

Title: Metrics of glycemic control but not body weight influence flavor nutrient conditioning in humans

Authors: Mary Elizabeth Baugh<sup>1,2</sup>, Monica L. Ahrens<sup>3</sup>, Amber K. Burns<sup>1,2,4</sup>, Rhianna M. Sullivan<sup>1,5</sup>, Abigail N. Valle<sup>1,6</sup>, Alexandra L. Hanlon<sup>7</sup>, Alexandra G. DiFeliceantonio<sup>1,2,8</sup>

Affiliations:

<sup>1</sup>Fralin Biomedical Research Institute at VTC, Roanoke, Virginia, United States of America

<sup>2</sup>Center for Health Behaviors Research at Fralin Biomedical Research Institute at VTC, Roanoke, Virginia, United States of America

<sup>3</sup> Department of Statistics, Blacksburg, Virginia, United States of America

<sup>4</sup>Translational Biology, Medicine, and Health, Fralin Biomedical Research Institute at VTC, Roanoke, Virginia, United States of America

<sup>5</sup> School of Neuroscience, Virginia Tech, Blacksburg, VA

<sup>6</sup> Department of Biological Sciences, Virginia Tech, Blacksburg, VA

<sup>7</sup>Center for Biostatistics and Health Data Science, Department of Statistics, Blacksburg, Virginia, United States of America

<sup>8</sup>Department of Human Nutrition, Foods, and Exercise, Virginia Tech, Blacksburg, Virginia, United States of America

Corresponding Author:

Alexandra G. DiFeliceantonio, PhD  
1 Riverside Circle, Suite 104  
Roanoke, VA 24016  
540-526-2285  
[dife@vt.edu](mailto:dife@vt.edu)

Keywords: carbohydrates, flavor nutrient conditioning, food reward, metabolism

Highlights: 3-5 bullet points

- Utilizing pooled data from 2 similar preliminary studies, we found that neither BMI nor waist-to-hip ratio were correlated with changes in flavor liking in our flavor-nutrient learning paradigm, but markers of metabolic health were negatively correlated with flavor-nutrient learning.
- Our findings highlight a variability in individual responses to flavor-nutrient learning paradigms.
- Taken in the context of existing evidence, our data emphasize the importance of investigating contributions of phenotypic factors in future research in the context of food preference and eating behaviors.

Funding Sources: Research reported in this publication was supported in part by the National Center for Advancing Translational Sciences and the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under Award Number

UL1TR003015 (AGD) and R01DK132389 (AGD). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abstract (250 words)

The modern food landscape, marked by a rising prevalence of highly refined, ultra-processed, and highly palatable foods, combined with genetic and environmental susceptibilities, is widely considered a key factor driving obesity at the population level. Gaining insight into the physiological and behavioral mechanisms that shape food preferences and choices is crucial for understanding obesity's development and informing prevention strategies. One factor influencing habitual eating patterns, which may impact body weight, is flavor-nutrient learning. Research suggests that post-oral signaling is diminished in both animals and humans with obesity, potentially affecting flavor-nutrient learning. By analyzing pooled data from two similar preliminary studies, we found that markers of glycemic control—specifically fasting glucose and HbA1C—rather than BMI, were negatively correlated with changes in flavor liking in our flavor-nutrient learning task. These findings contribute to the expanding body of research on flavor-nutrient learning and underscore the variability in individual responses to these paradigms. Obesity is increasingly recognized as a complex and heterogeneous condition with diverse underlying mechanisms. Together, our findings and existing evidence emphasize the importance of further investigating how phenotypic factors interact to shape food preferences and eating behaviors.

## **Introduction**

Obesity and its associated cardiometabolic conditions, including type 2 diabetes, are increasing in prevalence across the globe [1,2]. The modern food environment, characterized by increasing levels of highly refined, palatable, energy-dense foods is commonly cited as a primary driver of obesity at the population level [3]. Understanding the physiological and behavioral determinants of food preference and selection are likely important keys to understanding the pathogenesis of obesity to support population-level prevention efforts. Furthermore, understanding the mechanisms of these influences are also likely important in identifying potential targets for both behavioral and pharmacological treatments at an individual level.

While hedonic and orosensory properties of food play a role in eating behaviors, post-ingestive signals originating from the gut have also been identified as key physiological drivers of food choice [4]. One physiological driver of food choice, which likely plays a role in body weight, is flavor-nutrient learning (FNL). As a classical conditioning model, an initially arbitrary stimulus, such as the taste and olfactory sensation of a novel flavor, becomes a conditioned stimulus (CS) when it is associated with a biologically significant event (an unconditioned stimulus, US), such as the nutritional consequences of its ingestion. Used extensively in rodent models exploring drivers of appetite and eating behaviors [5,6], FNL has established post-ingestive signals related to nutrient availability for metabolism as an important mechanism linking neural substrates of reward with food preference and consumption behaviors [7,8].

The mesocorticolimbic dopamine system is central to eating behavior, motivation, and reward learning, including FNL [9–11]. Studies in mouse models have demonstrated that intragastric infusion of nutrients increases firing of ventral tegmental area dopamine neurons [12] and stimulates dopamine efflux in the striatum [13]. This dopamine signaling is required for the

acquisition of preferences through FNL [14–16]. There is some indication that these dopaminergic signals may be disrupted in obesity. For example, findings from positron-emission tomography studies suggest a negative quadratic relationship between striatal dopamine 2 receptor binding potential and body mass index (BMI) [17], indicating individuals with more severe obesity have lower dopamine receptor availability and possibly higher dopamine tone[18]leading to a reduction in dopamine-mediated reward sensitivity. Additionally, some evidence in animal models and humans suggests that post-oral signals of nutrient availability, which contribute to FNL, may be blunted in states of obesity [19,20]. Taken together, these data suggest FNL may be attenuated in states of established obesity; however, studies in both rodents and humans assessing the relationship between body weight status and FNL have yielded mixed findings [21–24].

Obesity is associated with varying degrees of altered glucose metabolism and insulin action; and though obesity is a primary risk factor, not all individuals with obesity will develop insulin resistance or type 2 diabetes[25,26]. Similarly, the development of insulin resistance may exist in the absence of obesity or precede excess weight gain in some individuals [27,28]. Because mechanisms of glucose sensing and metabolism in the periphery have been implicated as key to FNL[13,29–33], characterizing the relationships among weight status, glycemic control, and FNL is an important step in understanding how gut-brain interactions facilitate eating behaviors and influence long-term energy balance. Interestingly, central insulin, which is thought to primarily originate from the periphery [34], plays a key role in modulating dopamine activity in corticolimbic brain regions involved in food reward and motivated behaviors [35–37]. Reduced peripheral insulin sensitivity has been associated with reductions in insulin-mediated functional connectivity among these corticolimbic regions [36] and reductions in associative learning rate

[38]. Thus, peripherally circulating insulin levels could provide explanatory insight into observed differences in FNL outcomes.

Using data collected in 2 different preliminary studies with similar approaches, our overall objective was to assess the relationship between body weight status and behavioral food liking outcomes of FNL. We hypothesized that higher BMI would be associated with reduced FNL. Furthermore, given that obesity and insulin resistance are tightly linked [39] and glucose and insulin metabolism are likely important for FNL [22,29,31,32,40], we hypothesized that biomarkers of glycemic control may further characterize reward learning for carbohydrate-paired flavors in individuals across the BMI spectrum.

## **Materials and Methods**

### Overview of Study Designs

Data presented here are from 2 preliminary studies with similar study designs. Data collection for each study occurred from February 2022-May 2023 and May 2023-November 2023, respectively. The protocols for both studies were approved by the Virginia Tech Review Board (#21-964 and #19-927). Both studies were randomized cross-over designs, in which all participants completed all exposures for the conditions utilized in the respective study. **Figure 1** illustrates the general study design followed by both studies. The study designs and stimuli utilized were adapted from previous reported studies [29,31].

### Participants

For both studies, individuals aged 18-45 years were recruited to participate. Participants with BMI 18.5-30 kg/m<sup>2</sup> were recruited for Study 1, and participants with BMI 18.5-40 kg/m<sup>2</sup> were recruited for Study 2. All participants reported not taking medications known to influence study measures, including antiglycemic agents, thyroid medications, antidepressants, and

antipsychotics. They also reported not using tobacco or nicotine products and no history of cardiometabolic disease diagnosis. All participants provided verbal and written informed consent prior to participation in the studies.

## Pre-Test

### *Stimuli*

Stimuli used during the pre-test were 10 differently flavored, sweetened, non-caloric drinks containing demineralized water combined with sucralose (0.00862% w/v; Sigma-Aldrich MO, USA), citric acid (0.1% w/v; Sigma-Aldrich MO, USA), and one of the following flavors: 0.1% v/v acerola, 0.5% v/v bilberry, 0.1% v/v horchata, 0.1% v/v lulo, 0.2% v/v yuzu, 0.1% v/v papaya, 0.1% v/v chamomile, 0.1% v/v aloe vera, 0.1% v/v mamey, or 0.2% v/v maqui berry (Bell Labs Flavors and Fragrances, IL, USA). All drinks were sweetened to be equivalent to the sweetness of 75 kilocalories of sucrose as previously experimentally determined [31,41].

### *Procedure*

#### Internal State Measures

Hunger has been shown to affect preference ratings and FNL outcomes [42,43]. Therefore, participants were instructed to arrive at the laboratory feeling neither hungry nor full, and time since last food or drink consumption was recorded. At the start of the session, participants rated their current hunger, fullness, and thirst using visual analog scales (VAS) with ranges from 0 (e.g., "Not hungry at all") to 100 (e.g., "Very hungry"). For each participant, all lab-based drink exposure sessions and the post-test session were scheduled at approximately the same time of day to minimize potential within-subject differences in hunger ratings across sessions.

### *Anthropometrics*

During a screening and informed consent session, participants' height (wall-mounted stadiometer) and weight (Health O Meter ProPlus digital scale) were measured for calculation of BMI. In addition, waist and hip circumference were measured using Gulick tape measure following World Health Organization protocols [44] for the calculation of waist-to-hip ratio.

#### Perceptual Scale Training

After providing informed consent, participants were first trained in using the general labeled magnitude scale (gLMS) [45] and labeled hedonic scale (LHS) [46] for intensity and liking ratings, respectively.

#### Flavor Ratings

Participants were then presented with cups containing ~10 mL of each sweetened, flavored solution. Each of the 10 flavors was presented in 3 presentation blocks for a total of 30 presentations. The order of the 10 flavors was randomized within each presentation block, but order of the 30 presentations remained the same across all participants. Participants were instructed to take the entire solution into the mouth, swish it around, and expectorate into a sink. Next, participants rated the solution for intensity using the gLMS, liking using the LHS, and familiarity using a VAS anchored with "Not at all" and "Extremely". After tasting each solution, participants performed the same swish-and-spit procedure with deionized water and waited 30 seconds before tasting the next solution presentation. Flavors chosen to be paired with exposure session drink stimuli were individualized for each participant and were required to be similarly low in rated liking (i.e., below "Like Moderately" but greater than "Neutral") averaged across the 3 presentations for each participant. If flavors matching this criterion could not be identified, participants were excluded from further participation.

#### Drink Exposure Sessions

Participants completed drink exposure sessions in a randomized, cross-over order, in which all participants were subject to all conditions.

### *Experimental Drink Stimuli*

Flavors identified during the flavor ratings procedure based on rated liking unique to each participant were paired with 355 ml caloric (CS+) and non-caloric (CS-) drinks to be used as experimental stimuli during blood draw and indirect calorimetry lab session and at-home consumption. Drinks used a base of deionized water mixed with citric acid (0.1% w/v; Sigma-Aldrich, MO, USA). The caloric (CS+) drink contained 5.4% w/v sucrose (3.94 kcal/g; Domino Foods, Inc., NY, USA). The non-caloric (CS-) drink contained 0.00862% w/v sucralose (Sigma-Aldrich, MO, USA). Sweetness was matched between the drinks using previously published dose-response sweetness curves [41]. Drinks were colored with blue, red, or green food coloring (McCormick & Company, Inc., MD, USA), and drink color assignment was randomized for each drink condition for each participant.

### *Procedure*

Participants consumed the CS+ and CS- drinks 6 times each. For each drink condition, participants reported to the lab for 1 blood draw and 1 indirect calorimetry session. For the remaining 4 drinks, participants were instructed to consume 1 drink at home one hour before dinner the same day as the lab session and 1 drink one hour before lunch the following day (**Figure 1**). Drink compliance surveys were emailed to participants as reminders for drinks consumed outside of lab sessions.

### Blood Draw Session

Participants were asked to report to the blood draw session after fasting  $\geq 4$  hours, and time of last food or drink consumption was recorded. After IV catheter placement and a baseline blood

draw, participants were provided an experimental condition drink (i.e., CS+ or CS-) to consume within 5 minutes. Blood samples were then collected at 10, 15, 20, 30, 40, and 60 minutes after drink consumption. Participants rated hunger, fullness, and thirst on VAS at the start of the session, immediately after drink consumption, and 30 minutes after drink consumption.

#### Assessment of Blood Metabolites and Hemoglobin A1c

Blood glucose was measured in duplicate immediately after collection from whole blood using a point-of-care system (Hemocue Glucose 201 System, HemoCue, CA, USA). Blood was centrifuged and serum was stored at  $-80^{\circ}\text{C}$  for later insulin measurement using an enzyme-linked immunosorbent assay (ELISA; ALPCO, NH, USA). Hemoglobin A1c was assessed using an EDTA anticoagulated blood sample and a point-of-care analyzer (Afinion HbA1c, Abbott Laboratories, IL, USA).

#### Indirect Calorimetry Session

Participants were asked to fast for  $\geq 4$  hours (including caffeine) and refrain from exercise for 24 hours prior to the indirect calorimetry session. Upon arrival, participants reported the time of their last food or drink consumption. Indirect calorimetry was performed with a metabolic cart and canopy (TrueOne2400, Parvo Medics, Salt Lake City, UT) following best practices [47]. Prior to a 30-minute baseline measurement, participants rested quietly in a chair at a 30 degree recline for approximately 30 minutes. After the 30-minute baseline measurement, participants were provided a study drink to consume within 5 minutes. Gas exchange measurements were collected for 60 minutes following consumption of the drink. Metabolic rate was calculated using the modified Weir equation [48], and carbohydrate oxidation was calculated using tables based on the non-protein respiratory quotient proposed by Peronnet and Massicotte [49]. Participants

rated hunger, fullness, and thirst on VAS at the start of the session, immediately after drink consumption, and approximately 60 minutes after drink consumption.

### Post-Test

Participants were asked to fast for  $\geq 4$  hours prior to the session and rated hunger, fullness, and thirst levels using VAS upon arrival to the lab. Then, following the same swish-and-spit procedure used during the pre-test session, they tasted and rated intensity (gLMS), liking (LHS), and wanting (VAS) for each flavor consumed during their drink exposure sessions. These flavor solutions were prepared in the same manner as for the pre-test session (i.e., sweetened but containing no calories). As in the pre-test session, flavors were presented 3 times each, and ratings were averaged across the 3 presentations for statistical analyses.

### Statistical Analyses

Summary statistics for participant characteristics were calculated as means and standard deviations, and counts and percentages for continuous and categorical variables, respectively. Comparisons of pre-test perceptual ratings of liking, familiarity, and intensity for flavors paired with CS+ and CS- conditions were assessed with paired t-tests. Comparisons of change in rated liking and differences in post-test wanting ratings between CS+ and CS- conditions were assessed with paired t-tests. Changes in metabolic rate and respiratory quotient were determined following guidelines suggested by Fullmer et al. [47]. Resting metabolic rate and respiratory quotient were calculated as 5-minute means for which the coefficient of variation of  $VO_2$  was  $\leq 5\%$ , after discarding the first 5 minutes of data collection. Data collected during the first 5 minutes after drink consumption were additionally discarded for postprandial assessments. Slopes and areas under the curve for metabolic rate, RQ, were calculated as the rate of change from baseline to peak value and using the trapezoidal rule, respectively. Blood

insulin and glucose values from the session with the longer fasting period for each individual were used as baseline measures. To eliminate potential effects of flavor-flavor learning, eliminate mere exposure effects, and isolate the effects of learning for calorie-paired flavors specifically, perceptual ratings and metabolic responses for the CS- condition were subtracted from CS+ condition values for each individual, and then associations between perceptual ratings and metabolic health and adiposity variables were assessed using Pearson correlations. Linear mixed effects models testing interactions between drink condition and weight status or metabolic response variables on perceptual rating were also conducted and did not differ in outcome compared with Pearson correlations. Additionally, in separate models, fasting time was included as a covariate to adjust for potential effects of hunger or metabolic state, which did not influence the observed outcomes. Therefore, for parsimony and interpretability, results from correlation tests are presented here. For all statistical tests, the significance level was set to .05.

## **Results**

### Participants demographics

Participant characteristics at baseline for the overall sample and each study are shown in **Table 1**. Overall mean age for participants across the two studies was  $29 \pm 7$  years, and overall mean BMI was  $25.7 \pm 4.8$  kg/m<sup>2</sup>. Baseline blood glucose and serum insulin were  $81.5 \pm 7.9$   $9.0 \pm 4.7$ , respectively, and hemoglobin A1c was  $5.0 \pm 0.3\%$  in the overall sample. Participants in Study 2 had significantly higher body weights and waist-to-hip ratios compared with participants in Study 1. This was expected, as Study 2 aimed to intentionally recruit individuals with BMI 18-40 kg/m<sup>2</sup> but Study 1 limited BMI to  $\leq 30$  kg/m<sup>2</sup> for participation.

### Metabolic Measures

Confirming our experimental stimuli produced the expected metabolic responses, we observed that metabolic rate, respiratory quotient, blood glucose, and serum insulin were all elevated following consumption of the CS+ compared with the CS- experimental drink (**Supplemental Figure 2A & 2B**). Furthermore, the CS- experimental drink induced minimal changes from baseline in each metabolic measure.

#### Internal State and Compliance Measures

Subjectively rated hunger, fullness, and thirst at baseline were similar across all lab sessions (**Supplemental Figure 1**). Though participants were instructed to fast  $\geq 4$ h, there was a nonstatistically significant interaction between drink condition and session on fasting time ( $t = 1.80$ ,  $p = 0.08$ ), where participants reported fasting prior to indirect calorimetry sessions approximately 1 hour longer ( $t(74) = 1.31$ ,  $p = 0.20$ ) before consumption of the CS+ compared with CS- drink but 1 hour shorter ( $t(74.2) = -1.24$ ,  $p = 0.22$ ) prior to blood draw sessions (**Supplemental Figure 1A**).

Compliance in consuming the drinks 1 hour before lunch and dinner at home was 96%. In the 8 cases of non-compliance, only 1 participant consumed less than the entire drink on 1 occasion; in all other cases of non-compliance, participants consumed the drink outside of the specified time of 1 hour before a meal, up to 2 hours before or after the specified time.

#### Perceptual Ratings

During the pre-test, there were no statistically significant differences between flavors paired with CS+ and CS- drink conditions in rated liking ( $t(25) = 1.479$ ,  $p = 0.15$ ), familiarity ( $t(25) = -0.281$ ,  $p = 0.78$ ), or intensity ( $t(25) = 0.979$ ,  $p = 0.33$ ; **Figure 1**), as expected. At post-test we observed no overall significant differences in change in liking ( $t(25) = 0.179$ ,  $p = 0.86$ ) or post-test wanting ( $t(25) = 0.971$ ,  $p = 0.34$ ) ratings between CS+ and CS- drink conditions (**Figure 2**).

### Perceptual Ratings Associated with Characteristics of Weight Status & Glycemic Control

Although we did not observe overall effects of conditioning, there was substantial variation in responses to the conditioning paradigm (**Figure 2**). We sought to determine if this variation could be explained by characteristics of adiposity and metabolic health. Among those tested, only baseline blood glucose ( $r = -0.39$ ,  $p = 0.05$ ) and hemoglobin A1c ( $r = -0.43$ ,  $p = 0.03$ ) were correlated with change in rated liking (**Figure 3**), such that greater baseline blood glucose and HA1c were associated with smaller changes in liking of the CS+ after conditioning. These observed relationships remained when tested in a linear mixed effects model controlling for fasting time and including study as a random intercept. In contrast, no measures of adiposity or metabolic health were correlated with post-test rated wanting. No measures of dynamic metabolic responses, including areas under the curve for blood insulin and glucose, to consumption of condition drinks were correlated with changes in rated liking or post-test wanting ratings (**Supplemental Tables 1 & 2**).

### **Discussion**

Pooling data from 2 preliminary studies, we assessed the relationship between body weight status, as measured by BMI and WHR, and glycemic control, as measured by blood glucose, blood insulin, and hemoglobin A1c with behavioral outcomes using a FNL paradigm. We specifically recruited participants across a range of BMIs to examine if body weight status altered flavor nutrient learning as is suggested by findings that people with obesity have a blunted response to nutrients. We found that markers of glycemic control, namely baseline blood glucose and hemoglobin A1c, rather than BMI or WHR, predicted behavioral outcomes in our FNL paradigm.

Obesity has been associated with blunted response to post-ingestive nutrients. Specifically, in response to a fat stimulus **delivered directly to the gut**, inhibition of hypothalamic AgRP neurons was reduced after onset of high fat diet-induced obesity in mice [19]. In humans, van Galen et al. [20] observed attenuations in functional magnetic resonance imaging (fMRI)-assessed blood oxygen level-dependent (BOLD) signal after the onset of intragastric infusions of carbohydrate and fat and in single photon emission computed tomography (SPECT) imaging radiotracer binding after intragastric fat infusion in healthy weight participants, but not participants with obesity. Furthermore, Perszyk et al. [50] noted a supra-additive effect of foods containing fat and carbohydrate in combination on willingness-to-pay, thought to be dopamine mediated, in individuals with healthy weight but not those with obesity. Together, these observations have led to the postulation that FNL may be impaired in individuals with obesity. However, we did not observe a relationship between body weight status, assessed using BMI and WHR, and ratings of wanting or change in liking for calorie-paired flavor cues. In contrast, Ribeiro et al. reported similar increases in preference for calorie-paired flavors between individuals with obesity and healthy weight [23]. Of note, the group with obesity had substantially higher BMI ( $\bar{x} = 50.5 \pm 9.5$  kg/m<sup>2</sup>) than our data presented here. Interestingly, however, the increase in preference was observed only when *ad libitum* intake was considered as the behavioral measure; like our own data, Ribeiro et al. noted no change in pleasantness (i.e., liking) ratings for the CS+ flavor in the overall sample.

Historically, FNL paradigms in healthy weight humans have produced variable results in terms of post-ingestive preference learning [51], but FNL studies in rodent models without diet-induced obesity spanning several decades have reliably found that postingestive signals guide behavioral preferences [3,6,52]. Interestingly, FNL studies in rodent models of obesity have produced mixed findings in terms of acquisition of behavioral preference. Woods et al. [22]

observed that rats maintained on either a food restricted or ad libitum diet acquired a preference for a CS+ over CS- flavor after conditioning, yet diet-induced obese rats did not. On the other hand, Wald and Myers [21] observed that among outbred obesity-prone, obesity-resistant, and chow-fed control rats, obesity-prone rats who had gained the most weight during a high-fat, high-carbohydrate diet period acquired greater preference for the CS+ over the CS- flavor in a 2-bottle choice test compared with obesity-resistant and chow-fed animals. Interestingly, the obesity-prone rats acquired this learning at a much faster rate (i.e., within 1 training session) compared with the obesity-resistant and chow-fed groups. These differences in learning rates and conditioning responses suggest there may be underlying genetic- or trait-based differences that contribute to individual variability in food reward learning. Further supporting this notion is data linking expression of a Taq1A allele genetic variant with differences in striatal dopamine 2 receptor expression and dopamine signaling, the key signal driving reward and motivated behaviors [53]. The Taq1A polymorphism has also been shown to interact with the obesity promoting FTO gene allele to alter peripheral and central insulin insensitivity [54].

This is perhaps not surprising, as obesity and insulin resistance are clearly linked, and a majority of individuals with type 2 diabetes also have obesity [55]. Insulin, produced in the periphery by the pancreas, is a key neuroendocrine hormone in modulating dopamine action in striatal and prefrontal brain regions involved in reward learning and motivated behaviors [56]. Insulin receptors are highly expressed on dopaminergic neurons in the striatum [57], and available evidence suggests that peripheral insulin crossing the blood brain barrier is the primary source of central insulin action [34,37]. Among healthy weight individuals, elevations in peripherally circulating insulin after food consumption have been associated with attenuations in food cue reactivity in brain reward regions [35] as well as enhanced functional connectivity within mesocorticolimbic circuitry [36], presumably to regulate within-meal eating behaviors.

Insulin resistance has been associated with reduced food value encoding in brain reward regions [58] and increased activity in the prefrontal cortex [59]. Furthermore, learning rate during an associative learning task has been shown to be reduced in individuals with obesity and reduced insulin sensitivity [38]. In line with this evidence of insulin action on reward processing, we observed a negative association between markers of peripheral glucose and insulin metabolism, specifically baseline blood glucose levels and hemoglobin A1c, and conditioned preference, even within ranges considered clinically normal. In contrast, in cohorts of middle aged and older adults Epstein et al. observed that higher homeostatic model assessment for insulin resistance (HOMA-IR), hemoglobin A1c, fasting blood insulin, and fasting blood glucose were associated with increases in consumption of sugar-containing yogurt compared with non-nutritively sweetened yogurt [60,61]. The direction of our observed relationship is opposite of what was found in studies by Epstein et al.; however, participants in those studies had much higher insulin resistance than our sample here. It's therefore possible a non-linear relationship exists between nutrient responses driving flavor nutrient learning and markers of glycemic control. Furthermore, whether differences in ages between our sample and those of Epstein et al. contributed to differences in observed relationships between glucose metabolism and FNL outcomes is unclear.

Here, we chose to focus on subjective ratings of liking and wanting as behavioral outcomes as prior work in FNL in humans has linked markers of glucose metabolism specifically with changes in rated liking [31]. In this context, though correlative, our observations of the relationship between markers of glycemic control and changes in rated liking point toward these metabolic processes as important players in the increase of flavor liking through FNL. However, whether an increase in rated liking or pleasantness is the primary behavioral indicator of the acquisition of FNL is unclear, as with all learning paradigms measuring acquisition through

behavioral expression is imprecise. Choosing the right behavioral measurement is essential, and it might be possible that physiological measures correlate with some but not all behavioral ones. Some work in animal models suggests that motivation is a better measure of FNL and that liking, as assessed by oral taste reactivity, is not always associated with learned preference [6,62]. The findings from Riberio et al.[23] and Epstein et al.[60,61] discussed above are in line with this idea; both studies observed no difference in rated liking between CS+ and CS- conditions, yet intake of the CS+ condition was greater after the FNL paradigm. Future studies in humans incorporating assessments of motivation or incentive salience, brain response, and hedonic shifts will be important for disentangling the influence of body weight status and glycemia on FNL.

### Strengths and Limitations

Strengths of this study include the use of the CS- condition as a flavor and somatosensory control in our statistical analyses. Parsing out flavor-flavor learning, in which a flavor becomes preferred due to its association with an already preferred taste (e.g., sweetness), from FNL, in which the nutritional consequences of consumption drive the associative learning, can be a potential challenge for many studies with similar designs. Flavor novelty is a critical component of FNL paradigm design that commonly is not objectively assessed [51]. Rated familiarity was similar between CS+ and CS- flavors at baseline in our sample. Therefore, subtracting perceptual values of CS- flavors allowed us to control for taste and other somatosensory properties of our drinks to isolate the behavioral effects of the post-ingestive consequences of our CS+ condition. In addition, this methodological approach allowed us to rule out the mere exposure effect, in which liking or preference is increased simply because of repeated exposures.

There are several limitations to this study. First, as mentioned above, recent studies in FNL in humans have reported finding differences in behavioral task outcomes, specifically *ad libitum* intake, without concurrent differences in rated liking. In the present analysis, behavioral task outcomes, such as *ad libitum* intake, were not assessed. As these tasks could better reflect the constructs of motivation or incentive salience, they should be included in addition to measures of liking or hedonic changes in future studies. In addition, while hunger and fullness ratings were not different across sessions, it is possible that macronutrient composition of meals consumed on the day of lab sessions could have influenced preference outcomes. Second, our study samples were metabolically healthy overall; indeed, for one study included in this analysis, hemoglobin A1c levels greater than 5.7% were exclusionary from study participation, and in both studies, diagnoses of metabolic disorders were exclusionary criteria. Similarly, as participants were only instructed to fast  $\geq 4$  hours prior to blood draws sessions, we were unable to calculate proxies of insulin resistance, such as HOMA-IR or quantitative insulin sensitivity check index, which rely on blood insulin and glucose measures collected after  $\geq 8$ -hour fast. In addition, measures of body weight status were limited to only BMI and WHR for this analysis. As adipose tissue deposition site is strongly linked with insulin sensitivity and glycemic control [63,64], it will be important for future studies to assess body fat distribution using more precise measures. Lastly, while our measure of hemoglobin A1c as an assessment of glycemic control is useful for application in the clinic, future studies should consider more comprehensive assessment of blood glucose and insulin dynamics, such as glucose tolerance tests or clamps.

### Summary and future directions

Altered post-ingestive response to nutrients had been suggested as both a potential cause and consequence of obesity. Here, we found glycemic parameters within values considered clinically normal predicted changes in subjective experience in a FNL study. While these findings cannot

resolve the issue of cause or consequence, they provide support for blunted nutrient learning as fasting blood glucose and hemoglobin A1c increase. Future studies should consider incorporating more intrinsic behavioral measures of motivation or incentive salience as well as measured brain response to provide a more complete picture of the relationships among body weight status, clinical indicators of metabolic health, and FNL. Obesity is increasingly being recognized as a heterogeneous condition with diverse pathophysiology [65]. Together, the data presented here along with available evidence in the literature highlight a need to further characterize interactions between individual phenotypic traits on developing food preferences and eating behaviors.

## Acknowledgements

The authors would like to acknowledge Bridget Carter, Alexandra Basiliere, Arijit Pradhan, Ryan McMillan for their efforts in data collection and sample analysis for the results presented here.

## References

- [1] World Health Organization. Regional Office for Europe, WHO European Regional Obesity Report 2022, World Health Organization. Regional Office for Europe, Copenhagen, 2022. <https://iris.who.int/handle/10665/353747>.
- [2] P. Saeedi, I. Petersohn, P. Salpea, B. Malanda, S. Karuranga, N. Unwin, S. Colagiuri, L. Guariguata, A.A. Motala, K. Ogurtsova, J.E. Shaw, D. Bright, R. Williams, IDF Diabetes Atlas Committee, Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition, *Diabetes Res Clin Pract* 157 (2019) 107843. <https://doi.org/10.1016/j.diabres.2019.107843>.
- [3] H.-R. Berthoud, C.D. Morrison, K. Ackroff, A. Sclafani, Learning of food preferences: mechanisms and implications for obesity & metabolic diseases, *Int J Obes* 45 (2021) 2156–2168. <https://doi.org/10.1038/s41366-021-00894-3>.
- [4] I.E. de Araujo, M. Schatzker, D.M. Small, Rethinking Food Reward, *Annu Rev Psychol* 71 (2020) 139–164. <https://doi.org/10.1146/annurev-psych-122216-011643>.
- [5] A. Sclafani, Nutritionally based learned flavor preferences in rats., in: *Taste, Experience, and Feeding.*, American Psychological Association, Washington, DC, US, 1990: pp. 139–156. <https://doi.org/10.1037/10075-010>.
- [6] K.P. Myers, The convergence of psychology and neurobiology in flavor-nutrient learning, *Appetite* 122 (2018) 36–43. <https://doi.org/10.1016/j.appet.2017.03.048>.

- [7] W. Han, L.A. Tellez, M.H. Perkins, I.O. Perez, T. Qu, J. Ferreira, T.L. Ferreira, D. Quinn, Z.W. Liu, X.B. Gao, M.M. Kaelberer, D.V. Bohórquez, S.J. Shammah-Lagnado, G. de Lartigue, I.E. de Araujo, A Neural Circuit for Gut-Induced Reward, *Cell* 175 (2018) 665–678.e23. <https://doi.org/10.1016/j.cell.2018.08.049>.
- [8] H.E. Tan, A.C. Sisti, H. Jin, M. Vignovich, M. Villavicencio, K.S. Tsang, Y. Goffer, C.S. Zuker, The gut–brain axis mediates sugar preference, *Nature* 580 (2020) 511–516. <https://doi.org/10.1038/s41586-020-2199-7>.
- [9] W. Schultz, P. Dayan, P.R. Montague, A neural substrate of prediction and reward, *Science* 275 (1997) 1593–1599. <https://doi.org/10.1126/science.275.5306.1593>.
- [10] K.C. Berridge, T.E. Robinson, What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?, *Brain Research Reviews* 28 (1998) 309–369. [https://doi.org/10.1016/S0165-0173\(98\)00019-8](https://doi.org/10.1016/S0165-0173(98)00019-8).
- [11] R.D. Palmiter, Is dopamine a physiologically relevant mediator of feeding behavior?, *Trends Neurosci* 30 (2007) 375–381. <https://doi.org/10.1016/j.tins.2007.06.004>.
- [12] A.B. Fernandes, J.A. da Silva, J. Almeida, G. Cui, C.R. Gerfen, R.M. Costa, A.J. Oliveira-Maia, Postingestive Modulation of Food Seeking Depends on Vagus-Mediated Dopamine Neuron Activity, *Neuron* 106 (2020) 778–788.e6. <https://doi.org/10.1016/j.neuron.2020.03.009>.
- [13] X. Ren, J.G. Ferreira, L. Zhou, S.J. Shammah-Lagnado, C.W. Yeckel, I.E. de Araujo, Nutrient selection in the absence of taste receptor signaling, *J Neurosci* 30 (2010) 8012–8023. <https://doi.org/10.1523/JNEUROSCI.5749-09.2010>.
- [14] K. Touzani, R.J. Bodnar, A. Sclafani, Neuropharmacology of Learned Flavor Preferences, *Pharmacol Biochem Behav* 97 (2010) 55–62. <https://doi.org/10.1016/j.pbb.2010.06.001>.
- [15] A. Sclafani, K. Touzani, R.J. Bodnar, Dopamine and learned food preferences, *Physiol Behav* 104 (2011) 64–68. <https://doi.org/10.1016/j.physbeh.2011.04.039>.
- [16] K. Touzani, R. Bodnar, A. Sclafani, Activation of dopamine D1-like receptors in nucleus accumbens is critical for the acquisition, but not the expression, of nutrient-conditioned flavor preferences in rats, *Eur J Neurosci* 27 (2008) 1525–1533. <https://doi.org/10.1111/j.1460-9568.2008.06127.x>.
- [17] A. Horstmann, W.K. Fenske, M.K. Hankir, Argument for a non-linear relationship between severity of human obesity and dopaminergic tone, *Obesity Reviews* 16 (2015) 821–830. <https://doi.org/10.1111/obr.12303>.
- [18] V.L. Darcey, J. Guo, M. Chi, S.T. Chung, A.B. Courville, I. Gallagher, P. Herscovitch, R. Howard, M. La Noire, L. Milley, A. Schick, M. Stagliano, S. Turner, N. Urbanski, S. Yang, E. Yim, N. Zhai, M.S. Zhou, K.D. Hall, Striatal dopamine tone is positively associated with adiposity in humans as determined by PET using dual dopamine type-2 receptor antagonist tracers, *Mol Psychiatry* (2025) 1–10. <https://doi.org/10.1038/s41380-025-02960-y>.
- [19] L.R. Beutler, T.V. Corpuz, J.S. Ahn, S. Kosar, W. Song, Y. Chen, Z.A. Knight, Obesity causes selective and long-lasting desensitization of AgRP neurons to dietary fat, *eLife* 9 (2020) e55909. <https://doi.org/10.7554/eLife.55909>.
- [20] K.A. van Galen, A. Schranter, K.W. Ter Horst, S.E. la Fleur, J. Booij, R.T. Constable, G.J. Schwartz, R.J. DiLeone, M.J. Serlie, Brain responses to nutrients are severely impaired and not reversed by weight loss in humans with obesity: a randomized crossover study, *Nat Metab* 5 (2023) 1059–1072. <https://doi.org/10.1038/s42255-023-00816-9>.
- [21] H.S. Wald, K.P. Myers, Enhanced flavor-nutrient conditioning in obese rats on a high-fat, high-carbohydrate choice diet., *Physiol Behav* 151 (2015) 102–110. <https://doi.org/10.1016/j.physbeh.2015.07.002>.
- [22] C.A. Woods, Z.R. Guttman, D. Huang, R.A. Kolaric, A.I. Rabinowitsch, K.T. Jones, S. Cabeza de Vaca, A. Sclafani, K.D. Carr, Insulin receptor activation in the nucleus

- accumbens reflects nutritive value of a recently ingested meal, *Physiology & Behavior* 159 (2016) 52–63. <https://doi.org/10.1016/j.physbeh.2016.03.013>.
- [23] G. Ribeiro, A.B. Fernandes, F.P.M. Oliveira, J.S. Duarte, M. Oliveira, C. Limbert, R.M. Costa, D.C. Costa, A.J. Oliveira-Maia, Postingestive reward acts through behavioral reinforcement and is conserved in obesity and after bariatric surgery, *PLOS Biology* 22 (2024) e3002936. <https://doi.org/10.1371/journal.pbio.3002936>.
- [24] M.D. Meyer, V.B. Risbrough, J. Liang, K.N. Boutelle, Pavlovian conditioning to hedonic food cues in overweight and lean individuals, *Appetite* 87 (2015) 56–61. <https://doi.org/10.1016/j.appet.2014.12.002>.
- [25] T. McLaughlin, F. Abbasi, C. Lamendola, G. Reaven, Heterogeneity in the Prevalence of Risk Factors for Cardiovascular Disease and Type 2 Diabetes Mellitus in Obese Individuals: Effect of Differences in Insulin Sensitivity, *Archives of Internal Medicine* 167 (2007) 642–648. <https://doi.org/10.1001/archinte.167.7.642>.
- [26] N. Stefan, H.-U. Häring, M.B. Schulze, Metabolically healthy obesity: the low-hanging fruit in obesity treatment?, *The Lancet Diabetes & Endocrinology* 6 (2018) 249–258. [https://doi.org/10.1016/S2213-8587\(17\)30292-9](https://doi.org/10.1016/S2213-8587(17)30292-9).
- [27] S. Lee, M.E. Lacy, M. Jankowich, A. Correa, W.-C. Wu, Association between obesity phenotypes of insulin resistance and risk of type 2 diabetes in African Americans: The Jackson Heart Study, *Journal of Clinical & Translational Endocrinology* 19 (2020) 100210. <https://doi.org/10.1016/j.jcte.2019.100210>.
- [28] K. Morino, K.F. Petersen, S. Dufour, D. Befroy, J. Frattini, N. Shatzkes, S. Neschen, M.F. White, S. Bilz, S. Sono, M. Pypaert, G.I. Shulman, Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents, *J Clin Invest* 115 (2005) 3587–3593. <https://doi.org/10.1172/JCI25151>.
- [29] I.E. de Araujo, T. Lin, M.G. Veldhuizen, D.M. Small, Metabolic regulation of brain response to food cues, *Curr Biol* 23 (2013) 878–883. <https://doi.org/10.1016/j.cub.2013.04.001>.
- [30] M.M. Kaelberer, K.L. Buchanan, M.E. Klein, B.B. Barth, M.M. Montoya, X. Shen, D.V. Bohórquez, A gut-brain neural circuit for nutrient sensory transduction, *Science* 361 (2018). <https://doi.org/10.1126/science.aat5236>.
- [31] M.G. Veldhuizen, R.K. Babbs, B. Patel, W. Fobbs, N.B. Kroemer, E. Garcia, M.R. Yeomans, D.M. Small, Integration of Sweet Taste and Metabolism Determines Carbohydrate Reward., *Curr Biol* 27 (2017) 2476–2485.e6. <https://doi.org/10.1016/j.cub.2017.07.018>.
- [32] L. Zhang, W. Han, C. Lin, F. Li, I.E. de Araujo, Sugar Metabolism Regulates Flavor Preferences and Portal Glucose Sensing., *Front Integr Neurosci* 12 (2018) 57. <https://doi.org/10.3389/fnint.2018.00057>.
- [33] S. Zukerman, K. Ackroff, A. Sclafani, Post-oral appetite stimulation by sugars and nonmetabolizable sugar analogs, *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* 305 (2013). <https://doi.org/10.1152/ajpregu.00297.2013>.
- [34] A. Kleinridders, W. Cai, L. Cappellucci, A. Ghazarian, W.R. Collins, S.G. Vienberg, E.N. Pothos, C.R. Kahn, Insulin resistance in brain alters dopamine turnover and causes behavioral disorders, *Proc Natl Acad Sci U S A* 112 (2015) 3463–3468. <https://doi.org/10.1073/pnas.1500877112>.
- [35] N.B. Kroemer, L. Krebs, A. Kobiella, O. Grimm, S. Vollstädt-Klein, U. Wolfensteller, R. Kling, M. Bidlingmaier, U.S. Zimmermann, M.N. Smolka, (Still) longing for food: Insulin reactivity modulates response to food pictures, *Human Brain Mapping* 34 (2013) 2367–2380. <https://doi.org/10.1002/hbm.22071>.
- [36] S. Edwin Thanarajah, S. Iglesias, B. Kuzmanovic, L. Rigoux, K.E. Stephan, J.C. Brüning, M. Tittgemeyer, Modulation of midbrain neurocircuitry by intranasal insulin, *Neuroimage* 194 (2019) 120–127. <https://doi.org/10.1016/j.neuroimage.2019.03.050>.

- [37] S. Kullmann, D. Blum, B.A. Jaghutriz, C. Gassenmaier, B. Bender, H.-U. Häring, G. Reischl, H. Preissl, C. la Fougère, A. Fritsche, M. Reimold, M. Heni, Central Insulin Modulates Dopamine Signaling in the Human Striatum, *J Clin Endocrinol Metab* 106 (2021) 2949–2961. <https://doi.org/10.1210/clinem/dgab410>.
- [38] R. Hanssen, L. Rigoux, B. Kuzmanovic, S. Iglesias, A.C. Kretschmer, M. Schlamann, K. Albus, S. Edwin Thanarajah, T. Sitnikow, C. Melzer, O.A. Cornely, J.C. Brüning, M. Tittgemeyer, Liraglutide restores impaired associative learning in individuals with obesity, *Nat Metab* 5 (2023) 1352–1363. <https://doi.org/10.1038/s42255-023-00859-y>.
- [39] B.B. Kahn, J.S. Flier, Obesity and insulin resistance, *J Clin Invest* 106 (2000) 473–481.
- [40] L.A. Tellez, X. Ren, W. Han, S. Medina, J.G. Ferreira, C.W. Yeckel, I.E. De Araujo, Glucose utilization rates regulate intake levels of artificial sweeteners, *Journal of Physiology* 591 (2013) 5727–5744. <https://doi.org/10.1113/jphysiol.2013.263103>.
- [41] G.E. DuBois, D.E. Walters, S.S. Schiffman, Z.S. Warwick, B.J. Booth, S.D. Pecore, K. Gibes, B.T. Carr, L.M. Brands, Concentration—Response Relationships of Sweeteners, in: *Sweeteners*, American Chemical Society, 1991: pp. 261–276. <https://doi.org/10.1021/bk-1991-0450.ch020>.
- [42] S. Mobini, L.C. Chambers, M.R. Yeomans, Effects of hunger state on flavour pleasantness conditioning at home: flavour-nutrient learning vs. flavour-flavour learning, *Appetite* 48 (2007) 20–28. <https://doi.org/10.1016/j.appet.2006.05.017>.
- [43] K.M. Wall, M.C. Farruggia, E.E. Perszyk, A. Kanyamibwa, S. Fromm, X.S. Davis, J.R. Dalenberg, A.G. DiFeliceantonio, D.M. Small, No evidence for an association between obesity and milkshake liking., *Int J Obes (Lond)* 44 (2020) 1668–1677. <https://doi.org/10.1038/s41366-020-0583-x>.
- [44] Waist circumference and waist-hip ratio: report of a WHO expert consultation, n.d. <https://www.who.int/publications/i/item/9789241501491> (accessed May 28, 2025).
- [45] L.M. Bartoshuk, V.B. Duffy, B.G. Green, H.J. Hoffman, C.-W. Ko, L.A. Lucchina, L.E. Marks, D.J. Snyder, J.M. Weiffenbach, Valid across-group comparisons with labeled scales: the gLMS versus magnitude matching, *Physiol Behav* 82 (2004) 109–114. <https://doi.org/10.1016/j.physbeh.2004.02.033>.
- [46] J. Lim, A. Wood, B.G. Green, Derivation and Evaluation of a Labeled Hedonic Scale, *Chem Senses* 34 (2009) 739–751. <https://doi.org/10.1093/chemse/bjp054>.
- [47] S. Fullmer, S. Benson-Davies, C.P. Earthman, D.C. Frankenfield, E. Gradwell, P.S.P. Lee, T. Piemonte, J. Trabulsi, Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals, *J Acad Nutr Diet* 115 (2015) 1417–1446.e2. <https://doi.org/10.1016/j.jand.2015.04.003>.
- [48] J.B.D.B. Weir, New methods for calculating metabolic rate with special reference to protein metabolism, *J Physiol* 109 (1949) 1–9. <https://doi.org/10.1113/jphysiol.1949.sp004363>.
- [49] F. Péronnet, D. Massicotte, Table of nonprotein respiratory quotient: an update, *Can J Sport Sci* 16 (1991) 23–29.
- [50] E.E. Perszyk, Z. Hutelin, J. Trinh, A. Kanyamibwa, S. Fromm, X.S. Davis, K.M. Wall, K.D. Flack, A.G. DiFeliceantonio, D.M. Small, Fat and Carbohydrate Interact to Potentiate Food Reward in Healthy Weight but Not in Overweight or Obesity, *Nutrients* 13 (2021) 1203. <https://doi.org/10.3390/nu13041203>.
- [51] M.R. Yeomans, Flavour-nutrient learning in humans: an elusive phenomenon?, *Physiol Behav* 106 (2012) 345–355. <https://doi.org/10.1016/j.physbeh.2012.03.013>.
- [52] A. Sclafani, From appetite setpoint to appetite: 50 years of ingestive behavior research, *Physiol Behav* 192 (2018) 210–217. <https://doi.org/10.1016/j.physbeh.2018.01.001>.
- [53] E. Montalban, R. Walle, J. Castel, A. Ansoult, R. Hassouna, E. Foppen, X. Fang, Z. Hutelin, S. Mickus, E. Perszyk, A. Petitbon, J. Berthelet, F. Rodrigues-Lima, A. Cebrian-Serrano, G. Gangarossa, C. Martin, P. Trifilieff, C. Bosch-Bouju, D.M. Small, S. Luquet,

The Addiction-Susceptibility *TaqIA/Ankk1* Controls Reward and Metabolism Through D2 Receptor-Expressing Neurons, *Biological Psychiatry* 94 (2023) 424–436.  
<https://doi.org/10.1016/j.biopsych.2023.02.010>.

- [54] M. Heni, S. Kullmann, E. Ahlqvist, R. Wagner, F. Machicao, H. Staiger, H.-U. Häring, P. Almgren, L.C. Groop, D.M. Small, A. Fritsche, H. Preissl, Interaction between the obesity-risk gene *FTO* and the dopamine D2 receptor gene *ANKK1/TaqIA* on insulin sensitivity, *Diabetologia* 59 (2016) 2622–2631. <https://doi.org/10.1007/s00125-016-4095-0>.
- [55] R.A. DeFronzo, E. Ferrannini, L. Groop, R.R. Henry, W.H. Herman, J.J. Holst, F.B. Hu, C.R. Kahn, I. Raz, G.I. Shulman, D.C. Simonson, M.A. Testa, R. Weiss, Type 2 diabetes mellitus, *Nat Rev Dis Primers* 1 (2015) 1–22. <https://doi.org/10.1038/nrdp.2015.19>.
- [56] J. Gruber, R. Hanssen, M. Qubad, A. Bouzouina, V. Schack, H. Sochor, C. Schiweck, M. Aichholzer, S. Matura, D.A. Slattery, Y. Zopf, S.L. Borgland, A. Reif, S.E. Thanarajah, Impact of insulin and insulin resistance on brain dopamine signalling and reward processing - An underexplored mechanism in the pathophysiology of depression?, *Neurosci Biobehav Rev* 149 (2023) 105179.  
<https://doi.org/10.1016/j.neubiorev.2023.105179>.
- [57] D.P. Figlewicz, S.B. Evans, J. Murphy, M. Hoen, D.G. Baskin, Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat, *Brain Res* 964 (2003) 107–115. [https://doi.org/10.1016/s0006-8993\(02\)04087-8](https://doi.org/10.1016/s0006-8993(02)04087-8).
- [58] L.J. Tiedemann, S.M. Schmid, J. Hettel, K. Giesen, P. Francke, C. Büchel, S. Brassens, Central insulin modulates food valuation via mesolimbic pathways, *Nat Commun* 8 (2017) 16052. <https://doi.org/10.1038/ncomms16052>.
- [59] S. Kullmann, M. Heni, R. Veit, K. Scheffler, J. Machann, H.-U. Häring, A. Fritsche, H. Preissl, Selective insulin resistance in homeostatic and cognitive control brain areas in overweight and obese adults, *Diabetes Care* 38 (2015) 1044–1050.  
<https://doi.org/10.2337/dc14-2319>.
- [60] L.H. Epstein, M.J. Biondolillo, A. Rizwan, H. Ghanim, P. Dandona, W.K. Bickel, R.A. Paluch, Insulin Resistance and Glycated Hemoglobin in Obesity Are Associated With Preference for Sugar-Sweetened Yogurt: A Pilot Study, *Psychosom Med* 85 (2023) 289–293. <https://doi.org/10.1097/PSY.0000000000001171>.
- [61] L.H. Epstein, A. Rizwan, S. Rashid, W.K. Bickel, H. Ghanim, Glucose response to sugar challenge moderates the effect of insulin resistance on reinforcing value of sugar-sweetened yogurt, *Appetite* 193 (2024) 107160.  
<https://doi.org/10.1016/j.appet.2023.107160>.
- [62] A. Sclafani, K. Ackroff, Nutrient-conditioned flavor preference and incentive value measured by progressive ratio licking in rats, *Physiology & Behavior* 88 (2006) 88–94.  
<https://doi.org/10.1016/j.physbeh.2006.03.009>.
- [63] I.M. O’Shaughnessy, T.J. Myers, K. Stepniakowski, P. Nazzaro, T.M. Kelly, R.G. Hoffmann, B.M. Egan, A.H. Kissebah, Glucose metabolism in abdominally obese hypertensive and normotensive subjects, *Hypertension* 26 (1995) 186–192.  
<https://doi.org/10.1161/01.hyp.26.1.186>.
- [64] D.G. Carey, A.B. Jenkins, L.V. Campbell, J. Freund, D.J. Chisholm, Abdominal fat and insulin resistance in normal and overweight women: Direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM, *Diabetes* 45 (1996) 633–638.  
<https://doi.org/10.2337/diab.45.5.633>.
- [65] I.J. Neeland, P. Poirier, J.-P. Després, The Cardiovascular and Metabolic Heterogeneity of Obesity: Clinical Challenges and Implications for Management, *Circulation* 137 (2018) 1391. <https://doi.org/10.1161/CIRCULATIONAHA.117.029617>.



Table 1. Participant characteristics at baseline for the overall sample and separated by study. Data are presented as mean (standard deviation) or frequency (%).

	<b>Whole Sample (n=26)</b>	<b>Study 1 (n=11)</b>	<b>Study 2 (n=15)</b>	<b>p- value</b>
Sex				
Male, n	7 (27%)	1 (9%)	6 (40%)	0.19
Race, n				0.27
Asian	2 (8%)	2 (18%)	0 (0%)	
Black or African American	2 (8%)	1 (9%)	1 (7%)	
White	18 (69%)	6 (55%)	12 (80%)	
More than one race	2 (8%)	1 (9%)	1 (7%)	
Other	1 (4%)	0 (0%)	1 (7%)	
Unknown/Preferred not to disclose	1 (4%)	1 (9%)	0 (0%)	
Ethnicity, n				0.47
Not Hispanic or Latino	23 (89%)	9 (82%)	14 (93%)	
Hispanic or Latino	2 (8%)	1 (9%)	1 (7%)	
Prefer not to disclose	1 (4%)	1 (9%)	0 (0%)	
Age, yrs	29 (7)	27 (7)	30 (6)	0.34
Body Weight, kg	72.3 (17.3)	64.30 (12.1)	78.2 (18.4)	<b>0.04</b>
BMI, kg/m <sup>2</sup>	25.7 (4.8)	23.6 (2.9)	27.2 (5.4)	0.06
Waist-to-Hip Ratio	0.79 (0.08)	0.74 (0.04)	0.84 (0.08)	<b>0.001</b>
Hemoglobin A1c, %	5.0 (0.3)	5.1 (0.3)	5.0 (0.3)	0.66
Baseline Blood Glucose, mg/dL	81.5 (7.9)	78.2 (6.4)	84.0 (8.3)	0.07
Baseline Serum Insulin, $\mu$ IU/mL	9.0 (4.7)	10.0(5.7)	8.5 4.2)	0.50



Figure 1. Study design for both randomized, crossover pilot studies (A). In a pre-test, participants rated flavors for liking, familiarity, and intensity and completed measurements of height, weight, waist circumference, and hip circumference. Participants whose flavor ratings passed inclusion criteria were randomized to each experimental drink 6 times within 1 week in counterbalanced order. During one drink exposure session, blood was drawn at baseline and at 7 time points after drink consumption. In another session, indirect calorimetry with a metabolic cart was performed at baseline and for 1 hour after drink consumption. In a post-test, participants rated liking and wanting for flavors previously paired with experimental drinks. Pre-test mean rated liking (B), familiarity (C), and intensity (D) were similar between flavors selected for pairing with experimental drinks across Study 1 (dashed lines) and Study 2 (solid lines).

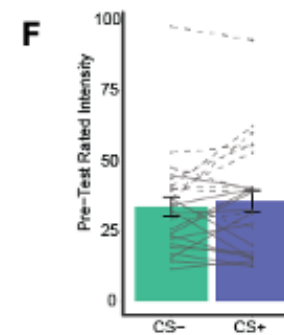
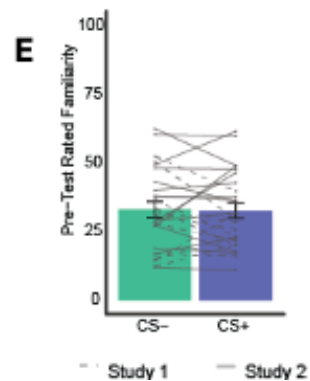
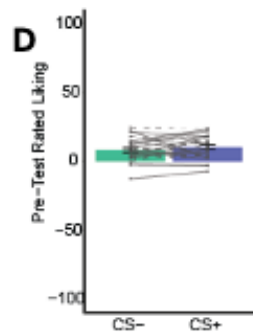
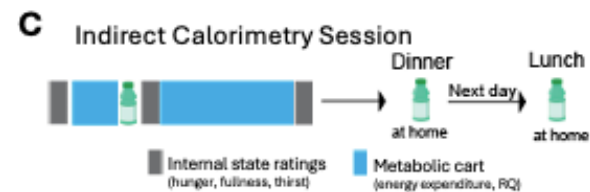
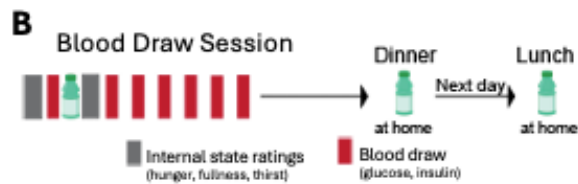
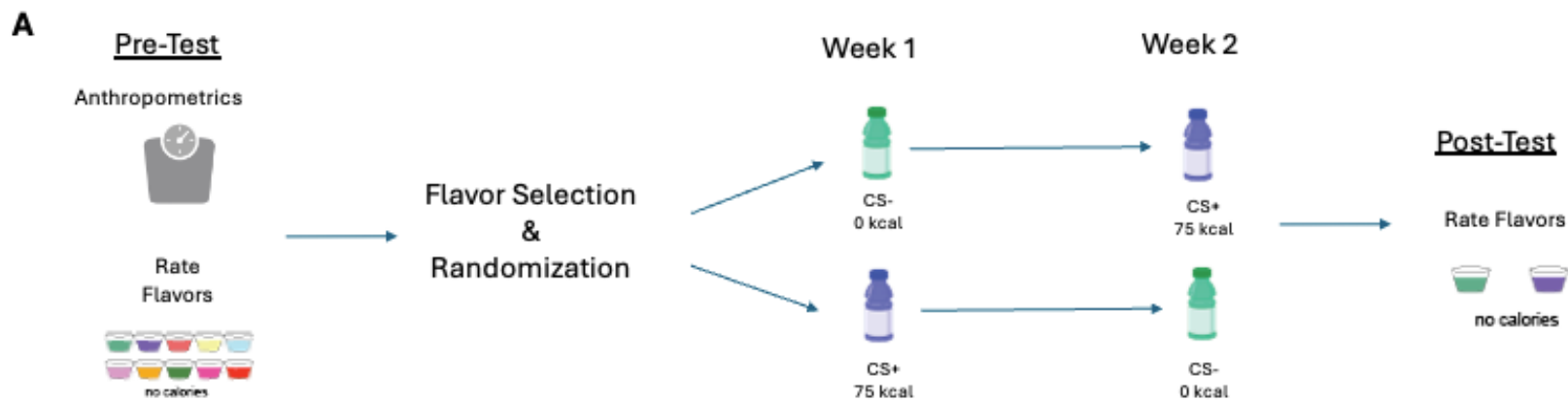


Figure 2. Change in rated liking from pre-test to post-test (A) and post-test rated wanting (B) were similar for flavors previously paired with 0-kcal (CS-) and 75-kcal (CS+) experimental drink conditions. Data presented as mean and standard error. Dashed lines represent individual participant ratings from Study 1 and solid lines represent individual participant ratings from Study 2.

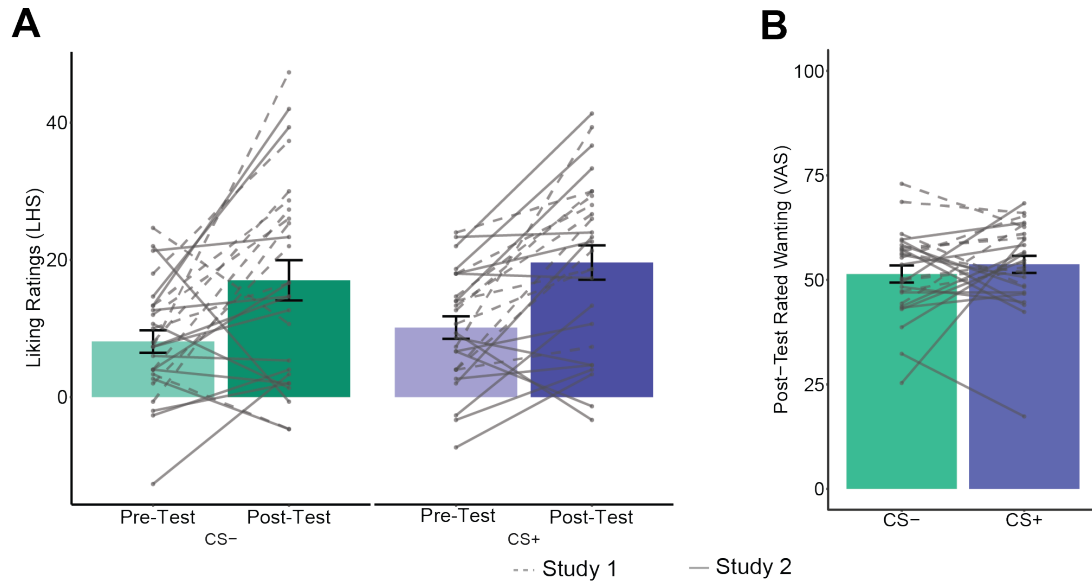
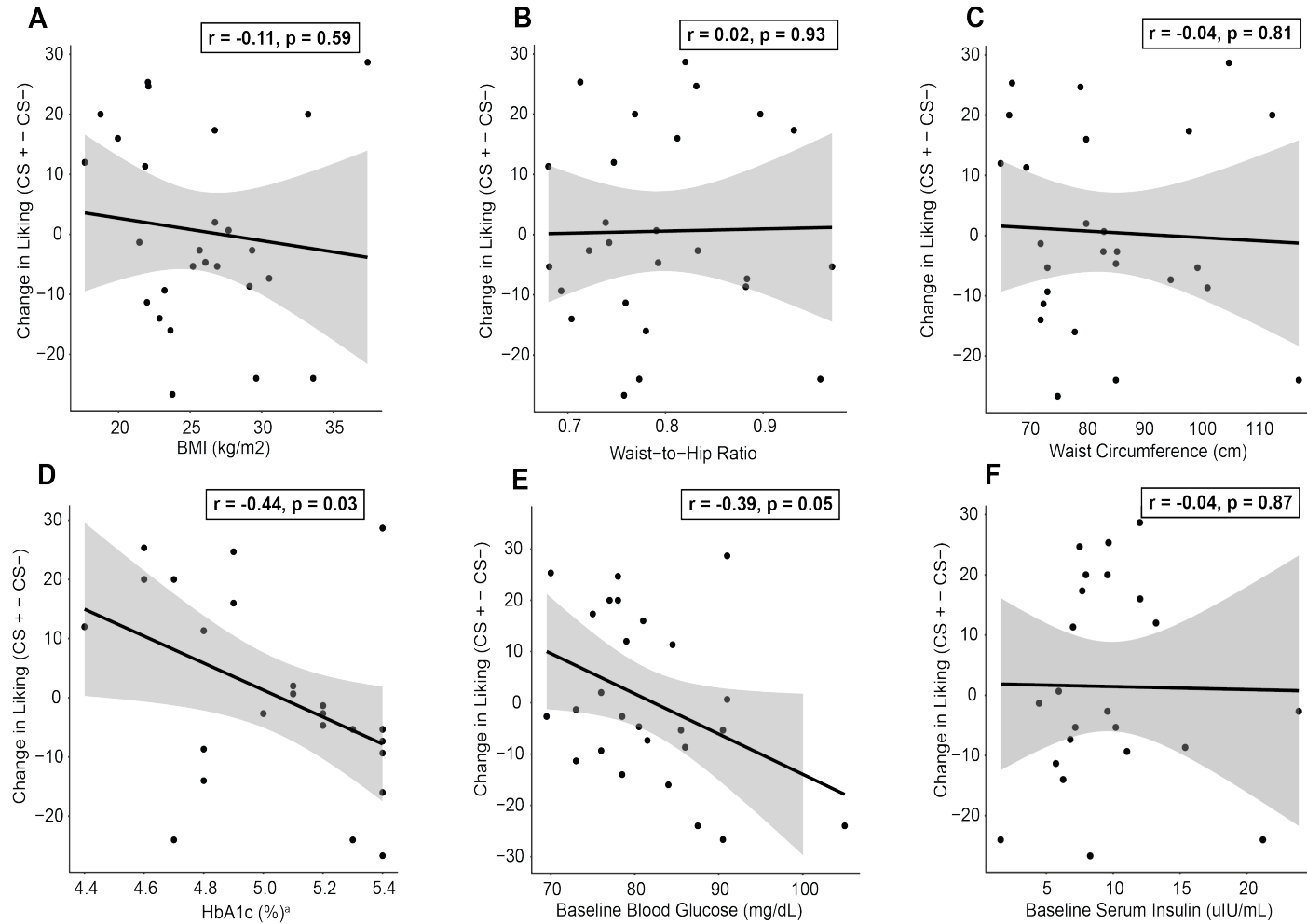


Figure 3. Correlations between body mass index (A), waist-to-hip ratio (B), waist circumference (C), hemoglobin A1c (D), baseline blood glucose (E), and baseline serum insulin (F) and change in liking ratings for the flavor previously paired with the 75-calorie CS+ experimental drink, after controlling for taste and somatosensory sensation by subtracting change in liking ratings for the 0-calorie CS- experimental drink flavor.



<sup>a</sup> n = 24 for HbA1c

Supplemental Table 1. Dynamic metabolic response correlations with change in liking for the CS+ flavor after accounting for changes liking ratings for the CS- flavor.

<b>Variable (CS+ - CS-)</b>	<b>Correlation</b>	<b>p-value</b>
Plasma Glucose AUC	0.12	0.56
Plasma Glucose Slope	0.16	0.45
Serum Insulin AUC	0.15	0.51
Serum Insulin Slope	0.06	0.79
Metabolic Rate AUC	-0.03	0.89
Metabolic Rate Slope	0.18	0.37
RQ AUC	-0.17	0.43
RQ Slope	-0.06	0.77

AUC, area under the curve; RQ, respiratory quotient

Supplemental Table 2. Dynamic metabolic response correlations with post-test wanting ratings for the CS+ flavor after accounting for post-test wanting ratings for the CS- flavor.

<b>Variable (CS+ - CS-)</b>	<b>Correlation</b>	<b>p-value</b>
Plasma Glucose AUC	0.15	0.47
Plasma Glucose Slope	0.12	0.57
Serum Insulin AUC	0.17	0.45
Serum Insulin Slope	0.12	0.60
Metabolic Rate AUC	-0.14	0.52
Metabolic Rate Slope	0.02	0.94
RQ AUC	-0.04	0.86
RQ Slope	-0.11	0.57

AUC, area under the curve; RQ, respiratory quotient

