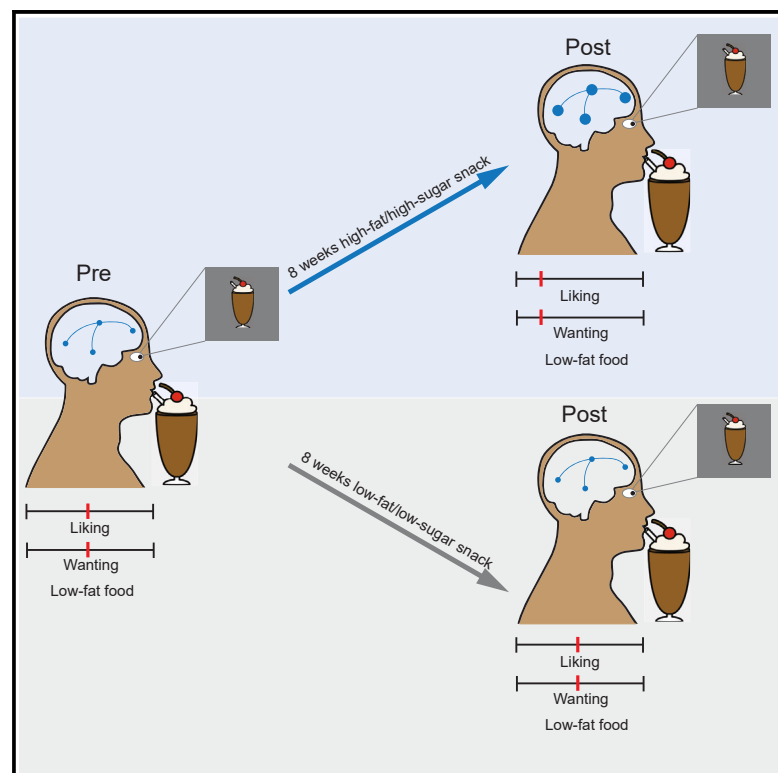


### Habitual daily intake of a sweet and fatty snack modulates reward processing in humans

#### Graphical abstract



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#### In brief

Edwin Thanarajah et al. show that daily snacking on an unhealthy food reduces preference for low-fat food and rewires brain reward circuits to enhance response to a palatable food and upregulate neural computations supporting learning beyond ingestive behavior. Effects are observed despite no change in body weight or metabolic health.

#### Highlights

- Daily consumption of a high-fat/high-sugar snack alters reward circuits in humans
- Preference for low-fat food decreases while brain response to milkshake increases
- Neural computations that support adaptive associative learning are also enhanced
- Effects are observed despite no change in body weight or metabolic health



## Clinical and Translational Report

## Habitual daily intake of a sweet and fatty snack modulates reward processing in humans

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## SUMMARY

Western diets rich in fat and sugar promote excess calorie intake and weight gain; however, the underlying mechanisms are unclear. Despite a well-documented association between obesity and altered brain dopamine function, it remains elusive whether these alterations are (1) pre-existing, increasing the individual susceptibility to weight gain, (2) secondary to obesity, or (3) directly attributable to repeated exposure to western diet. To close this gap, we performed a randomized, controlled study (NCT05574660) with normal-weight participants exposed to a high-fat/high-sugar snack or a low-fat/low-sugar snack for 8 weeks in addition to their regular diet. The high-fat/high-sugar intervention decreased the preference for low-fat food while increasing brain response to food and associative learning independent of food cues or reward. These alterations were independent of changes in body weight and metabolic parameters, indicating a direct effect of high-fat, high-sugar foods on neurobehavioral adaptations that may increase the risk for overeating and weight gain.

## INTRODUCTION

All organisms must procure energy to survive. Consequently, many strategies have evolved to optimize the detection, acquisition, use, and storage of energy sources. For example, environmental signals become associated with nutritional outcomes and are then subsequently employed by organisms as sensory “feedforward” cues that anticipate future consumption and restoration of energy balance.<sup>1–8</sup> A previously neutral sign of your favorite pastry shop, for instance, becomes associated with donut consumption—the sign (or “cue”) is imbued with the power to shape future complex behaviors to acquire another donut, even in the absence of hunger.

The critical internal signals that shape this sensory association learning are generated during nutrient ingestion and are conveyed subliminally to the central nervous system so that the nutritional value of foods and cues predicting this value can be learned.<sup>9</sup> For instance, when intestinal cells sense fat, a signal is generated and conveyed by the vagus nerve to the brain to regulate dopaminergic function, value encoding, and motivational drive.<sup>10,11</sup> Similarly, the ability of sugar consumption to recruit dopamine-responsive striatal circuitry and evoke motivated behavior is contingent upon the generation of signals produced when cells use glucose for fuel, i.e., glucose oxidation.<sup>12</sup> Accordingly, in humans the magnitude of an fMRI response to a calorie-predictive food cue is proportional to the metabolic signals



generated when consuming that food or beverage.<sup>13–15</sup> For instance, de Araujo et al.<sup>14</sup> demonstrated that immediate brain response to a non-caloric beverage was closely related to alterations in plasma glucose that the beverage induced when consumed with calories; in other words, the stronger neuronal signal reflects greater available energy.

This fundamental link between the sensory feedback and the energetic properties of food has important implications for understanding the processes by which the modern food environment promotes obesity. First, there is extensive evidence that sensory association learning, and the consequent power of a cue to control behavior (i.e., food cue reactivity), varies considerably across individuals and is associated with risk for weight gain.<sup>16,17</sup> Second, many modern processed foods are high in energy density and frequently contain both fat and sugar, which interact to potentiate reinforcement beyond the energetic value.<sup>18,19</sup> Modern processed foods are therefore potent reinforcers and, as with drugs of abuse, animal models have shown that their frequent consumption rewires brain circuits,<sup>11,20–27</sup> even in offspring born to mothers consuming a high-fat diet (HFD) during lactation.<sup>28</sup>

Also akin to addictive drugs, there is evidence that this rewiring promotes further consumption of highly palatable energy-dense foods. Providing rats with extended access to an HFD results not only in weight gain but also in adaptations to dopamine signaling and function as well as persistent decreased preference for chow following the withdrawal of the HFD.<sup>25</sup> Similarly, maintaining mice on an HFD blunts the vagal afferent feedback signal generated during fat ingestion,<sup>11,29</sup> resulting in decreased striatal dopamine release in response to intragastric infusion of lipids and reduced preference for low-fat foods.<sup>11</sup> HFDs can also blunt hypothalamic response to food cues associated with a lasting devaluation of nutritionally balanced standard chow—even in a calorie-restricted state.<sup>27</sup> Notably, effects where preference is shifted away from low-fat foods emerge as early as 24 h after starting the HFD and can occur in the absence of weight gain or change in metabolic markers.<sup>27</sup> Likewise, maintaining mice on a saturated (palm oil) versus isocaloric monounsaturated fat (olive oil) diet blunts striatal dopamine signaling and the reinforcing effects of amphetamine; both effects are unrelated to caloric intake, weight gain, and plasma levels of leptin, insulin, and glucose.<sup>24</sup> Collectively these preclinical studies provide strong support for the notion that an HFD shifts preference away from low-fat foods.

Strikingly, HFDs, in the absence of weight gain, can also enhance incentive motivation responses to calorie-predictive cues in the context of reduced motivation for food-seeking itself, as assessed by progressive ratio testing.<sup>30</sup> A single high-fat meal can also produce lasting strengthening of excitatory synaptic transmission onto dopamine neurons in mice,<sup>26</sup> consistent with the critical role of dopamine in driving associative learning with food cues to promote food intake and incentive sensitization.<sup>1,31–34</sup> Thus, like addictive drugs, there is evidence for a causal role of diet (i.e., fat/sugar) in rewiring brain circuits to promote further seeking of energy-dense foods.

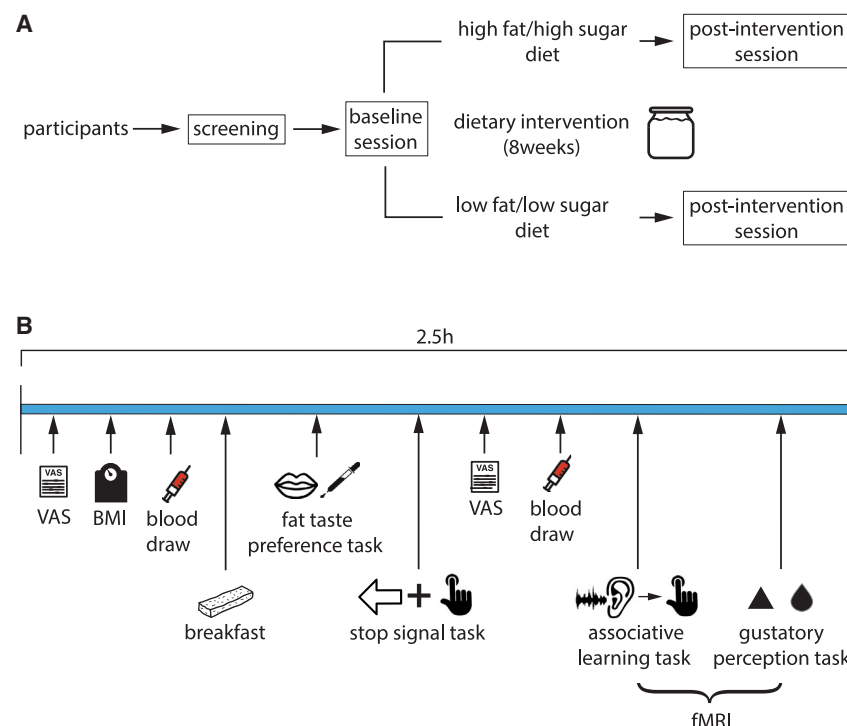
Whether such diet effects observed in animals translate to humans is untested. However, this is a critical question because it extends current models of obesity, which argue that genetic or trait-like factors predispose individuals for

weight gain in the obesogenic environment.<sup>35</sup> Specifically, if exposure to an HFD rewires brain circuits to impact preference and associative learning, then the cycle of overeating may begin with environmental exposure rather than (or in addition to) a predisposition.

Human prospective studies have demonstrated that HFDs can increase fat taste thresholds, i.e., decrease sensitivity,<sup>36,37</sup> likely relating to the reshaping of the transcriptome and epigenome of taste cells after HFD.<sup>38</sup> There is also evidence from neuroimaging studies that consuming sugar-sweetened beverages can impact cortico-striatal responses,<sup>39</sup> while increasing the ratio of saturated to monounsaturated fat can alter striatal responses during the performance of a working memory task.<sup>40</sup> In addition, there is strong evidence demonstrating that obesity is associated with altered striatal responses to food-related stimuli<sup>41</sup> and various reports that obesity is associated with alterations in associative learning.<sup>42</sup> Also, greater adaptive coding during reward learning predicts future weight gain.<sup>43</sup> Whether these effects are related to diet, adiposity, genetic predisposition, and/or metabolic dysfunction is unknown.

To address these gaps in current knowledge, this study (see [Figure 1](#) for study design) aimed to determine in healthy-weight individuals whether frequent exposure to a subtle high-fat/high-sugar (HF/HS) intervention over 8 weeks causes (1) shifts in fat preference, (2) alterations of neural response during exposure to palatable food, and (3) enhanced neural encoding of prediction errors (PEs) during an associative learning task. PEs are vital learning signals in computational theories of adaptive behavior,<sup>44,45</sup> represented in the brain by dopaminergic signaling<sup>46</sup> regulating motivation and reinforcing actions<sup>47,48</sup> through dopamine-dependent plasticity.<sup>49,50</sup> We therefore reasoned that if an HFD alters the neural encoding of PEs, then this would provide strong evidence that HFDs play a causal role in altering associative learning in humans. Notably, the learning task does not operate with explicit rewards and is not employing food rewards, so alterations in PE encoding would signify a fundamental and global change in learning circuits. Finally, the only requirement to the dietary manipulation was that participants consume two snacks daily (in addition to their regular diet) that were either HF/HS or equicaloric but low in fat and sugar (LF/LS; and high in protein), thus minimizing the possibility that the HFD exposure would lead to adiposity or changes in metabolic markers.

Consistent with animal work, we found that the HF/HS, but not the LF/LS, intervention reduced preference for a low-fat snack without affecting suprathreshold fat taste sensitivity. We also observed robust heightened neural responses to food predictive cues and the receipt of food. Finally, heightened neural PE coding in the HF/HS compared with the LF/LS group was observed, demonstrating the potency of HFDs to interact with preference formation and general learning about sensory associations independent of food cues or rewards. These effects occurred despite no changes in adiposity or markers of metabolic function and persisted when these markers were included as covariates in our analysis. Taken together, these findings demonstrate that, in humans, repeated exposure to energy-dense, HF/HS food, in the absence of body weight or metabolic change, can rewire brain circuits and shift dopamine-dependent associative learning and food preference.



**Figure 1. Study design**

(A) In this randomized, controlled design, healthy, normal-weight participants underwent baseline assessment after initial screening. Next, the participants were randomly assigned to dietary intervention with a high-fat, high-sugar (HF/HS) or a low-fat, low-sugar (LF/LS) yoghurt 2 times a day, in addition to their normal diet, for 8 weeks. Subsequently, all subjects were reassessed (post-intervention session).

(B) On the testing days participants arrived in the laboratory around the same time of the day after an overnight fast. BMI, hunger rating, and a blood draw (glucose, insulin, triglycerides and HbA1c levels) were assessed. Subsequently, the participants received a granola bar for breakfast and performed a fat and sugar concentration preference test and a stop signal task. After a second blood draw assessing glucose level, participants underwent fMRI acquisition during which they performed a food anticipation and consumption (milkshake) task and an associative learning task.

## RESULTS

### Metabolic state and general dietary pattern remained unchanged after HF/HS intervention

This study aimed to test the effects of frequent exposure to a HF/HS food snack on fat and sugar taste preference as well as brain responses to palatable food and sensory associative learning. Importantly, we sought to investigate these effects in comparison with an equicaloric LF/LS dietary intervention and without manipulating body weight and metabolic markers (Table 1).

Therefore, we first tested whether the intervention had an effect on body weight, metabolic state, or general dietary pattern. We performed linear mixed-effect models to test the

effect of intervention (HF/HS or LF/LS) and session (baseline, post-intervention) separately for each of the following parameters: body mass index (BMI), fat mass index (FMI), homeostasis model

assessment of insulin resistance (HOMA-IR), serum leptin levels, blood lipids (triglycerides, cholesterol), and self-reported fat and sugar intake using the Dietary Fat and Free Sugar-Short Questionnaire (DFS).<sup>51</sup> We found a slight decrease in the self-reported amount of food intake in DFS Questionnaire from baseline to post intervention (main effect of session:  $F_{(1,23)} = 6.47$ ,  $p = 0.018$ ), potentially indicating compensatory change in dietary pattern in both groups. Yet, BMI and FMI increased marginally across both dietary interventions (main effect of session on BMI:  $F_{(1,48)} = 4.74$ ,  $p = 0.034$ ; for FMI:  $F_{(1,48)} = 4.12$ ,  $p = 0.048$ ), confirming weight gain through increased calorie intake by the intervention snack in addition to the daily diet. Corresponding to the increase in fat mass, blood leptin levels showed an increase across both groups (main effect of session on leptin:  $F_{(1,40)} = 12.75$ ,  $p = 0.009$ ). Insulin resistance and blood lipids were not affected by the dietary intervention. But, importantly, none of these parameters were significantly different after the HF/HS compared with the LF/LS dietary intervention (Table 5). In other words, the HF/HS intervention did not have a differential effect on body weight or metabolic parameters.

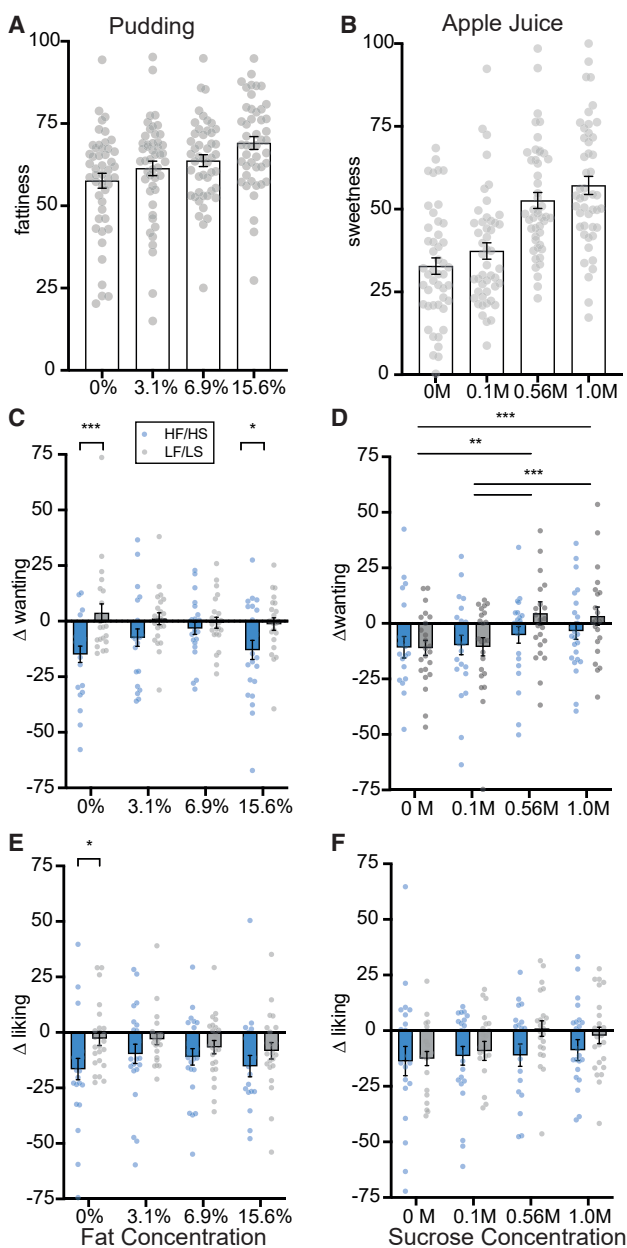
### Taste perception of fattiness and sweetness was preserved after HF/HS intervention

To assess the effect of diet intervention on taste perception and preference, participants rated puddings with varying fat concentrations (0%, 3.1%, 5.6%, 16.9%, weight by weight) and apple juice varying in sucrose concentration (0, 0.1, 0.56, 1 M added sucrose) for fattiness, creaminess, oiliness, sweetness, wanting, and liking. To this end, we used visual analog scales, the labeled hedonic scale,<sup>52</sup> and the general labeled magnitude scale.<sup>53</sup> First, we tested across both dietary intervention types, whether at baseline participants were able to detect the level of fattiness

**Table 1. Participant characteristics at baseline**

Parameter	HF/HS	LF/LS	p value
N	26	23	N/A
Gender (male/female)	9/17	8/15	N/A
Age (years)	26.29 (0.77)	25.04 (0.70)	0.181
BMI (kg/m <sup>2</sup> )	22.59 (0.51)	22.69 (0.56)	0.868
FMI (kg/m <sup>2</sup> )	5.83 (0.32)	5.83 (0.57)	0.999
HOMA-IR	1.90 (0.27)	1.72 (0.16)	0.558
Triglycerides (mg/dL)	95.80 (7.98)	83.87 (8.17)	0.302
HbA1c (%)	5.07 (0.07)	5.11 (0.04)	0.666
DFS total	55.50 (6.14)	60.93 (8.74)	0.26

Note: parameters were acquired at baseline prior to diet intervention and show means with standard error of the mean in parenthesis. BMI, body mass index; FMI, fat mass index; HOMA-IR, homeostasis model assessment of insulin resistance; DFS, = Dietary Fat and Free Sugar-Short Questionnaire.



**Figure 2. Effect of high-fat/high-sugar food on taste perception and preference**

(A and B) The participants were able to perceive changes in (A) fattiness and (B) sweetness, dependent on different fat and sucrose concentrations at baseline, i.e., before the diet intervention.

(C) Preference for the lowest (0%) and highest (15.6%) fat concentrations was reduced after HF/HS, but not after LF/LS intervention.

(D) Both HF/HS as well as LF/LS dietary interventions reduced the preference for lower sucrose concentrations (0 and 0.1 M).

(E) Liking for the lowest fat concentration was reduced after HF/HS dietary intervention, but not after LF/LS dietary intervention (relative to baseline).

(F) Liking of different sucrose concentrations remained unchanged after dietary intervention ( $\Delta$  rating = rating 8 weeks post intervention – rating at baseline; bars show mean  $\pm$  SEM, \*\*\* $p$  < 0.001, \*\* $p$  < 0.01, \* $p$  < 0.05) by linear mixed-effects models in R (version 3.6.1<sup>54</sup>) using the “nlme” package version 3.1-152.<sup>55</sup> Diet (HF/HS, LF/LS), and concentration were fitted as fixed effects, and subject was fitted as a random intercept. All post hoc analyses were

and sweetness for the different fat and sucrose concentrations. The participants reported a significant increase in perceived fattiness ratings across fat concentration levels ( $F_{(3,138)} = 16.84$ ,  $p < 0.0001$ ) and sweetness ratings across sucrose concentrations ( $F_{(3,138)} = 42.16$ ,  $p < 0.0001$ ; **Figures 2A and 2B**). In other words, the subjects were well able to perceive differences in fat and sugar content before starting the diet intervention.

Next, we assessed whether HF/HS compared with LF/LS intervention altered the taste perception of fattiness (interaction concentration  $\times$  diet:  $F_{(3,135)} = 0.39$ ,  $p = 0.75$ ) and sweetness (interaction concentration  $\times$  diet:  $F_{(3,135)} = 0.69$ ,  $p = 0.56$ ), relative to the respective baselines, and could not find a significant effect. Hence, the perception of increasing concentrations of fat and sugar was not influenced by the diet intervention.

### HF/HS intervention altered fat preference

Based on animal data,<sup>11</sup> we hypothesized that HF/HS intervention may decrease preference for low-fat food. Preference was quantified by subjective ratings on wanting and liking scales. For dietary-intervention-driven changes in wanting ratings, we found an interaction between concentration and dietary intervention ( $F_{(3,135)} = 2.56$ ,  $p = 0.039$ ). The post hoc analysis (corrected for multiple comparisons) revealed that the HF/HS food relative to the LF/LS food significantly decreased wanting for the lowest (0% fat:  $t = -3.85$ ,  $p = 0.0004$ ), but also the highest fat concentrations (15.6% fat:  $t = -2.416$ ,  $p = 0.02$ ; **Figure 2C**).

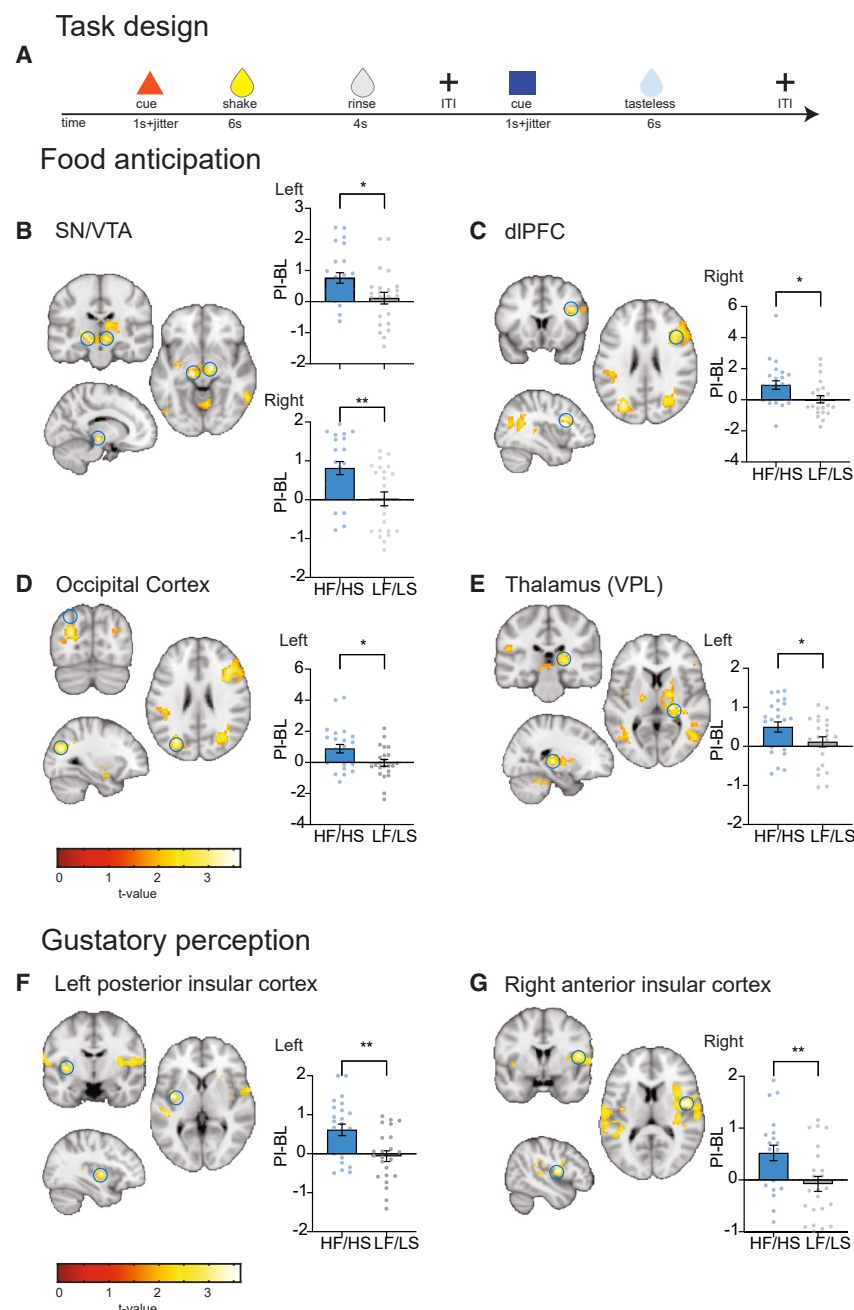
Notably, for dietary-intervention-driven changes in liking, we did not find a significant interaction between concentration or diet (interaction concentration  $\times$  diet:  $F_{(3,135)} = 1.20$ ,  $p = 0.31$ ). Still, we followed our *a priori* hypothesis and performed an analysis (*t* tests) to assess whether the HF/HS dietary intervention decreased liking of the lowest fat concentration. Indeed, we found that HF/HS relative to LF/LS dietary intervention significantly reduced the liking for the lowest fat concentration (0% fat:  $t = -2.52$ ,  $p = 0.015$ ; **Figure 2E**). Neither age, sex, change in BMI and fat mass, nor insulin resistance had a direct impact on wanting and liking scores or showed an interaction with intervention or concentration.

### HF/HS and LF/LS interventions reduced preference (wanting) for low sucrose concentrations

Recent animal work indicates that fat and sugar preference are differently modulated by an HF/HS diet<sup>20</sup>; for discussion, see de Araujo et al.<sup>9</sup> as well as Small and DiFeliceantonio.<sup>56</sup> Hence, we tested whether wanting of different sucrose concentrations was modulated by the HF/HS compared with LF/LS intervention and only found a significant main effect of concentration ( $F_{(3,135)} = 7.12$ ,  $p = 0.0002$ , interaction concentration  $\times$  diet:  $F_{(3,135)} = 1.36$ ,  $p = 0.258$ ). In other words, both HF/HS and LF/LS dietary interventions decreased wanting for the lower sucrose concentrations (0 and 0.1 M; **Figure 2D**) relative to baseline. We did not find any significant effects of concentration or dietary intervention on changes in liking (main effect concentration:  $F_{(3,135)} = 2.50$ ,  $p = 0.06$ , main effect diet:  $F_{(1,45)} = 1.28$ ,

corrected for the number of tests performed using the Holm-Sidak method. Unprocessed data underlying the display items in the manuscript are reported in **Data S1**.





**Figure 3. High-fat/high-sugar dietary intervention enhanced brain responses to milkshake anticipation and consumption**

(A) Trial structure of the milkshake task: milkshake cues were followed by milkshake and rinse, and tasteless cues were followed by the tasteless solution.

(B–E) The HF/HS intervention increased neural response to milkshake cues in (B) bilateral midbrain (substantia nigra/ventral tegmental area), (C) dorsolateral prefrontal cortex (dlPFC), (D) occipital cortex, and (E) the ventral posterolateral nucleus (VPL) of the thalamus.

(F and G) Neural responses to milkshake consumption were enhanced after the HF/HS dietary intervention in the (F) left posterior insular cortex and the (G) right anterior insular cortex. For the exact coordinates of the peak voxels, see [Tables 2](#) and [3](#). Significance threshold was set to  $p < 0.05$ , family-wise error (few)-corrected for multiple comparisons at the cluster level, with  $p < 0.001$  at the peak level. Error bars show mean delta contrast estimates (post-intervention – baseline)  $\pm$  SEM, \* $p < 0.05$  and \*\* $p < 0.01$  in the pairwise comparisons (post hoc). Statistical analyses were conducted using SPM12 in the framework of a general linear model (GLM) with flexible factorial designs. (Bars and error bars correspond to the mean and SEM of the contrast estimate at the peak of the cluster, inferred at the group level. Data points correspond to the individual contrast estimates at the same voxel).

Unprocessed data underlying the display items in the manuscript are reported in [Data S1](#).

$p = 0.26$ , interaction concentration  $\times$  diet:  $F_{(3,135)} = 1.02$ ,  $p = 0.38$ ; [Figure 2F](#)). Also, age, sex, change in BMI and fat mass, or insulin resistance did not show an impact on sucrose preference.

#### HF/HS intervention enhanced neural responses to food anticipation and consumption

In addition to the influence on taste perception, we hypothesized that the HF/HS versus the LF/LS intervention could affect neural responses to food anticipation and consumption in neurocircuits related to feeding and reward. Thus, we performed fMRI using the gustatory (milkshake) task. We sought to identify brain re-

gions that increased their activity in response to milkshake cues and milkshake consumption to a greater extent after the HF/HS than after the LF/LS dietary intervention (relative to the respective base-lines). To take into account the variance in participants' metabolic sensitivity and preference, we controlled for individual differences in insulin sensitivity and milkshake liking.

This analysis revealed that only after the HF/HS dietary intervention did the neural response to cues predicting milkshake increase in the midbrain (substantia nigra ventral tegmental area [SN/VTA]), the right

dorsolateral prefrontal cortex (dlPFC), the thalamus (ventral posterolateral nucleus [VPL]), and the bilateral occipital cortex ([Figure 3](#); [Table 2](#)). During milkshake consumption, we detected increased neural response in the left posterior insular cortex and the right mid to anterior insular cortex extending into the overlying operculum ([Figure 3](#); [Table 3](#)) after the HF/HS relative to the LF/LS dietary intervention. The opposite comparison (LF/LS condition  $>$  HF/HS condition) did not yield significant effects in response to cues or milkshake receipt. Also, the effect of the dietary intervention on the neural response to milkshake anticipation and consumption were not related to age, sex, change in BMI and fat mass, or leptin levels ([Tables S1–S4](#)).

**Table 2. Statistics for brain regions showing a greater increase of BOLD response to milkshake anticipation after HF/HS compared with LF/LS dietary intervention**

	Cluster level		Peak level				
	P(FWE-corr)	Size	P(FWE-corr)	T	x	y	Z
dIPFC, right	0.000	303	0.003	3.61	39	15	28
VTA/SN, left	0.000	446	0.003	3.61	-12	-22	-8
VTA/SN, right	N/A	N/A	0.010	3.37	10	-16	-8
Occipital cortex, left	0.004	118	0.027	3.19	-29	-73	25
Occipital cortex, left	0.000	425	0.031	3.17	58	-44	-17
Cerebellum, left	0.013	93	0.047	3.09	16	-44	-25

Note: statistics derived by a conjunction analysis testing the global null and identifying brain regions that (1) on average showed an increased activation in response to milkshake anticipation after the dietary intervention, and/or (2) showed a greater increase of activation after the HF/HS dietary intervention than after the LF/LS dietary intervention, relative to the respective baselines. dIPFC, dorsolateral prefrontal cortex; SN/VTA, substantia nigra/ventral tegmental area.

### HF/HS intervention enhanced neural responses to associative learning

Finally, we hypothesized that if HF/HS intervention modulates preference and alters neural responses to food consumption, then preference-forming processes must relate to responses in dopaminergic pathways underlying learning about cue-outcome associations more generally. Thus, we performed fMRI during an established associative sensory learning task that did not involve food-related stimuli. Specifically, we assessed the ability of participants to learn associations between auditory cues and subsequent visual outcomes. During the experiment, these associations fluctuated between being highly predictable and unpredictable, thereby requiring adaptive learning.<sup>57–59</sup> To assess differential effects of the dietary intervention on the neural correlates of learning, we analyzed our fMRI data to identify brain regions encoding adaptive PEs and tested whether the HF/HS food enhanced this neural encoding more strongly than the LF/LS dietary intervention (relative to the respective baselines). This analysis indeed revealed enhanced recruitment of neural circuits previously associated with adaptive PE encoding, following the HF/HS intervention as compared with the LF/LS intervention. The neural responses included the ventromedial prefrontal cortex (vmPFC), ventral striatum, posterior insular cortex, and the hippocampus, even when controlling for individual differences in insulin sensitivity (Figure 4; Table 4). Notably, the differential effects of the HF/HS intervention on the neural correlates of learning were not related to age, sex, change in fat mass, or insulin resistance (Tables S5 and S6).

### DISCUSSION

The current study demonstrates that short-term daily consumption of an HF/HS snack decreases preference for a low-fat food

**Table 3. Statistics for brain regions showing a greater increase of BOLD response to milkshake consumption after HF/HS compared with LF/LS dietary intervention**

	Cluster level		Peak level				
	P(FWE-corr)	Size	P(FWE-corr)	T	x	Y	Z
Anterior insular cortex, right	>0.001	614	0.002	3.68	47	-2	11
Poster insular cortex, left	>0.001	485	0.004	3.54	-38	-5	3
dIPFC, right	0.001	161	0.100	2.93	39	46	22
dIPFC, left	0.026	81	0.254	2.71	-46	32	17
Occipital cortex, left	0.004	123	0.297	2.67	-18	-98	-3

Note: statistics derived by a conjunction analysis testing the global null and identifying brain regions that (1) on average showed an increased activation in response to milkshake consumption after the dietary intervention, and/or (2) showed a greater increase of activation after the HF/HS dietary intervention than after the LF/LS dietary intervention, relative to the respective baselines. dIPFC, dorsolateral prefrontal cortex.

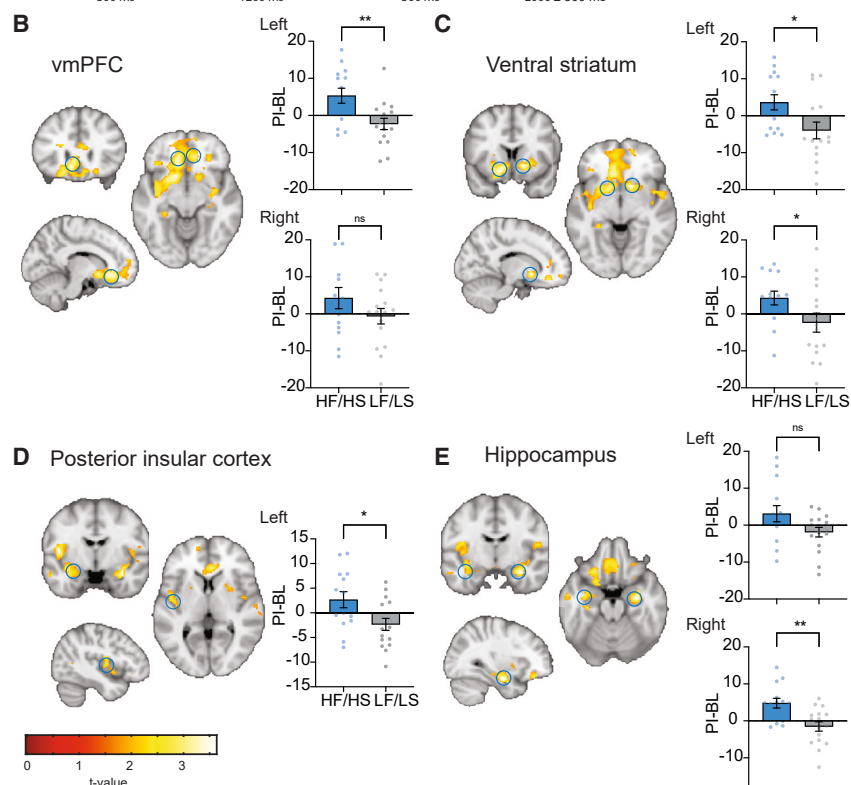
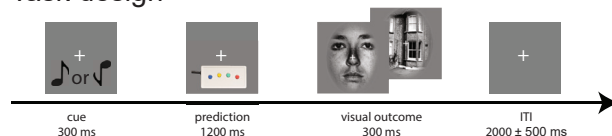
while simultaneously increasing brain response to an HF/HS palatable milkshake and enhancing neural computations that support adaptive associative learning. Moreover, and in line with recent preclinical data,<sup>11,27</sup> these effects are observed in the absence of changes in adiposity and metabolic markers in healthy-weight individuals, indicating a direct consequence of food on rewiring brain reward circuits. Although the underlying mechanisms remain unknown, these findings demonstrate that, like addictive drugs, habitual exposure to HF/HS food is a critical driver of neurobehavioral adaptations that may increase the risk for subsequent overeating and weight gain before the onset of changes in adiposity.

### Food, brain function and the pathophysiology of obesity

It is widely thought that common (polygenetic) obesity results as a function of gene × environmental interactions.<sup>35</sup> The assumption is that genetic traits and/or early life experiences result in a stable vulnerable phenotype where heightened hedonic processing overwhelms homeostatic signals and cognitive control to promote overeating and thus weight gain. Diet-induced weight gain and obesity are then thought to further increase the risk of overeating by influencing central reward circuits. Thus, the same behaviors that originally confer risk become even more influential (reviewed in Stice and Yokum<sup>60</sup>). Collectively, this produces models where innate risk leads to adiposity and metabolic dysfunction, further increasing risk.

The current findings suggest an additional possibility: an HF/HS diet contributes to the development of risk before the onset of obesity and independently of innate risk. In our sample of individuals with healthy BMI and metabolism, a short duration (8 weeks) daily exposure to an HF/HS snack versus an isocaloric LF/LS snack produced no specific effects on adiposity and metabolic markers but nevertheless shifted preference away from low-fat food and increased the sensitivity of brain reward circuits to food cues and stimulus-stimulus contingencies. The dietary intervention presumably introduced an additional calorie intake that resulted in a slight increase of body weight and fat mass in both groups. It is important to note, however, that the

## A Task design



groups (HF/HS versus LF/LS) did not differ in weight gain or self-reported eating behaviors, and this did not change as a function of intervention or correlate with perceptual or neural changes. We also note that although the LF/LS snack had increased protein, the neuronal responses increased after HF/HS dietary intervention rather than decreased after LF/LS intervention. Hence, we can assume the effects on behavioral and neuronal level to be solely attributable to the repeated exposition to HF/HS food.

These findings in humans parallel work in rodents highlighting the effect of diet in the absence of weight gain on blunting dopamine signaling<sup>11,20</sup> and affecting the same dopamine-dependent functions thought to be associated with initial risk, such as impulsivity,<sup>20</sup> preference,<sup>11,27</sup> and food cue reactivity.<sup>26</sup> Our results also align with prior neuroimaging work showing that habitual consumption of sugar-sweetened beverages can impact fronto-striatal responses to food cues,<sup>39</sup> and that increasing the ratio of saturated to monounsaturated fat can alter striatal responses during the performance of a dopamine-dependent working memory task.<sup>40</sup>

The effect of the HF/HS intervention on the preference for low-fat pudding is of particular note because it adds to a growing body of evidence that an HFD results in a devaluation of lower-fat foods. In rodents, an HFD reduces preference for low-fat

## Figure 4. High-fat/high-sugar dietary intervention enhanced brain activation related to associative learning

(A) The sensory-sensory associative learning task. Participants had to predict within 1,200 ms which visual stimulus (face or house) will follow an auditory cue (high or low tone).

(B–E) The HF/HS dietary intervention enhanced adaptive prediction error tracking in the (B) ventromedial prefrontal cortex (vmPFC), (C) ventral striatum, (D) left posterior insular cortex, and (E) hippocampus. For the exact coordinates of the peak voxels, see Table 4. Significance threshold was set to  $p < 0.05$ , FWE-corrected for multiple comparisons at the cluster level, with  $p < 0.001$  at the peak level. Error bars show mean delta contrast estimates (post-intervention – baseline)  $\pm$  SEM, \* $p < 0.05$  in the pairwise comparison (post hoc). Statistical analyses were conducted using SPM12 in the framework of a general linear model (GLM) with flexible factorial designs. (Bars and error bars correspond to the mean and SEM of the contrast estimate at the peak of the cluster, inferred at the group level. Data points correspond to the individual contrast estimates at the same voxel).

Unprocessed data underlying the display items in the manuscript are reported in Data S1.

emulsions<sup>11</sup> and produces a lasting devaluation of standard chow, which is associated with a reduced ability to diminish the negatively valenced hunger signal produced by agouti-related peptide (AgRP) neurons and with alternations in mesolimbic dopamine signaling.<sup>27</sup> Likewise, in humans, a longitudinal study of taste sensitivity and liking in adolescents found that

the amount of the daily intake of fat was positively associated with the liking of an HF/HS milkshake and negatively associated with the liking of the LF/LS milkshake over 4 years.<sup>61</sup> Because mesolimbic dopamine is not thought to influence food liking, we speculate that this shift in preference reflects diminished sensitivity of the gut-brain pathway to lipid reward. Alternatively, dietary fat intake has been shown to influence fat taste sensitivity, with high-fat intake producing decreased sensitivity associated with reduced expression of oral fat taste receptors.<sup>62</sup> This finding suggests that reduced oral sensation of fat might play a role in shifting preference away from low-fat food.<sup>63</sup> In the current study, we assessed fat taste perception by asking participants to rate the fattiness of puddings with different concentrations of fat. The dietary interventions did not influence these ratings. However, we did not assess fat-taste-threshold sensitivity. It is therefore possible that alterations in taste receptors or the tongue proteome<sup>38</sup> by an HFD may have contributed to the observed effect.

Collectively this emerging work suggests that frequent exposure to HF/HS snacks alone can alter physiology to create risk in non-dieting individuals who have maintained their regular diet as well as a healthy weight and metabolism by reducing preference for healthier food options while simultaneously



**Table 4. Statistics for the effects of HF/HS relative to LF/LS dietary intervention on neural tracking of associative learning**

	Cluster level		Peak level				
	P(FWE-corr)	Size	P(FWE-corr)	T	x	y	Z
Hippocampus, right	>0.001	5,563	>0.001	4.01	32	-10	-20
Ventral striatum, left	N/A	N/A	>0.001	3.97	-14	14	-10
Hippocampus, right	N/A	N/A	>0.001	4.01	34	-6	-18
Posterior insular cortex, left	N/A	N/A	>0.001	3.93	-48	-4	12
Ventral striatum, right	N/A	N/A	>0.001	3.93	14	12	-6
vmPFC, left	N/A	N/A	0.002	3.64	-6	28	-4
Hippocampus, left	N/A	N/A	0.013	3.32	-32	-2	-18
vmPFC, right	N/A	N/A	0.034	3.14	8	28	-16
Orbital cortex, right	0.010	210	0.016	3.29	28	36	-16

Note: statistics derived by a conjunction analysis testing the global null and identifying brain regions that (1) generally showed a significant correlation between their trialwise activity and the adaptive prediction error, and/or (2) showed a greater diet-induced increase of this neural tracking of learning after the HF/HS dietary intervention than after the LF/LS dietary intervention, relative to the respective baselines. vmPFC, ventromedial prefrontal cortex.

enhancing neural reward responses to palatable food. This insight is important because it partially removes the onus of blame from the individual to the environment. Specifically, the current findings raise the possibility that even healthy-weight individuals with minimal or no trait level risks, exposed to an unhealthy diet because of a lack of access to healthy foods, incur adaptations that promote overeating. It also follows that those with genetic risk might even be more susceptible. Consistent with this possibility, a recent human genetics study found that polygenetic risk for obesity was partially mediated by poor diet.<sup>64</sup> Thus, dietary exposure and the resulting neural adaptations may play a critical role in the strong association between socioeconomic status and BMI, given the established inverse association between food price and energy density.<sup>65</sup>

### Neural circuits

As predicted, the HF/HS compared with the LF/LS intervention induced changes in the neural response to food anticipation and consumption. Enhanced responses to food predictive cues in the midbrain and prefrontal cortex after the HF/HS dietary intervention is in keeping with a sizable preclinical literature showing that the consumption of energy-dense palatable food rich in fat and sugar rewires this reward circuitry to enhance incentive motivation,<sup>19,26,30,66,67</sup> an effect that can even transfer to the offspring of dams fed an HFD.<sup>28,68</sup>

Enhanced responses following the HF/HS versus the LF/FS intervention were also identified in sensory regions, including the visual cortex, thalamus, and insular cortex, which represent

the oral sensory features of foods.<sup>69,70</sup> It is likely that these effects reflect the enhanced saliency of the sensory cues.<sup>1,71</sup> However, the insular response extended from oral sensory regions to the more posterior insular cortex, which corresponds to the primary interoceptive cortex.<sup>72–74</sup> This area plays a key role in integrating signals from the body to control food intake,<sup>75</sup> such as allowing satiety signals like stomach distension to be integrated with oral sensory information.<sup>76</sup> The insula is also implicated in computing “interoceptive predictions.”<sup>77</sup> Murine studies using two-photon imaging have identified spontaneous activity patterns in the insula that reflected the evolving internal state of the organism from the thirsty-to-quenched and hungry-to-sated states.<sup>4</sup> Upon presentation of cues predicting food or water, these activity patterns shift over a very short timescale to simulate the future state of quenched or sated (specific to the cue and respective state). Because the timescale was much faster than the physiological transition (seconds versus minutes), these findings imply that information obtained from the cue was used to predict the future interoceptive state.<sup>5,78,79</sup> It is, therefore, possible that the increased insular response to milkshake reflects a combination of enhanced sensory saliency and gut-brain signaling; testing this possibility is an important future direction.

In addition to altering brain response to food-related stimuli, the HF/HS intervention also enhanced PE tracking during a sensory association learning task that contained no food images and was unrelated to feeding.<sup>58,80</sup> Rather, the task was designed to assess fundamental dopamine-dependent sensory associative learning. The enhanced responses we observed in the hall-mark circuitry, indicating that the rewiring induced by the HF/HS intervention generalizes to impact the forming of sensory associations beyond the context of ingestive behavior.

### Mechanisms

It is well established that diet-induced obesity is associated with adaptations in dopamine neurons and their signaling actions in corticolimbic projection sites.<sup>81,82</sup> However, studies have only recently begun to disambiguate effects of diet, adiposity, and metabolic function. This work, which has been mainly accomplished in rodent models, provides evidence for multiple mechanisms that may account for the observed findings. A prolonged HFD results in a reduction of dopamine reuptake<sup>83</sup> and downregulation of dopamine D2 receptors (DRD2),<sup>25</sup> with at least one study showing decreased DRD2 expression in calorie-restricted but high-fat-fed animals that did not gain weight or show changes in metabolic markers.<sup>20</sup> Downregulation of DRD2 can be accompanied by increased compulsive<sup>25,84</sup> and impulsive<sup>20</sup> responses, with an enhanced incentive motivation for sucrose,<sup>85</sup> which would be consistent with the enhanced responses to food-related stimuli observed following the HF/HS compared with the LF/LS intervention. In addition, very brief 24-h exposures to HFD have been shown to strengthen excitatory synaptic transmission onto dopamine neurons.<sup>26</sup>

HFD in the absence of weight gain also depletes lipid messengers like oleoylethanolamide (OEA),<sup>86,87</sup> which results in diminished lipid-induced dopamine release and reduced preference for low-fat emulsions through vagal signaling.<sup>11</sup> Accordingly, acute administration of OEA rescues gut-brain signaling to restore dopamine release and preference for low-fat emulsions. We observed decreased preference for a low-fat pudding

**Table 5. Participants characteristics before and after dietary intervention with HF/HS and LF/LS dietary intervention**

Parameter	HF/HS BL	HF/HS PI	LF/LS BL	LF/LS PI	Main effect of group	Main effect of session	Interaction group × session
<b>Anthropometric data</b>							
BMI (kg/m <sup>2</sup> )	22.59 (0.51)	22.69 (0.50)	22.69 (0.56)	22.96 (0.67)	$F_{(1,49)} = 0.06$	$F_{(1,48)} = 4.74^a$ , p = 0.034	$F_{(1,48)} = 0.62$
FMI (kg/m <sup>2</sup> )	5.83 (0.32)	5.93 (0.32)	5.83 (0.57)	6.38 (0.71)	$F_{(1,49)} = 0.12$	$F_{(1,48)} = 4.12^a$ , p = 0.48	$F_{(1,48)} = 1.66$
<b>Laboratory parameters</b>							
HOMA-IR	1.90 (0.27)	2.07 (0.22)	1.72 (0.16)	1.98 (0.20)	$F_{(1,49)} = 0.25$	$F_{(1,48)} = 2.40$	$F_{(1,48)} = 0.10$
Triglycerides (mg/dL)	95.80 (7.98)	91.08 (7.06)	83.87 (8.17)	86.39 (7.50)	$F_{(1,49)} = 0.75$	$F_{(1,48)} = 0.042$	$F_{(1,48)} = 0.53$
HbA1c (%)	5.07 (0.07)	5.11 (0.06)	5.11 (0.04)	5.14 (0.05)	$F_{(1,49)} = 0.21$	$F_{(1,47)} = 2.84$	$F_{(1,47)} = 0.05$
Leptin (ng/mL)	5.41 (1.05)	8.43 (1.68)	10.10 (2.43)	10.33 (2.15)	$F_{(1,47)} = 0.0001$	$F_{(1,39)} = 13.11^a$ , p ≤ 0.001	$F_{(1,39)} = 0.12$
<b>Daily food intake</b>							
DFS	55.50 (1.77)	54.33 (1.67)	60.93 (2.33)	56.57 (2.05)	$F_{(1,44)} = 2.51$	$F_{(1,23)} = 6.48^a$ , p = 0.018	$F_{(1,23)} = 1.83$
<b>fMRI motion parameters</b>							
FDmax (milkshake task)	0.82 (0.10)	0.66 (0.11)	0.93 (0.14)	0.75 (0.11)	$F_{(1,49)} = 0.90$	$F_{(1,147)} = 1.45$	$F_{(1,147)} = 0.04$
FDmax (associative learning task)	1.15 (0.27)	1.06 (0.26)	1.99 (0.77)	1.45 (0.37)	$F_{(1,42)} = 0.29$	$F_{(1,42)} = 3.90$	$F_{(1,42)} = 0.52$

Note: descriptive statistics are presented on the left side and show means with standard error of the mean in parenthesis. Inferential statistics are presented on the right side, we report test statistics  $F$ . With (a) significant effects are indicated. In this case, the p-value is given. There were no significant interactions diet × intervention for any of the parameters. BMI, body mass index; FMI, fat mass index; DFS, Dietary Fat and Free Sugar Questionnaire; FDmax, maximal framewise displacement in mm as an index of motion during fMRI.

<sup>a</sup>Significant effect

following the HF/HS versus isocaloric LF/LS dietary intervention, suggesting that this effect translates to humans. If so, the enhanced blood-oxygen-level-dependent (BOLD) responses observed to the milkshake predictive cues in the midbrain, prefrontal cortex, thalamus, and occipital cortex, as well as the enhanced responses to milkshake consumption in the insular cortex, could reflect enhanced phasic responses to these sensory stimuli in the context of diminished tonic dopamine resulting from the HF/HS intervention.<sup>88</sup> This interpretation is also consistent with the observation of enhanced neural activation during associative learning, which is based upon phasic dopamine neuron firing in response to PEs.<sup>89,90</sup> This finding is also consistent with the enhanced neural tracking of adaptive PEs observed in the current study.

#### Adaptive versus dysfunctional adaptation: A continuum

Regardless of the mechanism, one important question to arise from these data is how, on an evolutionary scale, the observed effects are adaptive and whether, like in addiction, there is a continuum where escalating use and exposure results in damage and dysfunction.<sup>19</sup> Given that our intervention was minimal in terms of both the amount of HF/HS food consumed and the length of the exposure, it is likely that the observed changes reflect an adaptive response for metabolically healthy organisms. From an evolutionary perspective, upon encountering a food environment with increased availability of highly palatable energy-dense foods, adaptations to neural circuits that enhance learning about food availability (associative learning and increased responses to food sensations), while promoting intake of higher energy-dense options (decreased preference for low

fat), could reasonably lead to an adaptive advantage given the likelihood of the transient nature of such an opportunity. However, following prolonged exposure, longer-lasting changes to neural circuits might promote dysfunctional behavior leading to weight gain and metabolic dysfunction. This proposition is consistent with reports of the persisting devaluation of standard chow following long-term exposure to HFD, and with re-exposure after withdrawal inducing feeding and stronger inhibition of hypothalamic circuits, which could promote binge behaviors and relapse.<sup>27</sup> It is also consistent with the ability of a 21-day, limited-access HFD, in the absence of weight gain, to induce a robust decrease in perineuronal net (PNN) intensity in the prefrontal cortex of rats.<sup>91</sup> PNNs are specialized extracellular matrices primarily surrounding GABAergic interneurons. Because PNNs contribute to synaptic stabilization and integrity, this diminished activity could be associated with more permanent circuit dysfunction. Future work in humans and preclinical models is needed to further investigate the kinetics of brain adaptations to diet and their reversal.

#### Conclusions

Using an interventional study in healthy, normal-weight participants, we demonstrate that, independent of body weight gain and alterations in metabolic markers, exposure to HF/HS food (1) reduces preferences for low-fat food, (2) plays a critical role in up-regulating brain responses to anticipation and consumption of highly palatable, energy-dense food, and (3) has a generalized effect on the neuronal encoding of PEs in the context of associative learning and independent of food rewards. Taken together, repeated consumption of HF/HS

relative to isocaloric LF/LS food, and in the absence of changes in body weight or metabolic state, can rewire brain circuits and thereby induce neurobehavioral adaptations. Hence, changing the food environment and reducing the availability of energy-dense HF/HS food items is pivotal to combating the obesity pandemic.

### Limitations of the study

There are a number of limitations that can be considered in interpreting the current findings. We initially assessed 82 individuals for eligibility in the study but enrolled only 57, as many individuals did not meet study inclusion criterion. In particular, participants were required to have a healthy BMI and to rate the milkshake and yogurt as at least moderately wanted. It is possible that different effects might be observed in individuals who are under- or overweight/obese or in individuals who do not want to eat these food items. It is also possible that results might not generalize to other snack foods or to different lengths of intervention. Although we assessed oral sensory perception, we used a whole food (pudding) and did not measure responses to fat or sugar alone, and we did not assess taste thresholds. While our procedure was ecological, we might have missed more subtle changes in taste perception. We also did not assess dietary intake during the dietary intervention in detail (except for the DFS Questionnaire). Therefore, it is possible that the intervention type systematically altered dietary patterns and contributed to our results. If so, our findings would still be attributable to HF/HS snacking, but also include indirect effects of the intervention on diet. Finally, although the intervention did not change adiposity or the metabolic markers that we assessed (e.g., glucose, insulin), it is possible that other metabolic factors did change (e.g., nutrient partitioning) or that more comprehensive measures (e.g., clamp studies) might have revealed more subtle effects and therefore contributed to our results.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Participants
- **METHOD DETAILS**
  - Study design
  - Task designs
  - fMRI data acquisition
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
  - Statistical data analyses
  - Analysis of the fat and sugar concentration preference task
  - General fMRI data analyses
  - fMRI-analysis of the milkshake task
  - Analysis of the associative learning task
  - fMRI-analysis of the associative learning task

### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cmet.2023.02.015>.

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### AUTHOR CONTRIBUTIONS

All authors contributed to the work presented in this paper. D.M.S., M.T., and J.C.B. conceptualized the study; the experimental setup was designed by A.G.D., S.E.T., D.M.S. and M.T., while S.I. designed and established the associative learning task applied. Data acquisition was performed by A.G.D., K.A. and S.E.T. Statistical analyses were performed by S.E.T., B.K., and L.R.; the manuscript was written by S.E.T., B.K., M.T., and D.M.S. Required infrastructure was provided, setup, and controlled by K.A., R.H., and M.S. The supervision of the study was performed by M.T., O.A.C., and J.C.B.

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### INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
<a href="#">Data S1</a>	This paper	N/A
Software and algorithms		
Matlab2014b	Mathworks	2014b
R	R core team	3.6.1
Cogent2000 (Matlab toolbox)	<a href="http://www.vislabs.ucl.ac.uk/cogent_2000.php">http://www.vislabs.ucl.ac.uk/cogent_2000.php</a>	2000
Psychophysics toolbox (Matlab toolbox)	<a href="http://Psychtoolbox.org">Psychtoolbox.org</a>	3.0.11
Other		
50 ml Syringe	Braun, Melsungen, Germany	8728844F-06
Syringe pump	HLL Landgraf, Laborsysteme, Langenhagen, Germany	LA-100
Silicon beverage tubing	Lindemann GmbH, Helmstedt	SIS01990
Milkshake flavors (Banana, Chocolate, Vanilla, Strawberry)	Kaba, Mondelez Deutschland GmbH, Bremen, Germany	N/A
Galletta instant pudding mix	Dr. Oetker GmbH, Bielefeld, Germany	N/A
Amecke Applejuice	Amecke Fruchtsaft GmbH & Co KG, Menden, Germany	N/A
Ja! Milk	REWE	N/A
Ja! Cream	REWE	N/A
Ja! 10% Quark	REWE	N/A

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Dana Small ([dana.small@yale.edu](mailto:dana.small@yale.edu)).

#### Materials availability

This study did not generate new reagents.

#### Data and code availability

- The published article and supplemental information include the data used to generate the figures in the paper ([Data S1](#)).
- This paper does not report original code.
- The human data reported in this study cannot be deposited in a public repository per GDPR and IRB data protection policies. To request access, please contact Marc Tittgemeyer, Max Planck Institute for Metabolism Research, [tittgemeyer@sf.mpg.de](mailto:tittgemeyer@sf.mpg.de). Data provision may include processed and unprocessed data and will require a data-sharing agreement.

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

#### Participants

Eighty-two volunteers of healthy weight (50 female, age:  $25.67 \pm 0.42$  years, BMI:  $22.81 \pm 0.32$  kg/m<sup>2</sup>) were recruited for this study. Due to the long-term intervention, we expected a high dropout rate of up to 50%. Based on a power estimation (G\*Power Version 3.1) assuming a small effect size of Cohen's  $d = 0.25$ , an alpha (significance) value of  $p = 0.05$ , a reasonable power of 0.9, and a correlation among repeated measures of 0.5 for a (repeated measures) design with two groups and within and between-group interaction, we aimed to include a total sample of 46 participants and, considering a dropout rate of 50%, to recruit at least 70 participants.

All participants were recruited from the pre-existing database of volunteers maintained at the Max Planck Institute for Metabolism Research; participants were medication-free, non-smokers without any history of neurological, psychiatric, gastrointestinal or eating disorders, and without special diets or medical treatments. After initial screening, 21 subjects were excluded before study participation for the following reasons: BMI not within the range of normal weight, milkshake wanting less than moderate, feeling of discomfort during the practice MRI session, disliking of the yoghurt used for dietary intervention. Furthermore, 4 subjects dropped out because they could not arrange to come to the follow-up appointment. In total, fifty-seven participants proceeded to the testing sessions, completed baseline assessment and were randomly assigned either a high-fat/high-sugar (HF/HS) or low-fat/low-sugar (LF/LS) intervention (cf. [Figure S1](#)).

While 8 participants dropped out after their first session, forty-nine individuals (32 female, age:  $25.69 \pm 0.53$  years, BMI:  $22.64 \pm 0.37$  kg/m<sup>2</sup>) completed the whole study: 26 participants received the HF/HS intervention, 23 participants received the LF/LS intervention. Out of these, one participant showed significantly increased plasma insulin (98 mU/l) and glucose levels (109 mg/dl) at baseline on the first testing day compared to other testing days, most probably because the individual did not fast before testing as required. Therefore, only the baseline dataset of this participant was excluded from further data analysis ([Table 1](#)).

All participants gave written informed consent to participate in the experiment. The local ethics committee of the Medical Faculty of the University of Cologne approved the study (Cologne, Germany; No. 14-128). The study has been registered at [ClinicalTrials.gov](#) (NCT05574660).

## METHOD DETAILS

### Study design

The study implemented an intervention food (HF/HS or LF/LS) for 8 weeks in a single-blinded, randomized, controlled design. Each volunteer participated in one screening session and three testing sessions. In the screening session, the inclusion criteria were checked, and participants were prepared for and familiarized with preference tests and two different functional Magnetic Resonance (fMRI) paradigms to investigate brain signaling during food anticipation, consumption and associative learning. During the testing sessions, tests and fMRI tasks were carried out. After the first testing session (baseline), we started the dietary intervention. Participants were then tested at two further sessions, four weeks and eight weeks later.

### Screening

The participants were invited to a screening session before inclusion in the intervention study. Here, body weight and height were assessed, and the participants were familiarized with the different rating scales (see Task Designs). In addition, the preference for different milkshake flavors was tested, the two best-liked milkshake flavors and a control (“tasteless”) solution were chosen for later use during the fMRI session (see Task Designs). Only participants who liked the milkshake at least moderately were included in the study. Afterwards, a ~15-minute practice fMRI session was conducted to allow the participants to become familiar with the apparatus and practice swallowing while in a supine position. Finally, the participants were asked to choose their favorite flavor for the dietary intervention described in the following. Participants who did not like the yoghurt snack provided for dietary intervention were excluded.

### Dietary Intervention

All participants were randomly assigned to either the HF/HS or LF/LS intervention. Depending on intervention, participants were asked to consume either an HF/HS yoghurt (40.8 % kcal from fat, 45.6 % kcal from carbohydrates, 13 % kcal from protein of 79.5 total kcal) or an LF/LS yoghurt (17.1 % kcal from fat, 29.1 % kcal from carbohydrates, 51.9 % kcal from protein of 78 total kcal) two times a day for eight weeks in addition to their normal diet. In the screening, the participants had chosen their favorite flavor among four options (vanilla, lemon, strawberry, peach-passionfruit; Dr. Oetker GmbH, Bielefeld, Germany) and were allowed to switch flavors during the study to avoid fatigue and increase compliance. All participants returned to the laboratory every three days to return the empty containers and receive the yoghurt for the following three days.

### Testing Sessions

All participants were tested at baseline, four weeks, and eight weeks post-intervention. The complete assessments described below were only performed at baseline and eight weeks post-intervention. Hence, only these time-points were considered for further analyses. All sessions started around the same time (either at 8:00 am or 9:30 am). Before each session participants completed a German version of the Dietary Fat and Free Sugar-Short Questionnaire DFS,<sup>51</sup> online from home. In brief, this 23-item checklist assesses the monthly consumption frequency of fat and sugar-containing foods which have been validated against established extensive food frequency questionnaires<sup>92</sup>; the questionnaire was chosen to assess consumption of fat and sugar-containing foods before and over the course of the experiment.

On each testing day, participants arrived fasted and were asked to have the last meal before 10 pm of the previous day. At the beginning of each testing day, body weight and composition were assessed on a medical body composition analyzer (mBCA 515, seca GmbH & co KG, Hamburg, Germany). For the blood sampling, an intravenous catheter was inserted in the non-dominant forearm vein. Next, the participants were asked to rate their hunger, satiety, thirst, tiredness as well as their desire to eat on a 100 mm visual analogue scale (0 = “not hungry/sated/thirsty/tired at all/don’t want to eat at all” and 100 = “very hungry/sated/thirsty/tired/very much want to eat”). Adherence to overnight fasting and insulin sensitivity were tested by sampling blood glucose and insulin level at the beginning of each testing day. Insulin sensitivity was assessed by the homeostasis model assessment of insulin resistance HOMA-IR.<sup>93</sup> To evaluate metabolic changes induced by dietary intervention, we additionally measured triglycerides,

cholesterol, and HbA1c at baseline and eight weeks post-intervention. After the first blood draw, the participants received a granola bar for breakfast containing 190 kcal. The participants could choose between three different flavors (Canadian Maple Sirup, Oats & Dark Chocolate, Oats and Honey, Nature Valley, USA). Following breakfast, the participants performed the fat and sugar concentration preference task<sup>94</sup> and the stop-signal task (for a detailed description of all tasks, see *Task designs*, details on the stop signal task are given in the [supplemental information](#)). After a second blood draw assessing glucose level after standardized breakfast, participants underwent fMRI acquisition: At baseline and eight weeks post-intervention, brain signaling during anticipation and consumption of selected milkshakes was assessed, and an associative learning task was performed.

## Task designs

### **Fat and sugar concentration preference task**

To assess changes in fat and sugar preference and perception, two sets of stimuli were created that either varied in fat or sugar content. For the fat stimulus, four puddings with varying fat content, 0%, 3.1%, 6.9%, and 15.6 % weight by weight (w/w), were created by mixing Galetta instant pudding mix (Dr. Oetker GmbH, Bielefeld, Germany) with milk or cream of varying fat content. The sugar content was kept constant between the stimuli. Participants were asked during the initial screening which pudding flavor they would prefer, vanilla or chocolate. For the sugar stimulus, unsweetened apple juice (Amecke Fruchtsaft GmbH & Co KG, Menden, Germany) was mixed with added sucrose in 0 M, 0.1 M, 0.56 M, and 1 M concentrations. Ratings were conducted in two blocks, with pudding or juice variations in counterbalanced order across testing days and participants. Within each block, stimuli concentrations were presented 3 times each (i.e. a total of 12 presentations) in random order. For the fat stimulus, participants were passed a tasting spoon with approximately 5ml of the pudding on the tip. For sucrose, participants sipped approximately 5 ml of apple juice from a pre-portioned medicine cup. After each tasting, they completed ratings i) of fattiness, creaminess, oiliness, wanting on a visual analogue scale (VAS); or ii) sweetness on the generalized labelled magnitude scale gLMS<sup>95,96</sup>; and iii) liking on the labelled hedonic scale.<sup>52</sup> The participants were instructed not to swallow the stimulus but spit it out after the rating. Subsequently, the participants rinsed their mouths with water and waited for 30 seconds before the next tasting. All scales and timings were performed on the computer using Matlab (version 2014b, MathWorks®) employing the Psychophysics Toolbox extensions.<sup>97,98</sup>

### **Food anticipation and consumption (milkshake) task performed during fMRI**

To test the effect of the dietary intervention on brain responses to food anticipation (milkshake predicting cue) and consumption (milkshake delivery), we performed a task introduced by Small et al.<sup>99</sup> and Veldhuizen et al.<sup>100</sup> and further validated by Oren et al.<sup>15</sup>

Before the task, each subject first chose a tasteless solution (control condition) and two milkshakes. The stimulus selection was performed during the screening session and used for the testing sessions. For tasteless selection, four different dilutions (100%, 75%, 50% and 25%) of the original solution (25 mM potassium chloride and 2.5 mM sodium bicarbonate) were presented to the participant pairwise in a dropper and sampled. The solution, which was selected by the individual participant in two successive comparisons “as tasteless”, was later used in the testing session. Next, participants tasted four milkshake flavors (banana, chocolate, vanilla, strawberry) created from flavored powder (Kaba, Mondelez Deutschland GmbH GB, Bremen, Germany), whole milk, and cream. After each tasting, participants were instructed to rinse their mouths with water and wait one minute for the subsequent trial. Overall stimulus intensity as well as sweetness intensity, liking and wanting were tested. Two milkshake flavors rated as similarly liked and wanted by the individual participant were selected for further testing. Only participants who liked and wanted the milkshakes moderately or higher on the scales were included in the study.

The milkshake task was performed in the testing sessions while undergoing fMRI (two 8.35 min long scanning sessions). The participants repeatedly received either a milkshake or tasteless solution in a randomized order ([Figure 3A](#)). Each milkshake delivery (6 s long) was followed by a water rinse (4 s long). A red triangle or a blue square on a screen predicted the milkshake or the tasteless solution, respectively (3 s on average). The association between cue and stimulus remained constant across sessions and was counterbalanced across subjects. Participants were informed about the cue-delivery association at the beginning of the scan through standardized written instruction. The interval between cue and delivery was programmed with a random exponential jitter of two seconds on average. The intertrial interval was programmed with 6 s on average.

The delivery of the liquids during fMRI was performed with a customized setup. The participants were equipped with a custom-designed Teflon mouthpiece for fluid delivery to the tongue tip attached to the fMRI head coil. The whole setup consisted of four programmable syringe pumps (LA-100, HLL Landgraf Laborsysteme, Langenhagen, Germany), each with a 50 ml syringe (Braun, Melsungen, Germany) containing either one of the two selected milkshakes, tasteless solution, or water. The syringes were connected to the mouthpiece via a silicon beverage tubing (Lindemann GmbH, Helmstedt) with an inside diameter of 2 mm. The syringe pumps were controlled by scripts written in Matlab™ (version 2014b, Mathworks®) using the psychophysics toolbox extension vers. 3.0.11.<sup>97</sup>

### **Associative learning task performed during fMRI**

A short version of the sensory learning task as described in detail by Iglesias et al.<sup>59</sup> was performed to assess associative learning independent of food rewards, while undergoing fMRI. In brief, participants had to learn the predictive strength of an auditory cue (a low [352 Hz] or high [576 Hz] tone) and predict a subsequent visual stimulus (house or face). Following the auditory cue presented for 300 ms, participants signaled by button press the visual stimulus they expected (1200 ms); subsequently the visual outcome (face and house) was presented for 300 ms. The inter-trial interval (ITI) varied between 1.6 and 5.85 s (randomly sampled from an exponential distribution, with a mean of 2.5 s). Notably, the cue-outcome association strength changed over the 160 trials (volatility), allowing for an adaptive learning rate and, hence, continuous collection of brain response to adaptive prediction errors. The



probability sequence was fixed for each subject with changes in the cue-outcome contingency of 80:20 and was pseudorandom. The correctness of the response was not associated with a trial-wise monetary reward; participants only received a fixed monetary compensation for participating in the study, independent of their task performance. Before the task, participants underwent a psychophysical matching to adapt the volumes of the two auditory cues (high and low tone) to perceive both tones as equally loud cf. den Ouden et al.<sup>101</sup> The total duration of the task was 11 minutes. Stimulus presentation and response collection was controlled using Cogent2000 (<http://www.vislab.ucl.ac.uk/Cogent/index.html>).

### fMRI data acquisition

fMRI data acquisition was conducted on a Magnetom Prisma 3T whole-body scanner using a 64-channel head coil (Siemens AG, Medical Solutions, Erlangen, Germany). The fMRI data were acquired with an echo planar imaging sequence (TR = 2100 ms, TE = 30 ms, field of view = 220 × 220 × 96 mm<sup>3</sup>, voxel size = 2.8 × 2.8 × 2.8 mm<sup>3</sup>, 34 oblique axial slices, no distance factor, ascending interleaved in-plane acquisition). In addition, we acquired two images with reversed phase encoding directions (anterior-posterior and posterior-anterior) to estimate and correct the susceptibility-induced distortion (same sequence as above, three volumes per image). High-resolution structural images were acquired using a T1-weighted sequence (MDEFT, TR 1930 ms, TE 5.80 ms, field of view 256 × 256 × 160 mm<sup>3</sup>, voxel size 1 × 1 × 1.25 mm<sup>3</sup>, 128 sagittal slices, or MPAGE, TR 2300 ms, TE 2.32 ms, field of view 256 × 256 × 192 mm<sup>3</sup>, voxel size 0.9 × 0.9 × 0.9 mm<sup>3</sup>, 213 sagittal slices).

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Statistical data analyses

After allocation to intervention group, 7 participants dropped out because of appointment conflicts and 1 participant had an incidental finding in the baseline scan. Since none of the drop-outs were specifically related to the intervention, we performed a per-protocol analysis on the participants that completed the intervention to investigate the HF/HS diet intervention under optimal conditions. An intention to treat analysis of the clinical parameters can be found in the supplementary information (Table S7). All behavioral, blood, and anthropomorphic data were analyzed using linear mixed-effects models in R (version 3.6.1<sup>54</sup>) using the 'nlme' package vers. 3.1-152.<sup>55</sup> In general, diet intervention (HF/HS, LF/LS) and session (baseline [BL], post-intervention [PI]) were fitted as fixed effects, and subject was fitted as a random intercept (see respective results sections for model details). All post-hoc analyses were corrected for the number of tests performed using the Holm-Sidak method.

### Analysis of the fat and sugar concentration preference task

The fat and sugar concentration preference tasks were performed to test if HF/HS dietary intervention compared to LF/LS dietary intervention influenced fat and sugar preference or perception. Fat concentration preference and perception were evaluated using a series of puddings with varying fat content (0%, 3.1%, 6.9%, and 15.6%), and sugar concentration preference and perception were evaluated using apple juice with varying sucrose content (0 M, 0.1 M, 0.56 M, and 1 M). For further analysis, we calculated the average rating across the total 12 presentations for each one of the four different puddings and juices, respectively.

To verify that the participants were able to discriminate the different fat and sucrose concentrations, we tested perceived fattiness for puddings,

$$M_1 : (\text{fattiness} \sim \text{concentration} + (1|\text{subject}))$$

and sweetness for apple juices,

$$M_2 : (\text{sweetness} \sim \text{concentration} + (1|\text{subject})) \text{ across all subjects at baseline.}$$

To assess whether the intervention (HF/HS or LF/LS) had an effect on perception (fattiness and sweetness) as well as preference (fat and sugar "wanting" and "liking"), we first calculated the change of each rating (fattiness, sweetness, liking, wanting for all concentrations of all stimuli) from baseline to post-intervention session, i.e.

$$\Delta \text{rating} = \text{rating}_{\text{PI}} - \text{rating}_{\text{BL}}.$$

Subsequently, we tested the effect of dietary intervention and concentration on  $\Delta \text{rating}$ , separately for each stimulus type,

$$M_3 : (\Delta \text{rating} \sim \text{stimulus concentration} * \text{diet} + (1|\text{subject})).$$

Dietary intervention and concentration were fitted as fixed effects, and subject was fitted as a random intercept. All post-hoc analyses were corrected for the number of tests using the Holm-Sidak method.

### General fMRI data analyses

The data pre-processing was identical for the milkshake task and the associative learning task. All individual data sets were pre-processed before running statistical analyses using tools from the FMRIB Software Library (FSL version 5.09, [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). Non-brain tissues (e.g., scalp and CSF) were removed using an automated brain extraction tool.<sup>102</sup> Time series were realigned to correct for small head movements using MCFLIRT.<sup>103</sup> The susceptibility-induced distortions were estimated based on the images with reversed phase-encoding using FSL's TOPUP tool<sup>104</sup> and applied for distortion correction of the functional images. All further

analysis steps were conducted using Statistical Parametric Mapping (SPM), version 12 (r6225, Wellcome Trust Centre for Neuroimaging, London) implemented in Matlab (version 2014b, MathWorks®). The T1 image was normalized to the Montreal Neurological Institute (MNI) reference space using the unified segmentation approach, and the ensuing deformation parameters were applied to (previously co-registered) functional images. Finally, functional images were smoothed using an 8 mm full-width-half-maximum Gaussian kernel.

For both tasks, statistical analyses were conducted using SPM12 in the framework of a general linear model (GLM). At the single-subject level, conditions were modelled using a boxcar reference vector convolved with the canonical hemodynamic response function and its time derivative. For both tasks, the following confounds were included as nuisance regressors for each session: 24 motion parameters — six parameters relating to the current and the preceding volume, respectively, plus each of these matrices squared, see Friston et al.<sup>105</sup>, mean signal extracted from the ventricular cerebrospinal fluid, and a matrix with motion-outlier volumes —identified using the tool `fsl_motion_outliers`, `dvars` option targeting global intensity differences between subsequent volumes, at a threshold of 75th percentile + 2.5 \* interquartile range, see Power et al.<sup>106</sup> For both tasks the maximum framewise displacement (maxFD) as a measure of motion between slices did not differ between groups and interventions (Table 5). Low-frequency signal drifts were filtered using a cut-off of 128 s. At the group level, for both tasks, the significance threshold was set to  $p < 0.05$ , family-wise error (FWE) corrected for multiple comparisons at the cluster level, with an underlying threshold of  $p < 0.001$  at the peak level.

### fMRI-analysis of the milkshake task

At the single-subject level, milkshake cue, tasteless cue, milkshake delivery, tasteless delivery, and rinse were modelled as separate regressors. The GLM for each subject included four sessions (2 scanning sessions for baseline and post-intervention, respectively). Contrasts for milkshake cue and milkshake delivery were computed separately for BL and PI (by averaging across the two sessions for each testing day) and used in the group-level analyses.

Two separate flexible-factorial designs for milkshake cue and milkshake delivery were specified at the group level, respectively, with subject, dietary intervention (HF/HS, LF/LS), and session (BL, PI) as factors. To control for peripheral insulin sensitivity and food preference, HOMA-IR in the baseline condition and milkshake wanting ratings were specified as covariates. Thus, each of the two GLMs for milkshake cue and milkshake delivery included the following regressors:  $BL_{HF/HS}$ ,  $PI_{HF/HS}$ ,  $BL_{LF/LS}$ , and  $PI_{LF/LS}$  (with HOMA-IR and milkshake wanting ratings as covariates). A conjunction contrast<sup>107</sup> was computed to identify regions that i) showed increased activation after the intervention on average and ii) showed an interaction effect between dietary intervention and session. That is, we used the conjunction analysis to constrain the results to those brain regions that increased their activity after the diet intervention, and over and above showed a greater dietary intervention-induced increase for the HF/HS than for the LF/LS intervention, relative to the respective baselines.<sup>107,108</sup> In addition, we computed the same contrast in the opposite direction to reveal activations that were i) generally decreased after the dietary intervention and/or ii) showed an interaction effect between intervention and session. To enable a more precise interpretation of significant group-specific differences in dietary intervention-induced changes in brain activity (conjunction contrasts), we extracted the parameter estimates in the peak voxels of all significant clusters and tested, mainly for visualization purpose, for differences between BL and PI within each dietary intervention group (i.e., separately for the HF/HS and the LF/LS group).

### Analysis of the associative learning task

For the trial-by-trial analysis of behavioral data from the learning task, we considered the Hierarchical Gaussian Filter HGF<sup>109,110</sup>; to model individualized Bayesian hierarchical learning. Unlike other models to predict associations between cues and outcome, the HGF does not assume a fixed learning rate, but allows for online adaption of the learning rate as a function of volatility.<sup>57</sup> In brief, the HGF contains three different hierarchy levels: The first level models the occurrence of the auditory and visual stimuli (i.e. perception). The second level represents the conditional probabilities of the visual stimulus given the auditory cue. The third level tracks the change in the conditional probability (i.e. log-volatility).<sup>59,111</sup>

In this study, we hypothesized that the HF/HS diet intervention would modulate learning of sensory cue associations formed by dopamine neuron function in the mesoaccumbens pathway; hence, based on numerous evidence for adaptive prediction error coding in the ventral striatum and midbrain e.g.,<sup>80,112,113</sup>, and recent findings related to the HGF<sup>58</sup> suggesting that ‘low-level prediction errors’ (sensory prediction errors) activate the ventral striatum (signed prediction errors) and midbrain (absolute prediction errors) whereas high-level uncertainty tracking in the HGF rather relates to other neuromodulatory systems (cholinergic in particular), we ignored the third level of the HGF and restricted our behavioural analyses to the lower-level computational quantities recovered by the model.

We used an identical implementation of the HGF as it has been introduced by Iglesias et al.<sup>111</sup> using the HGF toolbox (vers. 1.0; <http://www.translationalneuromodeling.org/tapas>) and modelled the following two parameters:

- a .the choice prediction error (‘low-level choice prediction error’) about the visual outcome in a given trial is the difference in the correctness of the subject’s choice and the subjective expectation (in terms of the a priori probability) of this choice being correct.<sup>111</sup> Note, this is a signed prediction error: the choice prediction error is positive when the participant made a correct choice and negative when the participant was<sup>111</sup>;

- b .the (adaptive) learning rate (i.e. uncertainty about outcome probability) by which visual stimulus probabilities are updated corresponds to the precision-weight at the second level in the HGF for the exact definition of the precision-weights at different levels, see Mathys et al.<sup>109</sup>;

From these parameters we computed the (signed) adaptive precision error relating to the precision-weighted choice prediction error about visual outcome in the HGF, that is, the product of choice prediction error (a) and the adaptive learning rate (b). This is the principal model parameter we use in our fMRI analysis of the learning task.

### **fMRI-analysis of the associative learning task**

In the course of data analysis, we excluded subjects based on the following two criteria: 1) more than 20 % invalid trials due to missing responses or reaction times longer than 1500 ms, and 2) less than 65 % correct responses. These criteria led to the exclusion of 10 BL (6 HF/HS, 4 LF/LS) and 11 PI (7 HF/HS, 4 LF/LS) datasets. Consequently, the BL condition included 19 HF/HS and 18 LF/LS datasets and PI condition included 18 HF/HS and 18 LF/LS datasets.

At the single-subject level, a GLM for each of the two sessions (BL, PI) was specified with separate regressors for the trial events auditory cue (duration 300 ms), response (1200 ms) and visual outcomes (face and house, respectively, duration 300 ms). The BOLD-response to face and house, respectively, was additionally parametrically modulated by the subject-specific adaptive precision error obtained from the HGF. Invalid trials (missed and delayed responses), if present, were modelled on a separate regressor (with the three trial events –cue, response, and outcome– collapsed into one event of 1800 ms duration). Contrasts for the parametric modulation of BOLD-response to visual outcome by adaptive prediction error were computed separately at BL and PI (by averaging across face and house for each testing day) and used in the group level GLM.

At the group level, a flexible factorial design was specified with the factors subject, dietary intervention (HF/HS, LF/LS) and session (BL, PI), and HOMA-IR as a covariate; all variances set to unequal and dependency set to 1 for dietary intervention, otherwise to 0. Because every subject performed the learning task differently, the time courses of the adaptive prediction errors were heterogeneous. As our analyses focused on the correlation between the fMRI BOLD response and precisely this leaning parameter –that can easily be influenced by outliers<sup>114</sup>– we employed the correction for the resulting departures from sphericity by assuming unequal variance for the factor subject, making the inclusion of random subject blocks unnecessary.<sup>115–117</sup>

The GLM included the following regressors, all referring to the trial-by-trial encoding of the adaptive prediction error:  $BL_{HF/HS}$ ,  $PI_{HF/HS}$ ,  $BL_{LF/LS}$ , and  $PI_{LF/LS}$  (and HOMA-IR as covariate). A conjunction contrast testing the global null<sup>107</sup>; was computed to identify brain areas that were i) generally involved in adaptive prediction error encoding and/or ii) showed an interaction effect between dietary intervention and session. That is, we used the conjunction analysis to find those brain regions that were involved in prediction error encoding, and over and above this general role showed a greater dietary intervention-induced increase for the HF/HS than for the LF/LS intervention, relative to the respective baselines.