had gene expression or at least 50% of either lean or obese samples had expression) to ensure reporting of reliable data given the low expression of the taste genes. Thus, the rationale for screening 71 to 44 genes was to remove genes that have very low expression where accurate differences in gene expression between lean and obese is less reliable. Kruskal–Wallis tests were used to assess differences in taste genes displaying a 1.5-fold expression change.

Results

Fungiform papillae biopsies and transcriptome profiles from 36 individuals were collected and analysed to identify differences in gene expression in fungiform papillae between lean ($n = 23$) and obese ($n = 13$) Caucasian females (Table 1). No difference in the density of fungiform taste papillae were observed between the two groups (Table 1). Fig. 1 shows an overview of sample preparation, sequencing and data analysis. In total, 31,969 "genes" (annotated genes and unknown transfrags) were compared between

Table 1 Description of subjects

	Obese $n = 13$	Lean $n = 23$	p-value
Age (years)	41.1 ± 12.9	31.9 ± 10.3	0.02
Height (cm)	165 ± 8.7	168.4 ± 6.5	0.30
Weight (kg)	101.3 ± 19.3	62.7 ± 7.7	>0.001
BMI (kg/m^2)	37.2 ± 6.3	22.1 ± 2.2	>0.001
Papillae count (papillae/cm^2)	18.4 ± 5.4	16.0 ± 7.2	0.30

mean ± SD

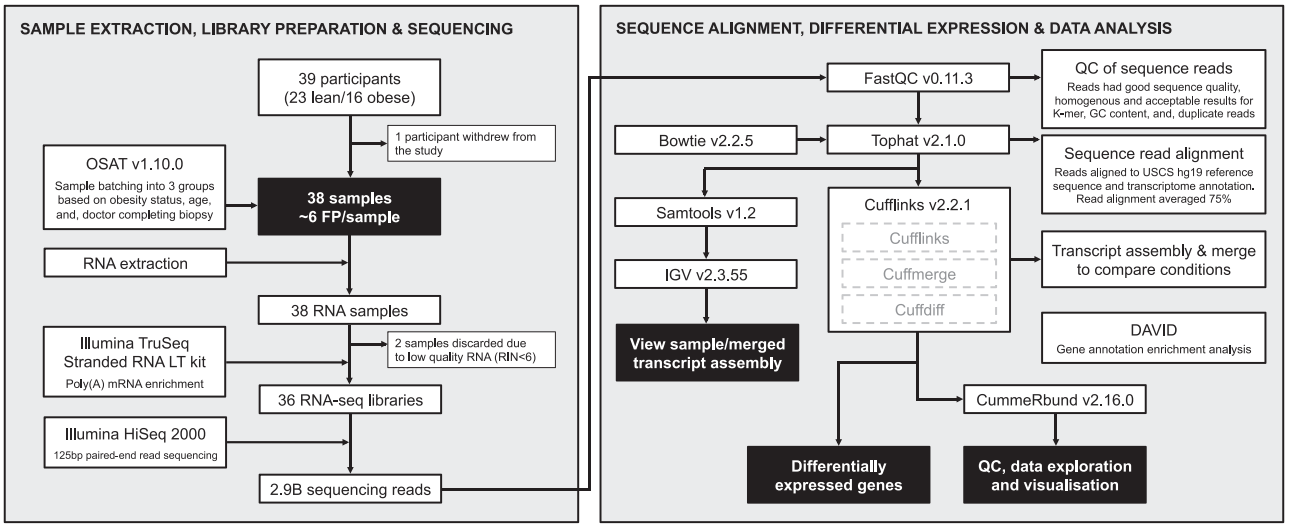


Fig. 1 Flowchart outlining the methodology employed to determine differential gene expression. Left hand box outlines the sample extraction, library preparation and sequencing, while the right hand box shows the data analysis pipeline of sequence alignment, determination of differentially expressed genes and data analysis. FP Fungiform papillae; RIN RNA integrity number; QC quality control

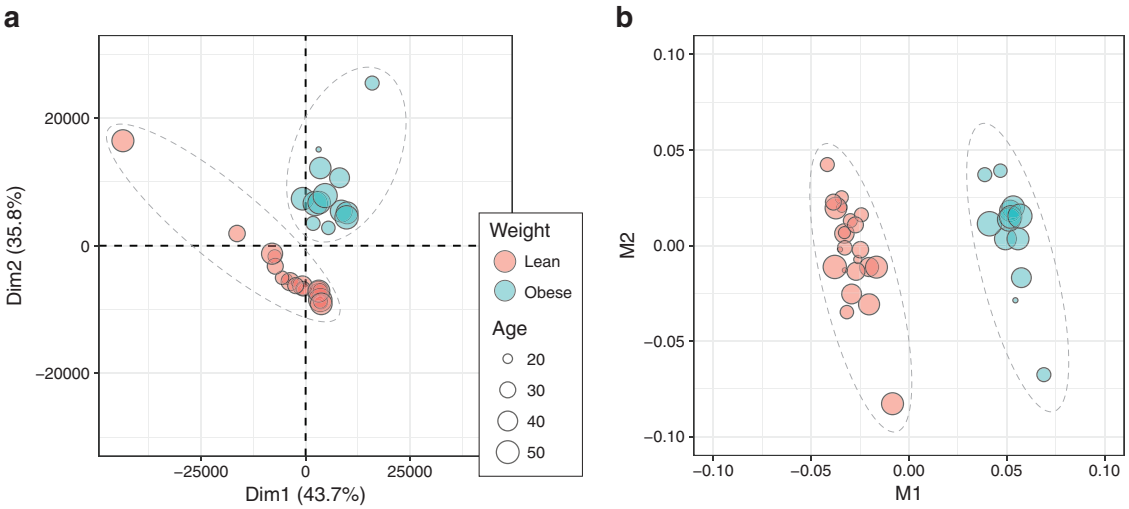


Fig. 2 Analysis of the global gene expression profile using dimensionality reduction methods of lean and obese fungiform papillae. Principal component analysis (PCA, PC1 and PC2) **a** and multidimensional scaling (MDS, M1 and M2) **b** separated based on the first and second dimensions. Colours and circle sizes represent weight status and age, respectively. Grey dashed circles outline groupings based on weight status

lean and obese, of which 26,871 showed gene expression (21,502 annotated hg19 genes and 5369 not annotated transfrags). Taste receptor genes were expressed in all samples, showing the collection of fungiform papillae from study participants did result in the collection of taste tissue.

Fungiform papillae transcriptome differs in obese individuals

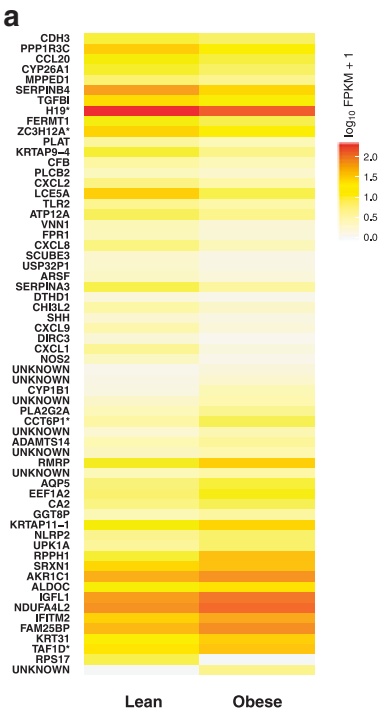
We first assessed the whole-transcriptome profiles of all samples using principal components analysis (PCA) and multidimensional scaling (MDS) to explore the main sources of variation within the data (Fig. 2). In both methods, lean and obese individuals clearly separated into two distinct groups (based in first and second dimensions) indicating obesity status was the key factor influencing variability in gene expression. This clustering highlights that obese have a unique fungiform papillae gene expression profile compared to lean individuals. Furthermore, there was no evidence for an age-related gradient or grouping of samples based on age in the plots (Fig. 2), confirming that obesity status was the biggest contributor to variance in transcriptome profiles over the study cohort.

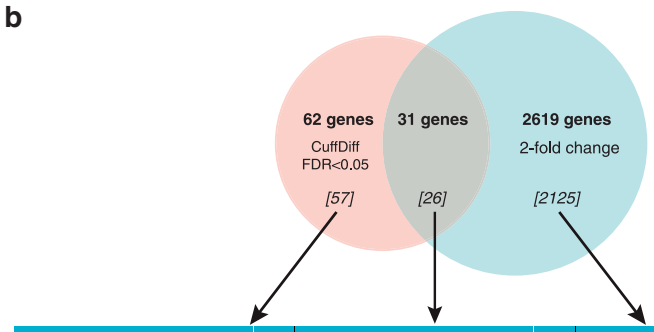
Two samples deviate slightly from each lean and obese group in the dimensionality plots (Fig. 2). Participant inclusion and study design were highly controlled ruling out likely

causes for these deviations (i.e. gender, smoking, medication) and further examination could not identify the putative cause (data not shown). These samples may deviate due to non-adherence to study protocol (e.g. not fasting for 2 h prior to fungiform papillae collection) or some other unknown/unmeasured factor. While they do deviate, their locations on PCA and MDS plots are congruous with samples of participants of the same weight status, and therefore, these samples were not excluded from further data analysis.

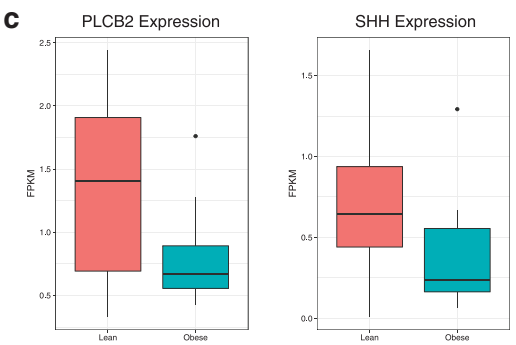
Differentially expressed genes and enriched biological pathways

CuffDiff analysis identified 62 genes with differential gene expression between fungiform papillae from lean and obese individuals (FDR < 0.05, $P < 0.0002$) (Fig. 3a, b, Table S1). Of these differentially expressed genes, 29 and 33 genes displayed decreased and increased gene expression, respectively, in the lean compared to the obese groups.





TERM	n	TERM	n	TERM	n
Inflammatory response (0006954)	11	Inflammatory response (0006954)	7	G-protein coupled receptor signalling pathway (0007186)	133
Chemotaxis (0006935)	6	Chemotaxis (0006935)	5	Detection of chemical stimulus involved in sensory perception of smell (0050911)	63
Cell chemotaxis (0060326)	5	Cell chemotaxis (0060326)	4	Chemokine-mediated signalling pathway (0070098)	23
Chemokine-mediated signalling pathway (0070098)	5	Chemokine-mediated signalling pathway (0070098)	4	Detection of chemical stimulus involved in the sensory perception of bitter taste (0001580)	15
Response to molecule of bacterial origin (0002237)	3	Positive regulation of neutrophil chemotaxis (0090023)	3	Cell-cell signalling (0007267)	40
Immune response (0006955)	7	G-protein coupled receptor signalling pathway (0007186)	5	Chemical synaptic transmission (0007268)	37
Positive regulation of neutrophil chemotaxis (0090023)	3	Movement of cell or subcellular component (0006928)	3	Neuropeptide signalling pathway (0007218)	22
Cellular response to lipopolysaccharide (0071222)	4	Immune response (0006955)	4	Sensory perception of smell (0007608)	28
Carbon dioxide transport (0015670)	2	Response to lipopolysaccharide (0032496)	3	Sensory perception of taste (0050909)	11
Regulation of cell proliferation (0042127)	4	Response to molecule of bacterial origin (0002237)	2	Cell chemotaxis (0060326)	16



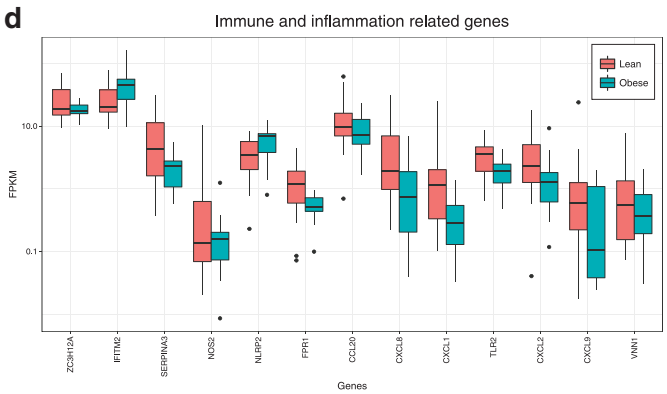


Fig. 3 Analysis of differentially expressed (DE) genes between lean and obese human fungiform papillae groups. **a** Heat map of the 62 DE genes identified through CuffDiff analysis ($p < 0.0002$, FDR < 0.05). **b** Overview of differential gene analysis and enriched biological terms. Venn diagram (top) showing the number of genes identified: through CuffDiff analysis (red circle), for an arbitrary cutoff of twofold change in gene expression (blue circle), and, that are found in both (overlap of two circles). The numbers in bold and in brackets represent the total number of DE gene regions and known annotated genes, respectively. The table displays the enriched biological gene ontology (GO) themes from the DE gene lists (the top 10 GO biological process terms order by significance of enrichment *p*-values) and the column n displays the number of term associated genes in the gene list. Numbers in brackets after the terms represent the GO identifier for that term. **c** Box plots of the expression of the genes *PLCβ2* and *SHH* with a known taste cell function. **d** Box plots of the 13 DE genes associated with the top 8 immune and inflammatory GO terms from the analysis of CuffDiff DE genes. The dots in the box plots show outliers (points outside 1.5 times the interquartile range from the first and third quartiles)

Specifically related to taste, the genes sonic hedge-hog (*SHH*) and phospholipase C beta 2 (*PLCβ2*) displayed lower gene expression in the obese compared to lean (Fig. 3c). SHH is a signalling hormone which is critical for the maintenance and renewal of taste cells and the patterning of taste papillae [43]. Likewise, PLCβ2 is a cell marker commonly used to identify type II taste cells and is involved in the transduction of sweet, umami and bitter tastes [44].

Analysis of the 62 differentially expressed genes for enriched biological function identified the top eight enriched pathways were associated with immune and inflammatory response (Fig. 3b). These 8 groups contained a total of 13 genes which represents 21% of the overall differentially expressed genes (Fig. 3d). Ten of these immune-related genes showed higher expression in lean compared to obese.

Genes displaying twofold expression change and enriched biological pathways

All genes having an arbitrary twofold expression change were assessed to identify more broadly the genes and enriched biological pathways involved in the observed separation of whole-transcriptome profiles between the lean and obese groups (Fig. 2). In total, 2619 genes had a twofold expression change (Fig. 3b, Table S1) (31 genes overlapped with the differentially expressed genes identified from CuffDiff).

Analysis for enriched biological pathways identified three common themes (Fig. 3b). The first theme includes terms associated with sensory perception and taste function. This included five of the top ten GO terms, including *'Sensory perception of bitter taste'*, *'Sensory perception of taste'* and *'GPCR signalling pathway'*. Cell and synaptic signalling is the second theme, which also is associated with taste cells (i.e. transduction of a sensory signal), and includes the terms *'Chemical and synaptic transmission'* and *'Neuropeptide signalling pathway'*, which are daughter terms to *'Cell-cell signalling'* and *'GPCR signalling pathway'*, respectively. Finally, terms associated with immune response (*'Chemokine-mediated signalling pathway'* and *'Cell chemotaxis'*) consistent with the enriched themes identified from the CuffDiff gene list.

Analysis of taste gene expression between lean and obese

Finally, the expression profile of taste receptors and genes associated with taste cells was assessed. The expression level of the different taste genes were highly variable, with a significant number of genes having no (i.e. TAS2R7, TAS2R8 and TAS2R9) or a very low (i.e. TAS2R38) expression level (average FPKM < 1).

Analysis of 44 taste genes identified seven additional genes which displayed a greater than 1.5-fold change in gene expression, of which four genes were significant (Kruskal–Wallis analysis p-value < 0.05) (Fig. 4). Additionally, a consistent direction in the gene expression was

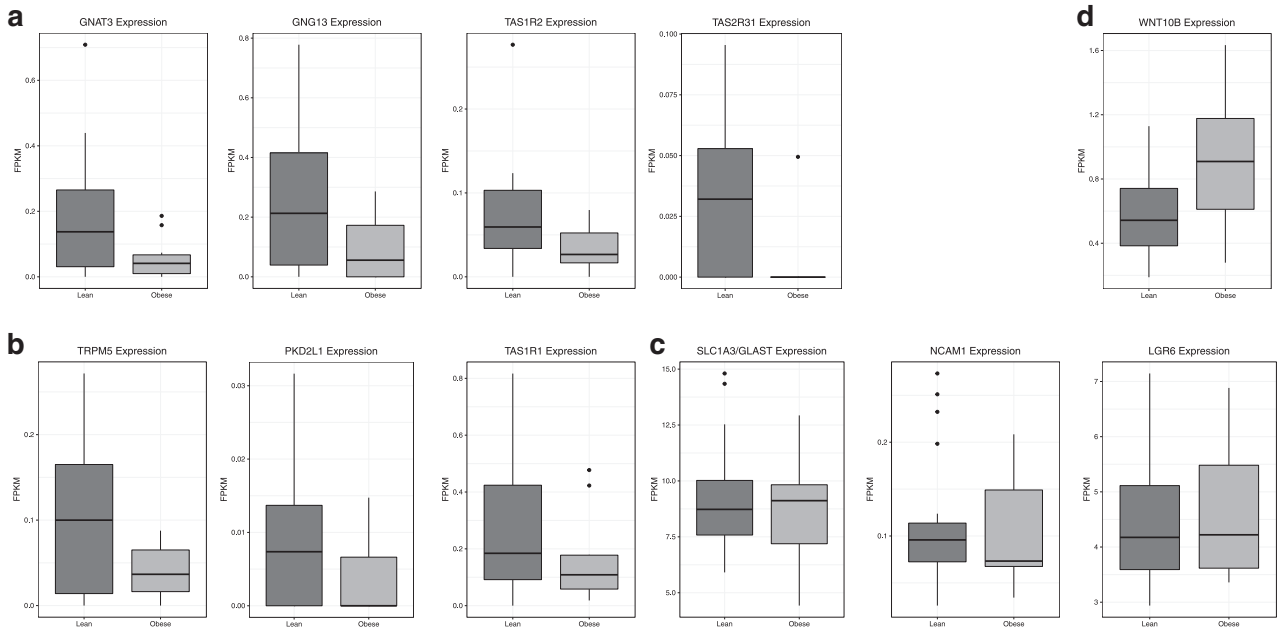


Fig. 4 Analysis of taste gene expression. Taste markers and receptors identified to have 1.5-fold increase in gene expression in lean compared to obese (in addition to PLCβ2 and SHH expression Fig. 3c) **a** having significant difference (Kruskal–Wallis test, p-value < 0.05) or, **b** not significant (p-value > 0.05). **c** Taste markers with similar expression profiles between lean and obese: SLC1A3/GLAST (type I taste cells), NCAM1 (type II taste cells) and LGR6 (taste stem/progenitor cell). **d** WNT10B gene expression with higher expression in obese (p-value < 0.05). The dots in the box plots show outliers (1.5 times the interquartile range from the first and third quartiles)

observed, with reduced expression in the obese compared to lean (9 genes or 20% with 1.5-fold change in gene expression, Fig. 4a, b). In addition to *PLCβ2* and *SHH* (Fig. 3c), this included: (i) further markers of type II taste cells and genes important in taste cell signal transduction: *GNAT3* (encoding α-gustducin), *GNG13* and *TRPM5*; (ii) type II taste receptors *TAS1R2*, *TAS2R31* and *TAS1R1* involved in the detection of sweet, bitter and umami taste, respectively; and, (iii) *PKD2L1* which has been associated with sour taste (Fig. 4a, b). The gene *WNT10B* was the only gene identified to have significantly higher gene expression in obese compared to lean (Fig. 4d).

Interestingly, the majority of the differentially expressed taste genes are of type II taste cell origin (*PKD2L1* is the exception) (Fig. 5). Analysis of markers commonly associated with type I and III taste cells, *GLAST* and *NCAM1* [44], showed no difference in gene expression (Fig. 4c, p-value > 0.05). This suggests change in taste gene expression profiles between lean and obese might be type II taste cell specific. Furthermore, analysis of *LGR6*, a marker for the stem/progenitor cells which develop into taste cells [45], showed no expression difference between lean and obese (Fig. 4c), suggesting that both groups have the same reserve and potential to regenerate taste cells and taste buds (Fig. 5).

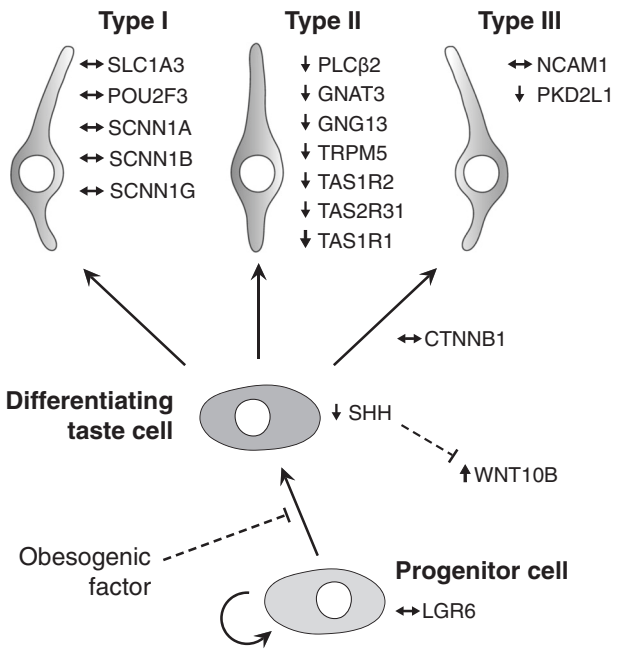


Fig. 5 Overview of taste associated gene expression at different stages of development/differentiation. Taste cells start out as self-renewing population (progenitor cell) which has been associated with the marker LGR6 [45], which differentiate into basal (type IV) taste cells and then into one of three mature taste cells (type I–III) based on the β-catenin level (encoded by *CTNNB1* gene) [67]. The genes with arrows identify genes having a 1.5-fold change in gene expression between lean and obese. Symbols ↔, ↓ and ↑ designate no difference in expression, lower expression in obese and higher expression in obese, respectively. We hypothesise that obesity, through an unknown obesogenic factor/mechanism, might be associated with reduced number of taste buds or taste cells

Discussion

In this study, we identify an altered global gene expression profile of fungiform taste papillae in obese individuals, which is distinct to the expression profile of lean individuals. Fungiform papillae are a collection of different cell types, including taste cells, epithelial cells, connective tissue, blood/immune cells etc. Therefore, the altered expression profiles and clear separation of lean and obese individuals in PCA and MDS plots reflects differences in gene expression over some, or, all of these different cell types.

Obesity is characterised by a systemic chronic subacute inflammatory process where adipose tissue and resident macrophages contribute to the production of pro-inflammatory cytokines [46]. This inflammatory process may explain our observed findings for differential expression of immune genes and the enrichment of immune/inflammatory biological terms, and, therefore accounts for a portion of the variability in the global expression profiles of fungiform papillae between lean and obese groups. Likewise, differences in the physiology of the taste buds/cells between lean and obese also contributes to the variability in the global gene expression profiles, given the number of taste genes identified and the enrichment for taste and cell signalling associated biological terms.

This study reports for the first time-specific down-regulation of many taste receptors in the obese, compared to the lean group. Of the taste genes identified in this study, we observe consistency for lower taste expression in the obese group, compared to the lean group, consistent with findings in animal studies in both oral and extra oral taste tissue [20, 47], or reduced taste cell responsiveness [48]. Only *WNT10B* was identified to have higher expression in obese.

Tastebuds are a collection of 50–100 taste cells and are composed of four different cell populations that have distinct function/role in taste perception. Type I and III cells are associated with salt and sour taste, respectively, type II cells are responsible for sweet, bitter and umami taste detection, and, stem/progenitor cells are involved in the replacement of taste cells [49]. The majority of taste genes showing expression differences between lean and obese were of type II taste cell origin, including commonly used type II taste cell markers *PLCβ2* and *GNAT3* (encoding α-gustducin) and taste receptors, while analysis of markers/receptors for type I and type III taste cells showed similar expression level between lean and obese groups (Fig. 5).

The expression of sour taste associated *PKD2L1* being the exception (type III cells). These findings suggest that obesity may exert specific changes in the gene expression of type II taste cells, however, further studies will be required to prove such an effect, especially given the taste cell markers analysed have been identified in rodent studies which may not always reflect human physiology [50].

Several possibilities may account for the observed reduced expression of taste genes in the obese compared to the lean. This could include (i) reduction in the number of taste cells/buds in obese (i.e. either through increased apoptosis of taste cells and/or reduced replacement of taste cells), or, (ii) the downregulation of specific taste genes within obese taste cells, with no change in the total number of taste cells. From our results, we hypothesise that obesity might be associated with reduced number of taste buds and/or taste cells. Supporting this is our finding of reduced *SHH* expression in the obese group and increased expression of *WNT10B*, with SHH being shown to negatively control and WNT10B levels i.e. decreased SHH increases WNT10B (consistent with the results presented) (Fig. 5) [51]. *SHH* is important for embryonic taste development and controls the renewal of taste cells and the number of tastebuds throughout life [43, 52]. Animal studies have shown that under or over expression of *shh* is associated with a loss-of-taste cells [43] or with additional ectopic development of tastebuds outside of normal papillae location [53], respectively. Additionally, use of hedgehog inhibitors in the treatment of human cancers is associated with taste loss [54, 55]. Therefore the reduced *SHH* in obese may be accompanied by reduced number of taste cells or an impairment in their replacement. A recent mice study provides additional evidence for alteration of number of tastebuds in obesity. This study identified a reduction in tastebud abundance in mice that were fed a high-fat diet driven by inflammatory factor TNF-α [56]. These results are complementary to the findings presented in the current study suggesting inflammatory factors produced in an obese state have an impact on tastebud apoptosis/renewal (Fig. 5).

The results of this study show clear differences in taste cell gene expression between lean and obese individuals. In particular, a significant reduction in *PLCβ2* expression was observed in obese and knockout studies in animals show a loss of PLCβ2 is associated with a loss/reduction in taste sensitivity/responsiveness [57, 58]. Therefore, it is unclear why human studies do not show similar clear associations between taste acuity and obesity status. A major limitation of these studies may be the small sample sizes which lack the statistical power to identify an association. This is highlighted by larger studies which have identified associations between obesity and taste acuity [7, 9]. Differences in the taste stimuli used could be another possibility for inconsistent associations. While, we observe an overall trend for reduced taste gene expression in obese compared to lean, not all of the taste receptors genes were equally impacted. Thus inconsistent results could be obtained through the use of different taste stimuli activating receptors that are differentially modulated by an obese state. Likewise, observed differences in gene expression may solely function to impact unconscious taste measures in obese like altered neural, satiety or hormonal responses [55, 59]. Further research is required to assess the impact that obesity has on taste cell expression and taste function.

An interesting finding from the study was the minimal impact of age on global fungiform papillae gene expression. The current age range of the study population was tightly controlled (18–55 years) and the upper age range is likely lower than age-related impacts on taste bud density or taste acuity [60]. Furthermore, participants were screened to ensure they did not suffer from other chronic diseases that maybe associated with taste loss or drugs that impair taste, which are common causes for sensory decline in the elderly [61]. Additionally, no information was collected on participant's oestrous cycle. It is unlikely that oestrous cycle has an impact on taste cells/buds given that (i) there are no reports of taste receptor cells expressing oestrous hormone receptors, and (ii) changes in food preferences over the oestrous cycle likely function to alter brain reward mechanisms [62]. However, the impact of oestrous cycle may be an interesting area to explore in future studies.

This study is the first to report the analysis of whole transcriptome of human fungiform papillae. Analysis of the whole-fungiform papillae was undertaken instead of analysing individual tastebuds (or taste cells) to observe a snapshot of the gene profile with no transcriptional changes introduced due to a delay in processing and storage of fungiform papillae. Further, the analysis of whole-fungiform papillae provides important additional information about the physiological environment of the papillae which may impact on the tastebud/cells function. Methods are available to isolate only taste cells/buds [63, 64] and these will be of interest to next determine the global gene expression of tastebuds or individuals taste cells.

Conclusion

In conclusion, we show a clear difference in the gene expression profile of human fungiform papillae between lean and obese individuals. The results suggest a distinctly different tastebud microenvironment exists in obese individuals which may contribute to changes in taste bud/cell function. Taste cells are constantly being replaced, with an

average lifespan of 7–24 days, dependant on cell type [65, 66]. Due to the quick turnover and replacement, taste buds and the differentiating/maturing taste cells would be highly responsive to this altered microenvironment. We hypothesise that obesity, via an obesogenic factor(s), might reduce the number of taste buds/cells, preferentially influencing type II taste cells. Of future interest is whether the observed obese expression profile can be *'reversed'* back to a lean profile. Indeed, human weight loss studies suggest that this might be possible. This research provides new evidence of a link between obesity and taste, deepening our understanding of obesity, and in the future, may contribute to help design strategies to combat obesity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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