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# FTO polymorphisms moderate the association of food reinforcement with energy intake☆,☆☆

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## HIGHLIGHTS

- Food reinforcement ($RRV_{food}$) predicts increased energy intake.
- FTO SNPs interacted with $RRV_{food}$ to predict additional variance in energy intake.
- FTO SNPs also moderated the association of $RRV_{food}$ with macronutrient and sugar intake.

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## ABSTRACT

Food reinforcement ($RRV_{food}$) is related to increased energy intake, cross-sectionally related to obesity, and prospectively related to weight gain. The fat mass and obesity-associated (FTO) gene is related to elevated body mass index and increased energy intake. The primary purpose of the current study was to determine whether any of 68 FTO single nucleotide polymorphisms (SNPs) or a FTO risk score moderate the association between food reinforcement and energy or macronutrient intake. Energy and macronutrient intake was measured using a laboratory ad libitum snack food consumption task in 237 adults of varying BMI. Controlling for BMI, the relative reinforcing value of reading ($RRV_{reading}$) and proportion of African ancestry, $RRV_{food}$ predicted 14.2% of the variance in energy intake, as well as predicted carbohydrate, fat, protein and sugar intake. In individual analyses, six FTO SNPs (*rs12921970*, *rs9936768*, *rs12446047*, *rs7199716*, *rs8049933* and *rs11076022*, spanning approximately 251 kbp) moderated the relationship between $RRV_{food}$ and energy intake to predict an additional 4.9–7.4% of variance in energy intake. We created an FTO risk score based on 5 FTO SNPs (*rs9939609*, *rs8050136*, *rs3751812*, *rs1421085*, and *rs1121980*) that are related to BMI in multiple studies. The FTO risk score did not increase variance accounted for beyond individual FTO SNPs. *rs12921970* and *rs12446047* served as moderators of the relationship between $RRV_{food}$ and carbohydrate, fat, protein, and sugar intake. This study shows for the first time that the relationship between $RRV_{food}$ and energy intake is moderated by FTO SNPs. Research is needed to understand how these processes interact to predict energy and macronutrient intake.



## 1. Introduction

Food reinforcement ($RRV_{food}$) refers to the motivation to eat, and is cross-sectionally related to increased energy intake in laboratory and usual intake situations [1,2], body mass index (BMI) and obesity in children and adults [3,4] and predicts body fat [5] and weight gain [5,6] in children and adults. The fat mass and obesity-associated (FTO) gene has been related to elevated BMI [7–9] and increased energy intake [10–13] in adults and children [14,15]. Animal models suggest that the FTO gene controls food intake through homeostatic mechanisms [16].

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Both hedonic and homeostatic processes are involved in the control of food intake [17]. While these are often considered autonomous or independent processes, they may interact to predict food intake. For example, food deprivation, a process that is related to homeostatic mechanisms and the biological need for energy intake [18], also has strong effects on $RRV_{food}$ and hedonic processes [19,20]. Understanding the relationships between homeostatic and hedonic controls of food intake may help to explain differences in eating behavior. The primary purpose of the current study was to determine whether FTO single nucleotide polymorphisms (SNPs) interact with $RRV_{food}$ to predict ad libitum energy intake in 237 adults of varying BMI. Since Frayling and colleagues' [7] initial report of an FTO variant associated with obesity, many FTO SNPs have been identified which are associated with food intake and body weight [13,21,22], but none on how food reinforcement may be moderated by FTO SNPs to influence food intake. The secondary purpose was to determine whether FTO SNPs interact with $RRV_{food}$ to predict macronutrient intake.

## 2. Methods

### 2.1. Participants

A sample of 237 participants (117 males, 120 females; 130 non-obese, 107 obese) from a study of genetic factors associated with food reinforcement was examined for single marker genetic associations. Details of the sample recruitment, inclusion/exclusion criteria [1] and findings [1,2,6,23–25] have been previously published. Participants were excluded from the study if they were taking medications associated with loss of appetite, were smokers, had diabetes, had previously been diagnosed with an eating disorder or psychiatric disorder (e.g. anxiety, depression, attention deficit hyperactivity disorder), were allergic to the ingredients in the study foods, were currently dieting, or did not rate at least a moderate liking (≥4 on a 9 point Likert type scale) for five out of the six study foods. Participants received a $50 gift certificate to local stores for completing the study. The study was approved by the University at Buffalo Health Sciences Institutional Review Board. Participant characteristics are shown in Table 1.

**Table 1**
Characteristics and predictions of characteristics with body mass index and energy intake (N = 237).

| Characteristic | | BMI (r) | Energy intake (r) |
|---|---|---|---|
| Age (years) | 34.5 ± 10.6 | 0.142* | −0.189** |
| BMI | 30.1 ± 7.6 | – | 0.125 |
| Restraint | 7.7 ± 4.7 | −0.006 | −0.263*** |
| Disinhibition | 6.3 ± 3.4 | 0.315*** | 0.099 |
| Hunger | 5.3 ± 3.2 | 0.054 | 0.168** |
| Sex (M/F) | 117/120 | − 0.152* | −0.350*** |
| Race/ethnicity | | | |
| Non-minority/minority | (176/61) | 0.039 | −0.088 |
| Laboratory intake (kcal) | | | |
| Energy intake | 601.2 ± 319.3 | 0.125 | – |
| Fat intake | 275.6 ± 147.2 | 0.121 | 0.997*** |
| Carbohydrate intake | 312.6 ± 164.6 | 0.122 | 0.998*** |
| Protein intake | 134.0 ± 68.1 | 0.192** | 0.908*** |
| Sugar intake | 167.4 ± 91.7 | 0.148** | 0.932*** |
| Food reinforcement task | | | |
| $RRV_{food}$ | 61.8 ± 132.4 | 0.141* | 0.419*** |
| $RRV_{reading}$ | 82.3 ± 114.6 | −0.005 | 0.011 |
| Liking of favorite food | 8.3 ± 0.9 | −0.031 | −0.003 |

Mean ± SD.
r = correlation coefficient, $RRV_{food}$ = highest fixed ratio schedule completed for food, $RRV_{reading}$ = highest fixed ratio schedule completed for reading time, liking ratings on a 9 point Likert type scale.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

### 2.2. Procedures

Participants visited the laboratory for two sessions, an ad libitum snack-eating session, and a food reinforcement session. Both experimental sessions were scheduled between the hours of 2 PM and 5 PM, during a normal period that individuals would consume additional energy outside of meal time. Participants were asked to refrain from eating or drinking, with the exclusion of water, for at least 3 h prior to the test session and to refrain from consuming the experimental foods in the 24 h prior to the test session. Upon initial arrival to the laboratory, participants read and signed consent forms, completed a same day and 24 h food recall, hunger questionnaires and were asked to provide a saliva DNA sample. Participants were asked to rinse their mouth with water and then spit into a plastic vial. Prior to the start of each session participants were provided with a preload of a Luna Sunrise Blueberry Bliss, Strawberry Crumble or Vanilla Almond Breakfast bar (Clif Bar & Company; Berkeley, CA, 42 g, 150 kcal, 4 g fat, 23 g carbohydrates, 7 g protein) to minimize the effects of hunger on energy intake and food reinforcement. Demographic information, height and weight measurements and three dietary habit questionnaires were administered.

### 2.3. Measurement

#### 2.3.1. Height and weight

The participant's weight and height were measured using a digital scale (Tanita Corporation of America Inc., Arlington Heights, IL) and a digital stadiometer (Measurement Concepts & Quick Medical, North Bend, WA).

#### 2.3.2. Ad libitum eating task

The ad libitum snack food consumption task was presented as a taste test. Participants were provided 210–305 kcal (42–60 g) servings of six palatable, high-energy-dense snack foods (amount of food presented (g) and energy density (kcal/g) shown in parentheses): Wavy Lay's Potato Chips (57 g, 5.4); Cooler Ranch Doritos (56 g, 5.4); M&M's (60 g, 5.0); Twix (48 g, 5.0); Kit Kat (42 g, 5.0); and Butterfinger (57 g, 4.5). Water was provided ad libitum. Participants were told that they could consume as much or as little of the food that they wanted as long as they tasted each food. Participants rated each food on a number of different characteristics including pleasurability, sweetness, blandness, flavorfulness, and bitterness using 9-point Likert-type scales. Food from the taste test was left in the room and participants were told that the food would be discarded after the session and they could continue eating if they choose to do so. When participants indicated that they were finished, they were asked to identify their favorite food from among the six available.

#### 2.3.3. Food reinforcement task

The reinforcing value of food was measured based on responding participants made for food or food alternatives on progressive ratio schedules of reinforcement. The experimental environment included two computer stations that participants could go back and forth between. At one station, participants could earn points toward food and at the other station they could earn points for time to spend reading *Time* and *Newsweek* magazines. This alternative activity was provided to reduce the likelihood that participants would engage in responding out of boredom. Participants were instructed on how to use the computer task and given a practice session.

The reinforcement task is similar to a slot machine with shapes that rotate on the screen and a point is earned each time the three shapes match in shape and color. For every five points earned, the subject was able to receive a 70–101 kcal (14–20 g) portion of his or her preferred snack food or 2 min of time to spend reading depending on which reward they were working for. The programmed reinforcement schedules for food and reading were progressive fixed ratio schedules with response requirements of 4, 8, 16, 32, 64, 128, 256, 512, 1,024,

2,048 and so forth for each point. Participants were instructed to perform one activity at a time (i.e. play the computer game, eat or read), and that the session would end when they no longer wished to earn points for access to food or time to spend reading. Water was provided ad libitum.

$RRV_{food}$ was defined as the highest fixed ratio schedule completed for food also known as the breakpoint or $P_{max}$ [1]. The $RRV_{reading}$ was used as a covariate and was defined as the highest fixed ratio schedule completed for reading time.

#### 2.3.4. Eating questionnaire

Participants completed the Three Factor Eating Questionnaire (TFEQ) [26], a validated instrument [27] with subscales that assess dietary restraint, hunger and disinhibition.

#### 2.3.5. Food liking, hunger

Subjective ratings of hunger and food hedonics were collected pre- and post intake of the pre-load and after both test sessions. For hunger, 1 indicated not at all hungry/not at all full and 10 indicated extremely hungry/extremely full, while for hedonics 1 indicated not liking at all and 9 indicated liking very much.

### 2.4. Genotyping

DNA was collected from saliva samples using a commercially available genomic DNA quick preparation kit (Oragene, DNA Genotek, Ottawa, Canada). DNA was extracted from the samples yielding 20 μL of DNA at a concentration of 100–130 ng/μL. After DNA purification, each sample was stored at −20 °C for later analysis.

#### 2.4.1. Genotyping and population stratification

384 SNPs were genotyped on an Illumina Golden gate platform (Illumina, San Diego, CA). The SNPs included 110 markers used to estimate individual (continental) ancestry. The FTO gene was chosen based on the association between FTO and obesity [7–9]. Sixty-eight FTO SNPs were chosen in two ways; as representatives for regions of high linkage disequilibrium (tag SNPs) using the software program TAGGER [28] and SNPs that were especially interesting due to previous associations or functional effects on gene transcription or performance [7–9]. All subsequent genetic analyses were performed using PLINK [29].

#### 2.4.2. Ancestry informative markers

Population stratification controls for individual differences that are correlated with the gene distribution of a subpopulation [30,31]. We genotyped a panel of 110 SNPs to estimate each individual's genetic proportion of European, Asian and African ancestry using the program STRUCTURE v2 [32]. Proportion African ancestry was used as a covariate in the current study.

#### 2.4.3. FTO risk score

Since the function of individual SNPs may interact with other SNPs to influence how FTO genes may interact with food reinforcement to predict energy or macronutrient intake, we created an FTO risk score based on 5 FTO SNPs (*rs9939609*, *rs8050136*, *rs3751812*, *rs1421085*, and *rs1121980*) that are related to BMI in multiple studies and provide data on which allele is the risk allele. The *rs9939609* risk allele (A) is related to increased body weight [9,14], BMI [7,9,14,33,21,34,35], waist circumference [21], hip circumference [9], body fat [21], and energy intake [10,13–15]. The *rs8050136* risk allele (A) is related to increased BMI [21,34,35], waist circumference [21], body fat [21,36], and energy intake [36]. The *rs3751812* risk allele (T) is related to increased BMI [21] and body fat [21]. The *rs1421085* risk allele (C) is related to increased BMI [8,21], waist circumference [21], and body fat [21]. The *rs1121980* risk allele (T) is related to increased BMI [33,21], waist circumference [21], and body fat [21]. Each FTO risk allele was treated equally in calculating the FTO score. For example using 5 FTO SNPs, if an individual was homozygous for all 5 risk alleles they would have a FTO risk score of 10 or if an individual was homozygous for all 11 non-risk alleles they would have a FTO risk score of 0. The individual FTO risk scores ranged from 0 to 10. The mean FTO risk score was $4.2 \pm 3.3$.

### 2.5. Analytic plan

The genetic dataset was cleaned by removing participants who were not successfully genotyped for at least 90% of the SNPs. Due to the diversity of our sample, both minor allele frequency (MAF) and Hardy–Weinberg equilibrium (HWE) proportions were examined within and across populations (European American, African American and other based on self-identified ethnicity). Screening within populations removed SNPs if HWE proportions $p < 0.001$. A MAF difference between populations greater than or equal to 30% was considered ancestry informative and was excluded from association analysis of the full population due to potential for false positive associations. Screening within populations removed SNPs if $MAF < 0.05$.

Zero order correlations were used to examine the relationship between BMI, energy intake and participant characteristics. Regression analyses were used to examine the interaction of FTO SNPs and the FTO risk score with $RRV_{food}$ to predict food intake (total energy intake, and carbohydrate, protein, fat intake, or sugar intake). Covariates to control for population stratification (proportion African ancestry as estimated using STRUCTURE), the $RRV_{reading}$ and BMI were included in all models. False discovery rate was used to control the family wise error rate for all 68 SNP association analyses [37].

Simple slopes were used to examine the moderating association of the models by calculating the slopes of the variants of the FTO SNPs at $RRV_{food} \pm 1$ standard deviation (SD) from the mean. The regression was graphed using the constant and the coefficients in the regression model to explore the features of the interaction between *rs12921970* and $RRV_{food}$. Data were analyzed using SYSTAT 11 (Systat Software, 2004).

## 3. Results

Characteristics of the sample are presented in Table 1. The final dataset included 64 of 68 FTO SNPs; 4 were removed based on minor allele frequency racial differences ≥30%. The remaining 64 SNPs had a minor allele frequency >0.05 and did not have any violations of Hardy–Weinberg equilibrium (<0.05).

$RRV_{food}$ explained 14.2% of the variance in total energy intake ($\beta = 0.371$, $p < 0.001$). Additionally, 6 FTO SNPs (*rs9936768*, *rs8049933*, *rs7199716*, *rs12921970*, *rs12446047*, and *rs11076022*) moderated the relationship between $RRV_{food}$ and energy intake to predict an additional 4.9–7.4% of variance of energy intake, for a total of 19.1–21.6% of the variance of energy intake (Table 2). The standardized regression coefficients for the individual SNPs (from each individual analysis) are also presented in Table 2 and the direction of the standardized regression coefficient of the individual SNPs are informative about whether the minor allele of each SNP would likely be considered a 'risk' allele (positive standardized regression coefficient) or a 'protective' allele (negative standardized regression coefficient).

The FTO risk score moderated the relationship between $RRV_{food}$ and energy intake to predict additional 2.2% ($\beta = 0.152$, $p = 0.016$) of variance of energy intake, for a total of 16.4% of the variance of energy intake. The FTO risk score did not improve the proportion of variance accounted for compared to any of the individual SNPs.

Table 2 demonstrates that in addition to energy intake, all of the FTO SNPs also moderated the relationship between $RRV_{food}$, fat intake, and carbohydrate intake (*rs9936768*, *rs8049933*, *rs7199716*, *rs12921970*, *rs12446047*, and *rs11076022*) and some of the FTO SNPs moderated the relationship between $RRV_{food}$ and protein intake (*rs7199716*,

**Table 2**
Standardized regression coefficients (β) and $R^2$ from hierarchical regression models for individual FTO SNPs predicting total energy intake, carbohydrate intake, fat intake, protein intake and sugar intake.[a]

| | *rs9936768* 52457064 | *rs8049933* 52651631 | *rs7199716* 52590749 | *rs12921970* 52422727 | *rs12446047* 52554803 | *rs11076022* 52708479 |
|---|---|---|---|---|---|---|
| *Total energy intake (kcal)* | | | | | | |
| $RRV_{food}$[b] | 0.371* | 0.371* | 0.371* | 0.371* | 0.371* | 0.371* |
| SNP | −0.111 | 0.024 | 0.129 | 0.096 | −0.039 | 0.063 |
| $RRV_{food} \times$ SNP | −0.323** | −0.295** | 0.318** | 0.294** | 0.344** | 0.306** |
| Total $R^2$ | 0.206 | 0.191 | 0.216 | 0.205 | 0.211 | 0.203 |
| *Carbohydrate intake (kcal)* | | | | | | |
| $RRV_{food}$ | 0.367* | 0.367* | 0.367* | 0.367* | 0.367* | 0.367* |
| SNP | −0.109 | 0.018 | 0.127 | 0.098 | −0.032 | 0.062 |
| $RRV_{food} \times$ SNP | −0.311** | −0.284** | 0.312** | 0.295** | 0.335** | 0.297** |
| Total $R^2$ | 0.201 | 0.186 | 0.213 | 0.205 | 0.206 | 0.199 |
| *Fat intake (kcal)* | | | | | | |
| $RRV_{food}$ | 0.375* | 0.375* | 0.375* | 0.375* | 0.375* | 0.375* |
| SNP | −0.114 | 0.029 | 0.132 | 0.094 | −0.038 | 0.064 |
| $RRV_{food} \times$ SNP | −0.332** | −0.305** | 0.318** | 0.293** | 0.349** | 0.311** |
| Total $R^2$ | 0.208 | 0.193 | 0.216 | 0.204 | 0.212 | 0.203 |
| *Protein intake (kcal)* | | | | | | |
| $RRV_{food}$ | 0.289* | 0.289* | 0.289* | 0.289* | 0.289* | 0.289* |
| SNP | −0.117 | 0.012 | 0.133 | 0.122 | −0.052 | 0.033 |
| $RRV_{food} \times$ SNP | −0.228 | −0.231 | 0.294** | 0.303** | 0.287** | 0.223 |
| Total $R^2$ | 0.172 | 0.164 | 0.203 | 0.206 | 0.183 | 0.165 |
| *Sugar intake (kcal)* | | | | | | |
| $RRV_{food}$ | 0.364* | 0.364* | 0.364* | 0.364* | 0.364* | 0.364* |
| SNP | −0.114 | −0.012 | 0.133 | 0.105 | −0.060 | 0.090 |
| $RRV_{food} \times$ SNP | −0.253 | −0.246 | 0.216 | 0.276** | 0.264** | 0.270** |
| Total $R^2$ | 0.199 | 0.189 | 0.202 | 0.214 | 0.199 | 0.207 |

* $p < 0.001$.
** $p < 0.000735$ (corrected using false discovery estimates for the 68 FTO SNPs studied) using PLINK.
[a] Controlled for proportion African, the highest reinforcement schedule of reading and BMI.
[b] $RRV_{food}$ = the highest reinforcement schedule completed for access to food or alternatives in the RRV task.

*rs12921970*, *rs11076022*), and sugar intake (*rs12921970*, *rs11076022*, *rs12446047*). While all of the SNPs were significant moderators of $RRV_{food}$ and energy intake at $p < 0.000735$ (corrected using false discovery estimates for the 68 FTO SNPs studied), only two SNPs (*rs12921970* and *rs12446047*) moderated the relationship between $RRV_{food}$ and energy intake plus all the macronutrients. For example, Fig. 1 demonstrates that *rs12921970* and *rs12446047* moderated the relationship between $RRV_{food}$ and total energy intake.

## 4. Discussion

High food reinforcement is related to increased energy intake and body weight [4,38–40], and predicts weight gain [5,6]. Rather than FTO having a main effect on energy intake as other investigators have shown [10,13–15,36], we have shown for the first time that the association of the $RRV_{food}$ with energy intake is moderated by FTO SNPs and a FTO risk score, suggesting that people with a higher motivation to eat may be impacted more by selected FTO alleles compared to individuals with a lower motivation to eat.

The reinforcing value of food influences energy intake by hedonic mechanisms in addition to the homeostatic control of food intake [17]. In the current study, food reinforcement was a strong predictor of energy intake (14.2%) and accounted for more variance in energy intake than any of the individual FTO SNPs or the FTO risk score suggesting that low food reinforcement is protective against any increased energy intake caused by the FTO SNPs [41].

While food reinforcement is related to food intake in part by hedonic mechanisms, the FTO gene is thought to moderate food intake though homeostatic mechanisms [16]. Using a mouse model, Olszewski and colleagues [16] showed deprivation upregulated FTO mRNA, while consumption of palatable fat or sugar did not alter FTO mRNA, indicating that the expression of the FTO gene is related to homeostatic energy intake. Animal models also provide evidence that the FTO gene is expressed in the arcuate nucleus of the hypothalamus, an area of the brain important for energy homeostasis [16,42].

While other investigators have demonstrated a relationship between FTO SNPs and energy intake [10–13], individual FTO SNPs may have relatively small genetic contributions to food intake and may be difficult to demonstrate in small populations. The FTO gene is only one of many genetic factors that play a role in energy balance and body weight. Many other human genes have been identified that are related to obesity, for example serotonin receptor, melanocortin 4 receptor, and opioid receptor genes and variants of these genes are related to increased BMI [7,43–45]. Segal and colleagues [46] analyzed genetic components and environmental components of BMI in monozygotic twins, dizygotic twins and same-age unrelated siblings and estimated that genetic components contribute about 65% [46] to variations in BMI.

Six FTO SNPs (*rs9936768*, *rs8049933*, *rs7199716*, *rs12921970*, *rs12446047*, *rs11076022*) interacted with food reinforcement to predict energy intake, fat intake, and carbohydrate intake, and three FTO SNPs (*rs12921970*, *rs11076022*, *rs12446047*) interacted with food reinforcement to predict protein intake (*rs7199716, rs12921970*, *rs12446047*) and sugar intake (*rs12921970*, *rs11076022*, *rs12446047*). Since *rs12921970* and *rs12446047* interacted with food reinforcement to predict energy intake, fat intake, carbohydrate intake, protein intake and sugar intake, *rs12921970* and *rs12446047* appear to have a robust relationship with food intake and would be good candidates for future studies to examine the relationships between food intake and FTO genes. These genes would also be good candidates as moderators of other predictors of food intake, for example snacking or fast food intake.

In the current study energy intake was measured using ad libitum food consumption of snack foods. A major limitation to the current study is that the food intake procedure included only high-energy-dense snack foods (Wavy Lay's Potato Chips, Cooler Ranch Doritos,

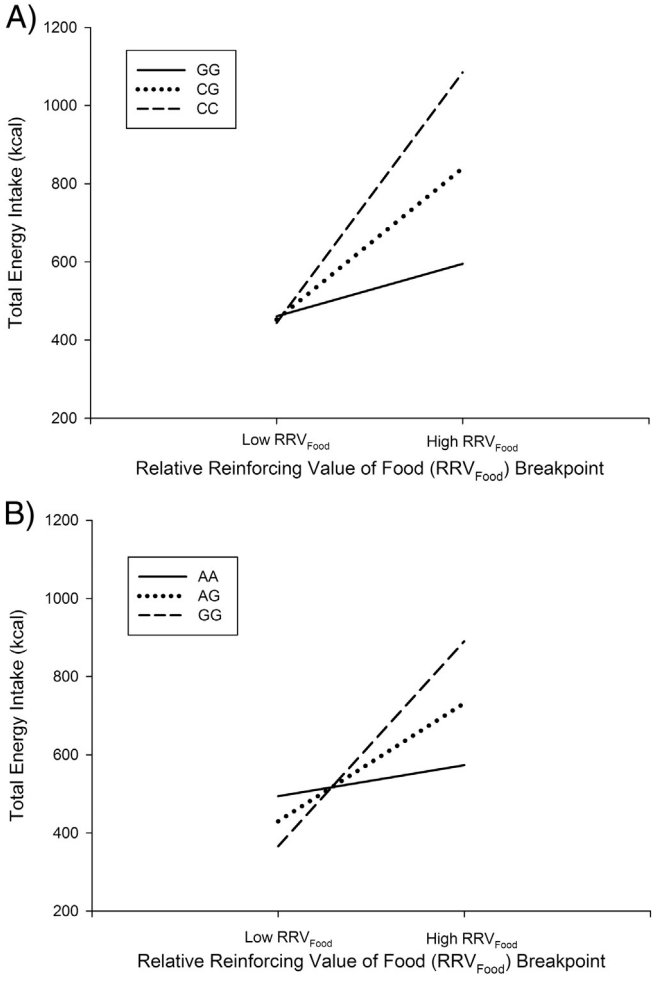


**Fig. 1.** The moderating relationship of the variants of the FTO SNPs (A) *rs12921970* and (B) *rs12446047* on food reinforcement ($RRV_{food}$) to predict energy intake. Low and high food reinforcement was defined by ±1 SD from the mean $RRV_{food}$.

M&M's, Twix, Kit Kat and Butterfinger). Since snack foods are typically highly palatable and reinforcing, the current findings may only apply to highly palatable foods with a high reinforcing value.

In summary, this study is consistent with previous findings that food reinforcement is related to increased energy intake during an ad libitum snack food eating task [3]. For the first time we demonstrate that the association of food reinforcement with energy intake is moderated by FTO SNPs. The pattern of the interaction suggests that low food reinforcement may reduce the impact of FTO risk alleles on energy intake. Rather than provide the same obesity treatment to everyone, interventions need to be developed that target individual subgroups of obesity, for example a treatment could target people with FTO risk alleles. Treatments with the goal of decreasing the reinforcing value of food would be very beneficial to people with FTO risk alleles and high food reinforcement.

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