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Maternal obesity regulates gene expression in the hearts of offspring

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Abstract *Background and aims:* Growing evidence suggests maternal obesity leads to adverse outcomes for offspring, including increased cardiovascular disease (CVD). Alterations in taste preferences of offspring from mothers consuming a high fat diet (HFD) have also been reported. Given recent reports describing cardiac taste receptors, we examined whether the expression of umami and bitter taste receptors is modulated by maternal obesity, and compared this with the physiological challenge of maternal exercise.

Methods and results: Female Sprague-Dawley rats were fed chow (C) or HFD (F) and half of each were provided with a running wheel to enable voluntary exercise (CE or FE), the others remaining sedentary (CS or FS). Two pups from each mother were killed at postnatal day 19.

Both lean and obese dams undertook similar amounts of exercise (8.1 ± 2.4 vs 5.1 ± 1.5 km). Maternal obesity increased offspring body weight, adiposity, net and weight-corrected heart ventricle weight, with no effect of exercise. Maternal obesity also increased offspring plasma leptin concentrations, which were normalised by maternal exercise. Cardiac ventricle mRNA expression of bitter taste receptors, β-adrenoceptor (Adrbk1) and angiotensin II receptor type 1a (Agtr1a) were significantly decreased in response to maternal obesity, with maternal exercise decreasing Agtr1a in FE offspring. No changes in umami receptors were observed. FTO mRNA expression was down-regulated by maternal HFD with an up-regulation in offspring of CE mothers.

Conclusion: Maternal obesity affected the expression of bitter taste receptors and other genes in the heart ventricle, potentially implicating these genes in the development of CVD associated with maternal obesity.

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Introduction

In recent decades obesity has become a global health epidemic, but it is increasingly recognised to have the potential to influence the next generation through effects on women of reproductive age [1]. Marked increases in maternal obesity have been observed in the last 20 years [2]. A large body of evidence supports programming effects of obesity during development, whereby maternal and early post-natal nutrition can affect the development of metabolic and neural pathways involved in energy homeostasis, leading to disease in adulthood [3]. Thus addressing the implications of maternal obesity for offspring outcomes

Acronyms: CVD, cardiovascular disease; Agtr1a, angiotensin II type 1a receptor; Agtr2, angiotensin II type 2 receptor; GPCR, G-protein coupled receptor; Tas1r, type 1 taste receptor; Tas2r, type 2 taste receptors; Adrbk1, β-adrenoceptor; PND, post-natal day; FTO, fat mass and obesity related gene; HFD, high fat diet; CS, chow sedentary; CE, chow exercise; FS, fat sedentary; FE, fat exercise.

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in terms of obesity, insulin resistance and cardiovascular disease (CVD) is important.

It is clear that the heart is significantly affected by maternal obesity with a well-established link between maternal obesity and CVD in offspring in both human and animal studies [4]. Offspring of obese and overweight mothers have a significantly increased risk of cardiovascular anomalies [5], and obesity during pregnancy has been linked to increased risk for CVD in adult human offspring [6]. However there is currently a limited mechanistic understanding of how maternal obesity causes foetal programming of CVD; altered sympathetic nervous activity and adrenoceptor expression, have been reported in offspring of obese dams [7]. Recently, it was shown that male offspring from obese mice dams had increased cardiac sympathetic dominance associated with an up-regulation of β1 adrenergic receptors at 12 weeks of age [8].

The renin-angiotensin system is also implicated in the development of CVD associated with obesity, angiotensin II being a primary contributor [9]. Angiotensin II exerts its effects on the heart and vessels largely through angiotensin receptor type 1 (Agtr1a). A recent study reported that over-nutrition through litter size adjustment in rats during lactation up-regulated Agtr1a in the myocardium [10].

Interventions to address the adverse effects of maternal obesity are needed. Studies in rats indicate that exercising the offspring can limit some of the detrimental effects of being gestated in an obese mother [11,12]. A different approach is promoting exercise during pregnancy. Whilst maternal exercise has been proposed as a means of limiting gestational weight gain, there are no clear guidelines [13], with few experimental studies directly investigating the potential of maternal exercise as an intervention. One recent study in rodents reported that whilst exercise had no effect on maternal weight, improvements in offspring glucose, leptin and triglyceride concentrations were observed [14].

Another fascinating aspect of maternal obesity is potential effects on offspring food preference. Maternal diet during gestation and lactation in humans can affect flavour preferences at weaning [15], and animal studies showed that a maternal 'junk food' diet leads to an increased preference for fat/sugar in offspring [16,17]. However at present it is unclear whether maternal obesity regulates specific taste receptors in offspring. Flavours are sensed by five taste receptors; sweet, umami and bitter G-protein coupled receptors (GPCR), and salty and sour ion channels. Whilst taste receptors were originally assumed to be exclusively expressed in the oronasal cavity, recently these taste receptors have been identified in extra-oral tissues [18]. Recently umami (type 1) & bitter (type 2) taste receptors were identified in both human and rodent hearts [19]. In mice the receptors were developmentally regulated, and bitter taste receptor expression was affected by nutritional status, suggesting these receptors may be involved in nutrient detection. Thus we were interested to test whether taste receptors in the heart are regulated by maternal obesity, a state of nutritional excess.

In light of the recent discovery of cardiac taste receptors, the present study examined the effects of maternal obesity and maternal exercise in rats on the expression of the β-adrenoceptor (Adrbk1), angiotensin II receptor type 1a (Agtr1a), and a subset of taste receptors (Tas1r1, Tas1r3, Tas2r126 and Tas2r143) in the cardiac ventricles of male offspring at weaning, post-natal day (PND) 19. As we had previously observed changes in fat mass and obesity related gene (FTO) expression in maternal obesity and exercise, FTO was measured. We were interested in exploring the possibility of relationships between physiological measures and changes in gene expression.

Methods

Maternal diet and tissue collection

All procedures involving animals were approved by the Animal Care and Ethics Committee, UNSW Australia (Approval No. 11/104B). Young female Sprague Dawley rats (n = 56) aged 6 weeks, 160−170 g, were obtained from the Animal Resources Centre, Perth, Australia. Rats were given either regular chow (Gordon's Stockfeeds, NSW, Australia) or commercial high fat diet (HFD) (Specialty feeds, NSW, Australia) in addition to Western foods (i.e. cakes, pies, dim sims, biscuits) *ad libitum* for 6 weeks to generate lean (n = 17) and obese (n = 15) dams. Half of each group were given an exercise wheel, whilst the other half were given a locked wheel and remained sedentary. Mating with chow fed males began 8−10 days after exercise was introduced. Rats remained on their respective diets throughout mating, gestation and lactation and exercise continued until the end of pregnancy. This generated four offspring groups; from lean sedentary (CS; n = 16), obese sedentary (FS; n = 17), lean exercised (CE; n = 14) and obese exercised (FE; n = 12) mothers.

Following birth, dams and pups were transferred to cages without exercise wheels. At PND1 litters were adjusted to 12 pups per dam. At PND19, 1−2 male pups per dam were culled under non-fasted conditions following injection of ketamine:xylazine 180:32 mg/kg and then decapitated. The heart ventricles were dissected, snap frozen and stored at −80 °C.

Real time quantitative polymerase chain reaction (RT-qPCR)

RNA was extracted using Tri-reagent (Sigma, USA) and treated with DNase to remove any genomic DNA contamination. One μg RNA was reverse transcribed to cDNA (Omniscript Reverse Transcription kit; Qiagen, Chatsworth, California, USA). An appropriate cDNA concentration for the genes of interest was determined using pooled samples. 384 well plates were run on a Roche LightCycler 480, software release 1.3.0.0705. Two housekeeper genes that exhibited the least variation and most stability across treatment groups (Ywhaz and Gapdh) were selected using Norm-Finder (Table 1). The geometric mean was used to

Table 1 Taqman probe sequences used for real time PCR.

Gene symbol	Gene name	NCBI gene reference	Applied biosystems assay
Reference genes			
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	NM_017008.3	Rn01775763_g1
Ywhaz	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	NM_013011.3	Rn00755072_m1
Genes of interest			
Adrbk1	Adrenergic, beta, receptor kinase 1	NM_012776.1	Rn00562822_m1
Agtr1a	Angiotensin II receptor, type 1a	NM_030985.4	Rn02758772_s1
FTO	Fat mass and obesity associated	NM_001039713.1	Rn01538187_m1
Tas1r1	Taste receptor, type 1, member 1	NM_053305.1	Rn01516038_m1
Tas1r3	Taste receptor, type 1, member 3	NM_130818.1	Rn00590759_g1
Tas2r126	Taste receptor, type 2, member 126	NM_139335.1	Rn00595098_s1
Tas2r143	Taste receptor, type 2 member 143	NM_001025061.1	Rn02585801_s1

Abbreviations: Gapdh, glyceraldehyde-3-phosphate dehydrogenase; Ywhaz, tyrosine 3-monooxygenase/tryptophan 5- monooxygenase activation protein, zeta polypeptide; Adrbk1, adrenergic, beta, receptor kinase 1; Agtr1a, angiotensin II receptor, type 1a; FTO, fat mass and obesity associated; Tas1r1, taste receptor, type 1, member 1; Tas1r3, taste receptor, type 1, member 3; Tas2r126, taste receptor, type 2, member 126; Tas2r143, taste receptor, type 2, member 143.

determine Ct values of the housekeeper genes and expression values for the genes of interest were calculated using ΔΔCT methodology.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.00 for Windows (GraphPad Software, La Jolla California USA); results are expressed as mean ± SEM. Data were analysed by two-way ANOVA (maternal diet x maternal activity), followed by post hoc LSD test and $P < 0.05$ was considered to be statistically significant. If data was not normal a $\log_{10}$ transformation was performed to normalise the data prior to analysis. Correlations were computed using Pearson correlation coefficients, and fitted with linear regression lines.

Results

Maternal obesity and exercise level

As shown previously using this model of maternal obesity [12], dams consuming the HFD gained more weight and were significantly heavier (lean dams 251.9 ± 3.1 g, versus obese dams 296.3 ± 5.3 g) prior to the commencement of exercise.

Both groups of dams showed similar modest levels of exercise (8.1 ± 2.4 versus 5.1 ± 1.5 km total distance run in lean and obese dams respectively). When dams were weighed at the end of the experiment HFD dams were significantly heavier than chow fed dams, irrespective of exercise level ($P < 0.01$).

Pup bodyweight, ventricle weight and leptin concentrations

Maternal HFD significantly increased the body weight of offspring at PND19 (Table 2 $P < 0.001$), with an interaction observed between maternal diet and maternal exercise (Table 2 $P < 0.05$). Maternal HFD was associated with an increase of over 150% in total white adipose tissue mass (Table 2 $P < 0.001$). Similarly, plasma leptin concentrations were more than double in the maternal HFD groups (Table 2 $P < 0.001$), however in offspring of mothers on a HFD, maternal exercise led to a significant reduction in plasma leptin compared to the offspring of sedentary mothers on the same diet (Table 2 $P < 0.05$).

There was a strong positive correlation between body weight and heart ventricle weight ($P < 0.0001$, $r = 0.9713$). Heart weight was higher in both FS and FE groups compared to CS and CE respectively, in terms of net weight (Table 2 both $P < 0.001$) and when corrected for body weight (both $P < 0.01$).

Ventricle mRNA expression

Two-way ANOVA revealed an overall effect of diet for both Tas2r126 and Tas2r143 mRNA expression ($P < 0.001$). Decreases in Tas2r126 and Tas2r143 expression, were apparent in FS ($P < 0.01$) and FE ($P < 0.001$) compared to lean maternal counterparts, as shown in Fig. 1. Moreover for Tas2r126 and Tas2r143, mRNA expression was negatively correlated with body weight (Fig. 2 A, B $P < 0.001$) and heart ventricle weight (Fig. 2C, D $P < 0.001$). Unsurprisingly given the correlation with body weight, a similar relationship with plasma leptin concentrations was observed ($P < 0.001$ Tas2r126 r-0.5929, Tas2r143 r-0.5444).

For Tas1r1 and Tas1r3 receptors no differences in mRNA expression were observed across treatment groups (Fig. 1, C and D).

FTO mRNA expression exhibited an overall effect of diet ($P < 0.001$) and of exercise ($P < 0.05$; Fig. 3A). Exercise in lean mothers increased offspring FTO expression compared to offspring of lean sedentary mothers ($P < 0.05$). Maternal HFD decreased FTO expression in both FS ($P < 0.05$) and FE ($P < 0.001$) compared to pups from mothers fed chow.

Adrbk1 mRNA expression showed an overall effect of diet (Fig. 3B $P < 0.05$) with maternal HFD decreasing expression. Agtr1a exhibited a down regulation with maternal HFD (Fig. 3C) in both the FS ($P < 0.01$) and FE

Table 2 Offspring body weight, total WAT, plasma leptin and heart ventricle net weight.

Group	CS (17)	CE (14)	FS (16)	FE (12)	Interaction
Body weight (g)	33.11 ± 0.78	30.44 ± 0.47	47.42 ± 1.64[a]	49.82 ± 1.75[a]	[b]
Total WAT (g)	0.26 ± 0.02	0.23 ± 0.02	0.66 ± 0.06[a]	0.71 ± 0.07[a]	–
Plasma leptin (ng/ml)	6.50 ± 0.52	6.20 ± 0.42	16.4 ± 0.93[a]	14.1 ± 0.91[a,c]	–
Ventricle weight (g)	0.14 ± 0.00	0.13 ± 0.00	0.21 ± 0.01[a]	0.23 ± 0.01[a]	–

WAT-white adipose tissue.
Data are expressed as mean ± SEM. First letter indicates maternal diet, chow (C) or HFD (F); second letter indicates maternal activity, sedentary (S) or exercise (E). Total WAT represents the sum of retroperitoneal, gonadal and visceral fat pads. Data were analysed by 2-way ANOVA with maternal diet and maternal exercise as factors, followed by post hoc.
[a] $P < 0.001$ maternal diet effect.
[b] $P < 0.05$ maternal diet-maternal exercise interaction.
[c] $P < 0.05$ maternal exercise effect.

($P < 0.001$) offspring compared to controls. Agtr1a expression also decreased with maternal exercise in FE ($P < 0.01$) compared to FS pups.

Two-way ANOVA revealed no significant interactions between maternal diet and maternal activity level for any of the genes tested.

Discussion

The major finding of this study was a significant down-regulation of type 2 bitter taste receptor expression (Tas2r143 and Tas2r126) in the cardiac ventricles of rats born to obese mothers. This effect of maternal obesity was selective for bitter type receptors as umami receptor expression was unaffected. Significant decreases in expression of FTO, β-adrenoceptor and angiotensin II type 1a receptors were also observed. Limited effects of maternal exercise were observed, with a significant increase in FTO in offspring of lean exercised mothers, and a decrease in Agtr1a in offspring from obese exercised mothers compared to obese sedentary mothers.

The decreases observed in Tas2r143 and Tas2r126 expression supports findings by Foster et al. [19] that these

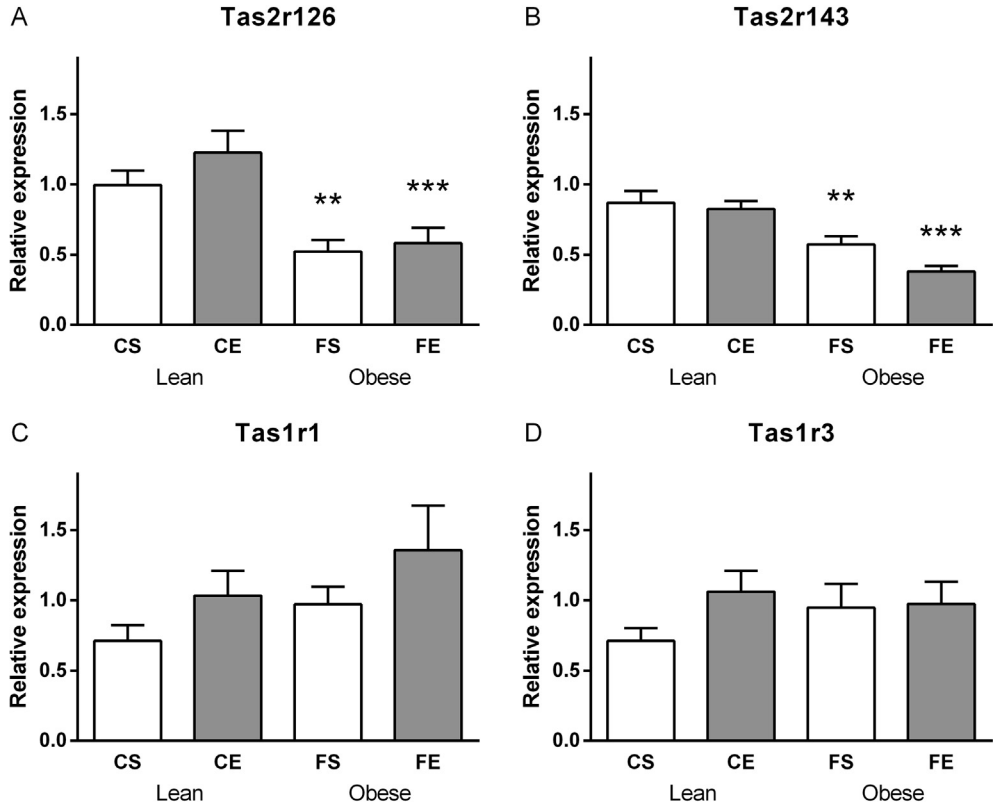

Figure 1 Heart ventricle mRNA expression of type 1 & 2 taste receptor genes in offspring. Data are expressed as mean ± SEM (n = 9–15) of offspring from sedentary (open bars) and exercise (closed bars) dams. The first letter indicates maternal diet; chow (C) or HFD (F) and the second letter indicates maternal activity; sedentary (S) or exercise (E). Data were analysed by two-way ANOVA (maternal diet x maternal activity) followed by post-hoc comparison. **, P < 0.01; ***P < 0.001 maternal diet effect.

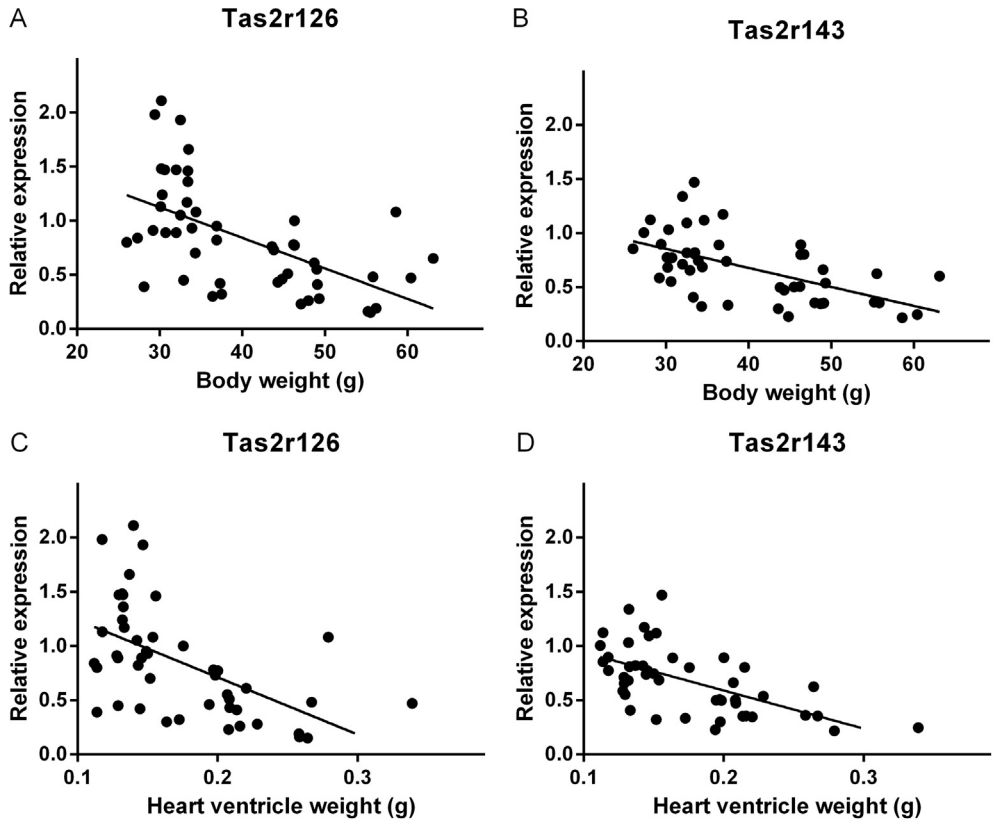

Figure 2 Relationship between Tas2r126 & Tas2r143 mRNA expression and body weight and heart ventricle weight. Significant correlations ($P < 0.001$, n 48–49) were observed between Tas2r126 and body weight ($r = -0.5726$) (A), Tas2r143 and body weight ($r = -0.5764$) (B), Tas2r126 and heart ventricle weight ($r = -0.5527$) (C) and Tas2r143 and heart ventricle weight ($r = -0.5982$) (D).

taste receptors are nutritionally regulated. Thus 48 h of starvation increased expression of these receptors in rat hearts [19] whilst we observed down-regulation in response to chronic nutritional excess. At the time of kill, day 19, the weanling offspring were heavier and fatter, and were consuming milk that would presumably be richer in fats and sugars. Offspring started to eat solid food from postnatal days 16–17 in their home cage. Thus the down-regulation of bitter receptors may be linked to altered dietary composition. Minimal research into the effect of diet on bitter taste receptor expression has been published. An evolutionary study implicated the amount of plants in the diet as a driving force in the interspecies differences in bitter taste receptor repertoires [20]. One study in mice, showed a diet-induced regulation of bitter taste receptor subtype in the gastrointestinal tract [21]. A low cholesterol diet increased bitter taste receptor 138 transcription, but not bitter taste receptor 108 in duodenum and jejunum, but not in ileum and colon [21]. The authors speculated that increases in Tas2r expression may act to prevent the consumption of toxic compounds that are high in plant based foods. A low dietary cholesterol intake has been linked to increased Tas2r expression in murine small intestine through increased SREBP-2 activity [22]. Whether dietary cholesterol intake affects the expression of bitter taste receptors in cardiac tissues is unknown, however it is a plausible mechanism driving changes in receptor expression observed in this incidence. In contrast, the finding by Foster and colleagues [19] of an up-regulation in bitter receptors in cultured cardiomyocytes under glucose deprivation may suggest that the down-regulation of bitter taste receptors in this study could be related to increased sugar intake.

Our results may suggest a nutrient sensing function of these cardiac receptors; however this is still an area of speculation. In other extra-oral tissues type 2 taste receptors are believed to play diverse roles including release of appetite regulating hormones in the brain [23] and the activation of protective airway reflex in response to inhaled toxins and irritants [24].

Interestingly recent work shows that bitter taste receptor agonists cause negative inotropy in murine hearts [25], with a concentration-dependent decrease in left ventricular developed pressure and an increase in aortic pressure. While the physiological implications of this finding are not clear, this is a potential mechanism that may contribute to increased CVD risk in offspring of obese mothers. Although strong negative correlations were observed between Tas2r126 and Tas2r143 mRNA expression and body weight, heart weight and plasma leptin concentrations, it is difficult to determine a causal effect.

The absence of umami taste receptor (Tas1r1 and Tas1r3) expression changes is in line with Foster and colleagues [19], who found no changes in Tas1r1 or Tas1r3

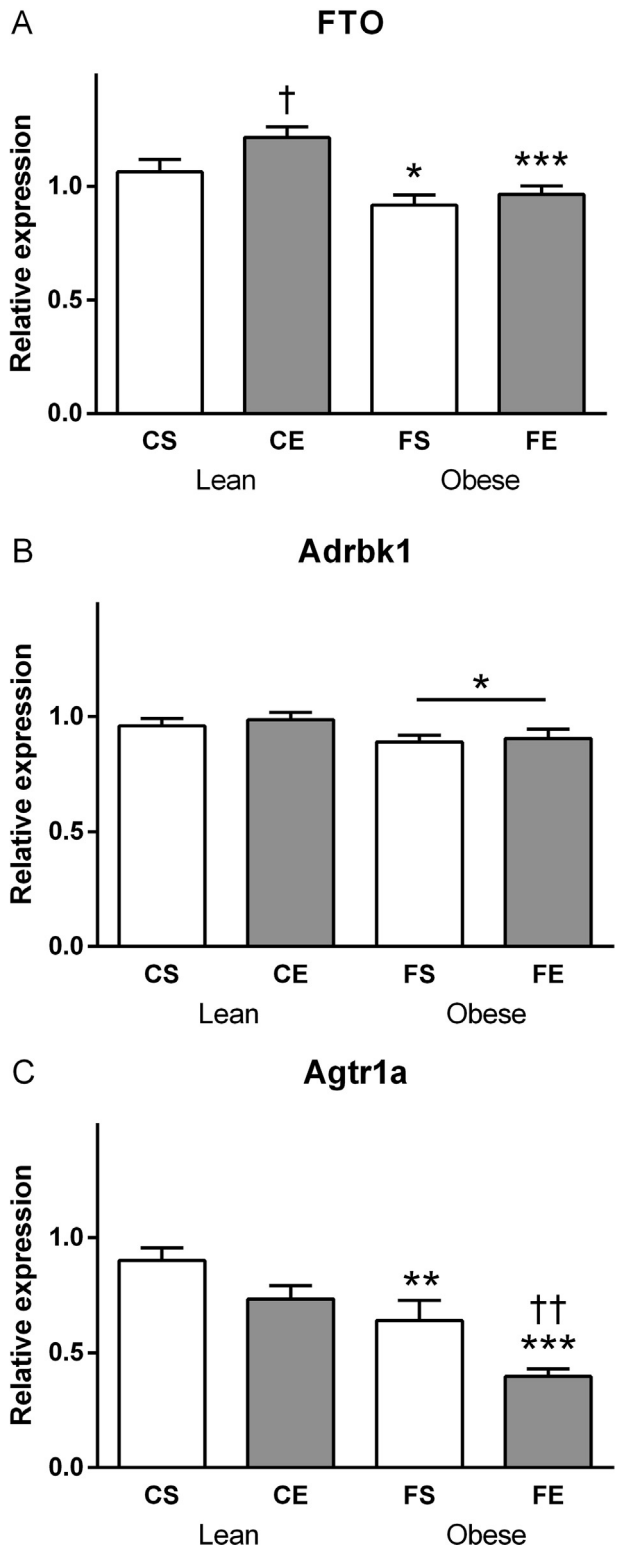

Figure 3 Heart ventricle mRNA expression of genes of interest in offspring. Data are expressed as mean ± SEM (n = 11–15) of offspring from sedentary (open bars) and exercise (closed bars) dams. The first letter indicates maternal diet; chow (C) or HFD (F) and the second letter indicates maternal activity; sedentary (S) or exercise (E). Data were analysed by two-way ANOVA (maternal diet x maternal activity) followed by post-hoc comparison. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ maternal diet effect; †, $P < 0.05$, ††, $P < 0.01$ maternal exercise effect.

expression following 48 h starvation in rats. It has previously been suggested that umami taste receptors may act as amino acid sensors in extra-oral tissues, with involvement in activation of the autophagy pathway [26]. However the functional role of umami receptors in the heart and whether nutrient intake affects their expression is yet to be clarified. It may be that umami receptors exert different physiological roles in the heart.

The role of taste receptors in the heart requires further investigation to better understand their physiological function. Once the ligands for these taste receptors in extra-oral tissues have been identified, their pharmacological potential as drug targets can be explored. It has already been hypothesised that type 2 taste receptors mediate off-target drug effects [27], however there is potential for them to become drug targets in their own right. As Foster and colleagues [19] observed an up-regulation of type 2 taste receptors in cultured neonatal rat ventricular myocytes subjected to 24 h glucose deprivation, it is important to direct future studies to elucidate the exact roles of the type 2 taste receptors in the heart, with a focus on glucose and other nutrients.

The decrease in Adrbk1 mRNA expression is in keeping with a report showing that maternal obesity increased offspring sympathetic activity at PND 30 [7], which would be expected to affect β-adrenoceptor expression. As these receptors are developmentally regulated, it is interesting to compare our findings to a recent study which found an up-regulation of Adrbk1 in the offspring of obese mice mothers at 12 weeks of age [8]. This suggests that the decrease we observed may be transient. In terms of Agtr1a a reduction in expression was also observed in response to maternal obesity. As another developmentally regulated gene this change in expression may be specific to this age, and it has been shown that post-natal over-nutrition up-regulates Agtr1a in myocardium [10]. It is interesting to note that a further down regulation of Agtr1a was observed in offspring of exercising mothers consuming a HFD. It would be interesting to examine angiotensin II levels in both the mothers and offspring to observe the relationship with receptor expression.

The reduction observed in cardiac FTO expression in offspring of obese mothers is thought-provoking as recent research using cultured neonatal rat cardiomyocytes shows that leptin led to an increase in FTO expression [28]. A study on sheep found no immediate effect of prenatal over-nutrition on FTO expression, but offspring from mothers with a restricted nutrient intake that were subsequently subjected to an 'obesogenic' environment and went on to become obese had markedly decreased cardiac FTO expression compared to either lean controls, or obese sheep from mothers that did not experience nutrient restriction [29]. Thus it would appear that cardiac FTO expression is affected by obesity and maternal programming, making it another interesting candidate gene for the CVD risk observed with maternal obesity. FTO expression was also regulated by exercise; our lab has previously shown an effect of exercise on FTO

expression in the hypothalamus [30], and here maternal exercise increased FTO expression in offspring of dams consuming chow.

Both the renin-angiotensin and sympathetic nervous systems are involved in the link between obesity and hypertension [31], with early work in rodents showing that chronic overeating increases sympathetic activity [32]. In humans, hypertension is related to elevations in plasma catecholamines however other factors appear to be involved, as removal of autonomic influences does not resolve increased systolic blood pressure in obese subjects [33].

A novel finding of this study was a reduction in cardiac FTO mRNA expression in response to maternal HFD. In contrast was FTO increased in offspring of exercised mothers consuming chow, which may hint at a role of this novel obesity gene in cardiac function. This is the first study to investigate the effect of maternal obesity on the expression of taste receptors in the hearts of offspring. The finding that maternal HFD selectively down-regulated both of the bitter taste receptors studied implies a response to the nutritional status of the weanling rat offspring; importantly no such reduction was seen in the umami taste receptor.

Conflict of interest

The authors declare no conflict of interest.

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