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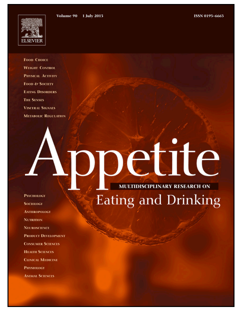

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Effect of metabolic state on implicit and explicit responses to food in young healthy females

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Abbreviations:
IAT, Implicit Association Test; VAS, visual analogue scale

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Abstract

Recent neuroscience research has delineated key psychological components of reward: wanting, liking and learning. Each component is further divided into explicit and implicit processes. While explicit processes are consciously experienced, implicit processes are not always directly accessible to conscious inspection. In the present study, we investigated the effect of metabolic state on implicit and explicit responses and their relationship in food context, especially when foods and visually matched non-food items are contrasted, and when foods in a sole food context but differing in energy content (high-energy - low-energy) or taste (sweet - savoury) were contrasted. Sixty healthy non-obese females participated in the study in fasted and fed states. Three Implicit Association Tests were used to assess implicit associations. Explicit liking and wanting ratings were assessed by visual analogue scales. In the implicit food–non-food context, food was preferred over non-food items both in fasted and fed states, though the strength of implicit associations declined significantly from fasted to fed state. However, the direction or strength of implicit associations was not significantly different between the metabolic states when comparing concepts within food context only, differing in energy content or taste. Instead, explicit responses reflected the change in the metabolic state in a manner consistent with alliesthesia and sensory-specific satiety. The results of the present study suggest that implicit associations are relatively resistant to acute change in the metabolic condition compared to explicit ratings, which shift more readily according to the fasted-fed continuum. The shift in the prevailing metabolic state was, however, reflected in the strength of implicit responses towards food in relation to non-food items, yet in the sole food contexts implicit associations were comparable between the fasted and fed states.

Keywords: Implicit Association Test, explicit responses, implicit associations, food, metabolic state

Introduction

Reward-driven behaviour is at the motivational core of almost all human action, and much of human behaviour can be explained by simple processes of approaching rewards, i.e., pleasure-inducing stimuli and avoiding unpleasant stimuli (Berridge & Kringelbach, 2008). Food and eating are amongst the most powerful natural sources of pleasure (Berridge & Kringelbach, 2008). Consummatory behaviour, along with basic homeostatic needs, is especially rewarding as it ultimately serves survival. Nevertheless, in affluent societies food consumption occurs for reasons other than energy deprivation – simply for pleasure (Lowe & Butryn, 2007), and this has become a significant motivational driver for food intake. This tendency is referred as “hedonic eating”, as a distinction to energy deficit-driven “homeostatic eating” (Monteleone et al., 2013).

Reward and pleasure are generated via active and complex processes that include several psychological components corresponding to distinguishable neurobiological mechanisms. Recent advances in neurobiology and affective neuroscience have delineated the psychological components of reward: motivation (wanting), emotion (liking) and learning (predictive associations and cognitions). Each component is further divided into explicit and implicit processes. While explicit processes are consciously experienced, implicit processes are not always directly accessible to conscious experience (Berridge & Robinson, 2003).

During recent decades, the scope and methods of investigating people’s attitudes, beliefs and behaviours have broadened beyond techniques of explicit self-report measurements. Subjective evaluations, frequently interpreted as indication of deliberative and conscious processes, are traditionally assessed by direct methods (e.g., questionnaires, interviews). However, these methods are prone to several limiting factors (for example social desirability, self-presentation, limitations in motivation or ability) (Hofmann et al., 2005, Nosek et al., 2011), including limited value in assessment of psychological attributes that are introspectively inaccessible or beyond conscious awareness. To avoid problems associated with direct methods and to explain variation in attitudes or behaviours not accounted for by explicit measures, researchers have adopted a wide range of alternative measurement instruments, i.e., computerised measurement techniques to infer cognitive processes (thoughts, feelings, behaviour) without directly asking participants about them (Gawronski & De Houwer, 2014; Hahn & Gawronski, 2017). These measurement tools are considered to tap more implicit / automatic / unconscious processes (Greenwald & Banaji, 1995; Fazio & Olson, 2003) and could be particularly suited to reflect spontaneous, uncontrolled behaviour (De Houwer & Moors, 2010). Hence, although it has been pointed out that both indirect and direct measurement outcomes can be valid indicators of behaviour (Fazio & Olson, 2003), the assessment of implicit associations is important, because it can provide useful information about a person’s relatively spontaneous associations as well as future choices and decisions (e.g., Galdi et al., 2008), especially when self-regulation resources are depleted (Gailliot et al., 2014; for a review see Muraven & Baumeister, 2000).

One of the most widely used implicit measurement technique is the Implicit Association Test (IAT) by Greenwald and colleagues (1998). The IAT procedure has been used to assess a variety of concepts, such as stereotypes (Agerström & Rooth, 2011), self-esteem and self-concept (Greenwald & Farnham, 2000), political behaviour (Galdi et al., 2008), consumer behaviour (Maison et al., 2004), mental health (Rüsch et al., 2007), and addiction (Wiers et al., 2002). In the domain of dietary and eating behaviour, the IAT has been employed to distinguish between different types of individuals, e.g., overweight / obese vs. normal-weight controls (Roefs & Jansen, 2002; Craeynest et al., 2007; Craeynest et al., 2008), low-emotional vs. high-emotional eaters (Ayres et al., 2011; Bongers et al., 2013), restrained vs. unrestrained eaters (Houben et al., 2010), with low vs. high in reward sensitivity (Ashby Stritzke, 2013), with high vs. low inhibitory self-control (Haynes et al., 2015) and to predict weight gain (Nederkoorn et al., 2010) and snacking behaviour and snack choice (Perugini, 2005; Richetin et al., 2007; Friese et al., 2008; Ayres et al., 2012; Eschenbeck et al., 2016; Trendel & Werle, 2016). Furthermore, due to the high flexibility of the IAT procedure, it can be modified to investigate various target concepts (e.g., products, individuals, objects, concepts) using different attribute dimensions (e.g., evaluative, semantic, behavioural), yet the design of the IAT requires a careful decision of the category labels and stimulus items to represent the concept of interest (Nosek et al., 2007). The rationale underlying the IAT test is based on the assumption that automatic associations underlie the investigated phenomena and facilitate or inhibit IAT responses. The original version of the IAT consists of two binary categorisation tasks that are combined in an association-congruent or an association-incongruent manner with the to-be-measured psychological attribute (e.g., attitude, stereotype). The outcome measure of the IAT test, the IAT effect, assumes that in the hypothesised association-congruent tasks responses should be faster and/or more accurate compared to those in association-incongruent tasks. This, in turn, is taken as an indication that the concepts are strongly associated in memory (Greenwald et al., 1998; Greenwald, Nosek, & Banaji, 2003). Applied to food context, if respondents react faster on congruent tasks (‘Food – Approach’ and ‘Non-food – Avoid’) compared to incongruent tasks (‘Food – Avoid’ and ‘Non-food – Approach’), it can be concluded that respondents have stronger association with foods than non-food items. The association is frequently termed also as a (implicit) preference (Greenwald et al., 1998; Lane et al., 2007), because the IAT measures relative strengths of associations and the “implicit preference” is used as a shorthand for stronger association of one of the two target concepts with positive valence, and/or weaker association of that concept with negative valence (Greenwald, Nosek, & Banaji, 2003).

Recently, some studies have attempted to verify the effect of different motivational factors, including metabolic state (for example fasted vs. fed state), on automatic associations. Ferguson and Bargh (2004) showed that thirsty participants had more automatic positivity towards relevant objects (such as water) than non-thirsty participants, whereas hungry or more deprived participants had a greater attentional bias or more positive immediate valence, respectively, to food-related words compared with less hungry participants (Mogg et al., 1998; Seibt et al., 2007). Furthermore, participants in the pre-lunch group were slower to associate food words with unpleasant words than participants in the post-lunch group (Stafford & Scheffer, 2008). Metabolic state can modulate the formation of implicit preferences also within a food category; hungry participants displayed a higher implicit preference for the approached food brand as compared to satiated participants, whereas explicit preferences remained unaffected (Zogmaister et al., 2016). Finlayson et al. (2008) examined the influence of hunger state on explicit and implicit processes, the latter measured using a computerised forced-choice procedure. The method comprised a series of 150 trials presenting two food stimuli from different food categories and within each pair of stimuli participants were asked to select the food they most wanted to eat at that moment. Based on the reaction time of each choice, the authors reported that metabolic state (i.e., fasted vs. fed state) modified changes in explicit liking and wanting in a manner consistent with sensory-specific satiety, whereas – on the contrary to the findings mentioned above – no relationship between hunger and implicit wanting was found. On the other hand, the forced-choice method has been criticised for not necessarily measuring the component of implicit wanting ~~and the authors reminded not to make too straightforward conclusions about the apparently interactively operating explicit and implicit reactions~~ (Havermans, 2011~~; Finlayson & Dalton, 2012~~). More recently, Kraus and Piqueras-Fiszman (2016) assessed the sensitivity of two indirect measurement procedures, i.e., motivational tendencies (approach vs. avoidance) and evaluative associations (positive vs. negative), towards two food products employing Recoding-Free IAT (IAT-RF) within participants assigned to hunger vs. reduced-hunger groups. They reported that responses from the motivational IAT-RF corresponded more clearly to the expected tendencies towards the products depending on the recent feeding manipulation than those from evaluative IAT-RF, and the authors suggested the former to be 'sensitive enough to detect motivational changes in approach-avoidance tendencies for either one of the two products'.

However, as described above, previous studies have used various implicit measurement techniques, designs, contexts, and stimuli, which could also have contributed to the mixed findings concerning the effect of metabolic state. Therefore, it is challenging to draw firm conclusions about the results of earlier research even though some studies suggest that motivational state, especially deprived condition, affects automatic or implicit responses. Furthermore, in many earlier studies researchers have used separate study populations in pre-post designs, which is not comparable to designs where responses of the same participants are collected before and after a planned intervention.

Therefore, in the present study our objective was to determine the effect of metabolic state on implicit and explicit food-related responses in healthy young females in a well-controlled pre-post design. We were especially interested in examining whether a metabolic state (i.e., fasted vs. fed condition) affects these responses when (1) foods and visually matched non-food items are contrasted and when (2) foods in a sole food context but differing in energy content (i.e., high-energy – low-energy) or taste category (i.e., sweet – savoury) are contrasted. Implicit associations were assessed with IAT tests tailored specifically for this study using images of food items as target stimuli and motivational approach-avoidance words as stimuli for the attribute categories. In fasted state, we expected to detect a stronger implicit association towards foods compared to non-food items and high-energy meals compared to low-energy meals because food and especially high-energy food signify source of energy and ultimately serves survival. In addition, we expected to see a stronger implicit association to savoury compared to sweet snack foods in fasted state, as was shown for example by Kraus & Piqueras-Fiszman (2016). In the fed state, the associations were expected be less pronounced yet replicate the direction of association as shown in the fasted state. Furthermore, in line with the concepts of alliesthesia (Cabanac, 1971), i.e., a relationship between person's internal state and perceived sensation of a given stimulus, and sensory-specific satiety (Rolls et al., 1981) we expected that the explicit liking and wanting responses especially those of wanting high- and low-energy meals and savoury snack foods would decrease due to a savoury pizza meal consumed between fasted and fed states, whereas liking responses would show less pronounced decrease compared to wanting ratings.

Materials and methods

Participants

A total of 60 healthy females participated in the study (Table 1). The inclusion criteria of the study participants were female gender, age between 20–40 years and body mass index (BMI) between 19–29 kg/m². Exclusion criteria were as follows: food allergies or intolerances, restrictive diet (e.g., vegetarian, gluten-free diet), frequent breakfast skipping, marked changes in diet during past six months to lose weight, chronic medication (except oral contraceptives), chronic disease (e.g., diabetes, eating disorder, celiac or neurological disease), and smoking. Participants were recruited in two separate phases via internet-based calls within students and personnel of the University of Eastern Finland, Savonia University of Applied Sciences and Kuopio University Hospital. In the first phase (the 1st cohort), 28 volunteers (age 27.6±6.0 years, BMI 23.0±2.5 kg/m^2 (mean±SD)) and in the second phase (the 2nd cohort) 32 volunteers (age 24.3±5.5 years, BMI 23.0±3.0 kg/m^2 (mean±SD), none had taken part in the 1st phase) participated in the second study. Participants' weight, height or BMI did not differ between those recruited in different phases, except for age that was higher among those participating in the study during the first phase (p=0.01). In all analyses, the data were analysed as one group (n=60).

The study was carried out in accordance with the guidelines laid down in the Declaration of Helsinki. The Ethical Committee of Northern Savo Hospital District, Kuopio, Finland approved all procedures involving human participants. Written informed consent was obtained from all participants.

Table 1. Characteristics of the study female participants (n=60).

Characteristic	Mean (SD)	Min - Max
Age (years)	25.8 (5.9)	20.0–40.0
Weight (kg)	64.2 (7.7)	50.4–83.1
Height (cm)	167.3 (6.1)	153.7–184.6
Body mass index (kg/m^2)	23.0 (2.8)	19.0–29.3

Study design

Before participating in the study, volunteers were interviewed to confirm their eligibility. At the end of the screening interview, study procedure and measurements were explained to the volunteers at a general level to familiarise them with the study protocol. However, due to the nature of implicit responses, detailed objectives of the study were not revealed to the volunteers, because prior information could have affected these responses and hence the reliability of the results (Harmon-Jones et al., 2007; Bongers et al., 2013). All participants were naïve to the IAT procedure.

Participants were instructed to keep their usual diet, exercise routines and sleep habits as constant as possible during the days prior to the study visit, refrain from heavy exercise 12 h before the study visit and avoid alcohol consumption for 24 h before entering the study. At the beginning of the study visit, participants' height and weight were measured, and duration of the fast as well as alcohol consumption during the previous day were checked.

Study visits were conducted at the Sensory Laboratory of the Institute of Public Health and Clinical Nutrition at the University of Eastern Finland between 9:00 and 13:00 hours. A visit included two computer-based IAT test sessions, one before and one after a lunch, i.e., in fasted and in fed state. The first test session (i.e., in fasted state) was performed 3 h after a habitual breakfast (the 1st study) or after an overnight (10–12 h) fast (the 2nd study). The length of the fasted time did not have a significant effect on the variables examined in the study (data not shown).

The second test session was performed 30 min after a lunch of participant's choice (pizza options: Hawaii, Tuna, Mozzarella and Vegetable; Dr. Oetker Suomi Ltd., Helsinki, Finland). During a 30 min period between the end of the pizza meal and the beginning of the second test session, participants sat and could read, play games, browse the internet, or do jigsaw puzzles.

Implicit association test, IAT

A computerised categorisation task, Implicit Association Test (IAT) (Greenwald et al., 1998) run by Inquisit software (version 4.0.6.0, Millisecond Software, LCC, Seattle, WA, USA) was used to examine implicit associations. We designed three separate IAT tests, (1) Food – Non-food, (2) High-energy – Low-energy, and (3) Sweet – Savoury, to assess overall implicit associations. Each IAT test included two binary categorisation tasks, one target and one attribute category pair, which were combined in an association-congruent and an association-incongruent manner. The calculated measurement outcome, the IAT score (D score, IAT effect), is based on reaction times (milliseconds) from the set of the classification tasks and provides information about spontaneous associations towards the two classes of target items used in the test. The raw IAT data were processed with a standard procedure included in the Inquisit software. The individual IAT score is obtained by computing the difference between the mean latency of the blocks and by dividing the result by the overall standard deviation (see Greenwald et al., 2003). The IAT score has a possible range of -2 to +2, which indicates the strength and also the direction of the association in the original IAT test ($D<0.15$ = little to no, $D>0.15$ = slight, $D>0.35$ = moderate, $D>0.65$ = strong association). Due to the comparative nature of the original IAT test, the resulting IAT score should not be interpreted as an absolute attitude or preference, but as a relative one indicating a comparative association between the target categories.

Because the nature and construal of the categories play a marked role in determining the IAT effect (Lane et al., 2007), the IAT target categories were labelled as Food – Non-food, High-energy – Low-energy and Sweet – Savoury to define the concepts of interest. Approach and avoid categories were used as an attribute category pair. The decision to use "Approach" and "Avoid" labels for the attribute category pair followed previous practices to assess indirectly motivational tendencies towards specific objects (e.g., Palfai & Ostafin, 2003), including food items (e.g., Kemps et al., 2013). Both the target and attribute categories were presented in the top left and top right corners of the screen and remained on the screen during the IAT test. Stimulus images and words were displayed successively in the centre of the screen. Participants were instructed to categorise the stimulus images and words as quickly and accurately as possible by pressing either of the two assigned response keys (left 'E' or right 'I') according to the category labels, while their individual performance (i.e., reaction time and accuracy of the categorisation (error rate)) was measured.

The IAT tests followed a fixed block structure and included seven different blocks divided into five practice blocks and two test blocks. After the separate practice blocks of target, attribute and combined block (20 trials in each), the first combined test block with 40 trials was presented. Then the categorisation task changed between the blocks, continued with two practice blocks (20 trials) and ended with the second test block (40 trials). The order of the association-congruent and -incongruent blocks was counterbalanced over participants.

Stimuli used in the IAT tests

Each IAT test included 16 different target stimuli, eight images per each target category, which are displayed in the Supplementary data. All foods in the Sweet – Savoury and High-energy – Low-energy IATs were presented on a white background and foods and non-food items in the Food – Non-food IAT on a grey background. Identical sets of 16 different attribute stimuli, 8 stimulus words per category, were used in all IAT tests in order to maximise equivalence among the IAT tests.

Images of food and non-food items

Images used in the Food – Non-food IAT test were selected from a larger set of previously designed stimulus images of food and non-food items (Kaurijoki et al., 2008). The images for the target categories were chosen so that they would closely match regarding shape, colour and overall presentation, but represent two different categories: foods (e.g., Golden Delicious apple) and non-food items (e.g., yellow tennis ball).

Images of high- and low-energy meals

Stimulus images for the high- and low-energy meal categories were designed and photographed at the University of Eastern Finland (UEF). The images were reprocessed with Adobe Photoshop Lightroom 6.3 and Adobe Photoshop CC (Adobe Systems Inc., 2014) to attain optimal brightness, contrast and overall uniformity among the images. The selection was based on the results from a pre-test, in which a set of images of high- and low-energy meals (n=33) was presented to female volunteers (n=30, age 23.8±4.2 y, 23.1±2.9 kg/m^2). They were asked to rate the pictured foods on a 10-point scale in terms of attractiveness, estimated energy content, and suitability for a meal at the time of assessment (morning and afternoon). Eight images, which received the highest ratings in each category (attractiveness, suitability, high and low energy content), were then selected for the high- and low-energy meal categories. The selected images in the high-energy category included foods such as hamburger, pizza and typical Finnish main meals, and in the low-energy category mainly salad-based meals. Two additional images in the high-energy (~~no. 61 (~~salami pizza~~)~~ and ~~86 (~~French fries and hamburger~~)~~) and two images in the low-energy (~~no. 482 and 526 (~~salad portions~~)~~) meal category were taken from the Food-pics database (Blechert et al., 2014).

Images of sweet and savoury snack foods

Similarly to the high- and low-energy meals, the design and selection of images for the Sweet – Savoury IAT test was produced at the UEF and pre-tested by the same group of female volunteers. They were asked to rate the foods in 32 images on a 10-point scale in terms of attractiveness, suitability for a snack food at the time of assessment (morning and afternoon), and whether the foods in the images fitted into a sweet or savoury snack food category. Eight images, which received the highest ratings in each category (attractiveness, suitability and sweet / savoury category), were selected for categories indicating typical sweet and savoury snack foods consumed in Finland. Five additional images in the sweet (~~no. 4 (~~cookie~~)~~, ~~no. 28 (~~piece of berry cake~~)~~, ~~no. 103 (~~piece of raspberry cake~~)~~, ~~no. 107 (~~piece of chocolate cake~~)~~ and ~~no. 287 (~~chocolate bar~~)~~) and two in the savoury (~~no. 110 (~~cashew~~)~~ and ~~no. 58 (~~ham sandwich~~)~~) snack food category were taken from the Food-pics database (Blechert et al., 2014).

Stimulus words

Stimulus words (verbs in Finnish) representing “Approach” (i.e., aspire, seek, favour, desire, choose, long for, need, take) and “Avoid” categories (i.e., refuse, avoid, restrict, reject, abandon, watch out, evade, withdraw) as attribute stimuli were chosen by the research group. The words were selected so that at first a list of appropriate words for both categories were created using a Finnish thesaurus of synonyms. From this list, eight most suitable synonyms were selected by the consensus of the researchers to best represent the everyday language for each category. Furthermore, although the IAT effect seems to be relatively unaffected by the small variation in average word length and by the number of stimuli representing each target and attribute category (unless only a minimal number of exemplars are used), the IAT effect is influenced primarily by the category labels with stimuli that affect the construal of the category (Nosek et al., 2005). The stimulus words were chosen so that any potential effect of word length could be controlled. In this study the mean length of the stimulus words was 6.5±1.6 and 7.4±1.8 letters in "Approach" and "Avoid" categories, respectively.

Explicit ratings - subjective sensations and food image ratings

Participants rated their subjective sensations of appetite (i.e., hunger, desire to eat, satiety, and fullness), test meal satisfaction, alertness and mood as well as gave their explicit liking and wanting ratings of the food images used in the High-energy – Low-energy meal IAT and Sweet – Savoury snack food IAT tests on an electronic visual analogue scale (VAS). The explicit ratings were not assessed for the images in Food – Non-food item IAT test due to their more experimental nature (i.e., visually comparable images for food and non-food items). Ratings were obtained before and after the lunch, i.e., in fasted and in fed state, each time after the implicit measurements. The data were collected with the Inquisit software (version 4.0.6.0, Millisecond Software, LCC, Seattle, WA, USA).

Each VAS contained unstructured horizontal 10 cm line with verbal anchors (in Finnish) at both ends expressing the weakest and the strongest statement (e.g., *Not at all hungry – Extremely hungry*, respectively). The explicit liking was assessed with a question *"How much do you like the food shown in the image?" (I do not like it at all – I like it very much)* and the explicit wanting with a question *"How much would you like to have the food shown in the image at the moment?" (Not at all – Very much)*. Participants were instructed to click a point on the horizontal line corresponding to their sensations and perceptions at the time of assessment. After clicking on the line, the program converted the selected point into a numeric form scaled from zero to ten.

Statistical methods

The data were analysed using a statistical software package IBM SPSS Statistics for Windows (version 25.0, Armonk, NY, IBM Corp, USA). Participants were included in the analysis of the IAT test results if the error rate was less than 10 percent. An error during an IAT test occurs when a given stimulus is categorised incorrectly. The correct categorisation of the test stimuli is predetermined by the investigators via the IAT script. The programme running the IAT test keeps a record of the errors during the test and provides a global error rate in the end. Consequently, one participant was excluded from the Food – Non-food IAT, one participant from the Sweet – Savoury IAT and four from the High-energy – Low energy IAT test analyses. Because several measures were not normally distributed, non-parametric methods were used. Mann-Whitney U test was used to discover any differences between the participants recruited in different phases. Wilcoxon Signed-Ranks Test was used to investigate the differences between the fasted and fed states. Spearman correlations were calculated to discover any relation of subjective hunger and satiety ratings with implicit associations, i.e., IAT scores, and explicit ratings and between IAT scores and explicit ratings in both metabolic states. The Type I error rate was controlled using the Bonferroni adjustment for multiple comparisons between explicit and implicit measures. Unless otherwise specified, the results are reported as means ± standard error (SE) with a value $p \leq 0.05$ (2-tailed) as a criterion for the statistical significance.

Results

Implicit responses

Food – Non-food IAT

The mean IAT score of the Food – Non-food test indicated that food was, on average, implicitly preferred to non-food items in both fasted and fed states (Figure 1). The mean score of the Food – Non-food IAT was higher in fasted state compared to fed state ($p<0.05$) indicating a greater implicit preference for foods compared to non-food items in fasted than in fed state.

High-energy – Low-energy IAT

The mean IAT score of the High-energy – Low-energy test indicated that low-energy meals were, on average, implicitly preferred to high-energy meals in both fasted and fed states (Figure 1). The mean IAT scores of the High-energy – Low-energy test did not differ between metabolic states.

Sweet – Savoury IAT

The mean IAT score of the Sweet – Savoury test indicated that sweet snack foods were, on average, implicitly preferred to savoury snack foods in both fasted and fed states (Figure 1). The mean IAT scores of the Sweet – Savoury test did not differ between metabolic states.

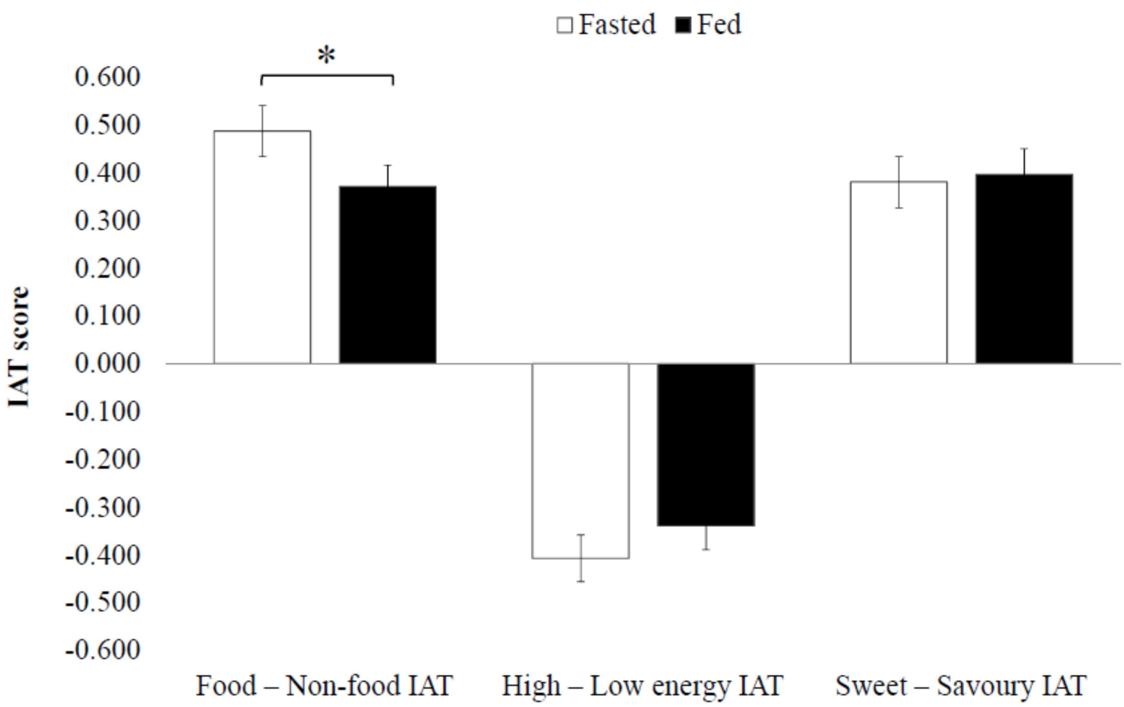

Figure 1. Mean Implicit Association Test (IAT) scores (with standard errors, SEM) of the Food – Non-food item, High-energy – Low-energy meal and Sweet – Savoury snack food tests in fasted and fed state. Positive scores indicate stronger implicit association (or preference) with food compared to non-food items in the Food – Non-food IAT test and with sweet compared to savoury snack foods in Sweet – Savoury IAT test. Negative scores indicate stronger implicit association (or preference) with low-energy meals than with high-energy meals in High-energy – Low-energy IAT test; Wilcoxon Signed-Ranks Test; *p<0.05 (two-tailed); Food – Non-food IAT (n=59), High-energy – Low-energy IAT (n=56) Sweet – Savoury IAT (n=59).

Explicit responses

Subjective sensations

Subjective sensations before and after the lunch are presented in the Table 2. Consumption of lunch produced a significant increase in satiety and fullness ratings and a decrease in hunger and desire to eat ratings. Mood ratings were higher in fed than in fasted state, which indicated an increased positive mood after lunch. Alertness ratings did not differ between metabolic states.

Table 2. Ratings of subjective sensations (mean (SD)) in fasted and fed state (n=60 females)

Subjective sensation[a]	**Fasted state**	**Fed state**	**p-value**[b]
Hunger	5.4 (2.3)	0.8 (1.3)	p<0.001
Desire to eat	6.3 (2.2)	1.8 (1.9)	p<0.001
Satiety	2.2 (2.1)	8.4 (1.4)	p<0.001
Fullness	1.8 (1.8)	7.9 (1.6)	p<0.001
Mood	7.6 (2.1)	8.2 (1.5)	p=0.038
Alertness	6.2 (2.2)	6.0 (2.1)	p>0.05
Test meal satisfaction	-	7.4 (1.7)	-

[a]Measured using an electronic visual analogue scale (VAS) with 'Not at all' as left and 'Extremely' as right verbal anchor, except for mood, where left anchor was 'Bad' and right anchor 'Good'; [b] Wilcoxon Signed-Ranks Test.

Explicit liking and wanting ratings

Liking. When comparing the mean liking ratings within the fasted or fed states, no differences were found between the liking ratings of high-energy vs. low-energy meals or sweet vs. savoury snack foods in either fasted or the fed state.

When comparing the mean liking ratings between the fasted and fed states, the liking ratings of high-energy meals, sweet snack foods and savoury snack foods were higher in fasted compared to fed state (p<0.01; Figure 2). The liking ratings of low-energy meals did not differ between the metabolic states.

Wanting. When comparing the mean wanting ratings within the fasted or fed states, no differences were found between the wanting ratings of high-energy meals vs. low-energy meals in the fasted state. In the fed state, the wanting ratings of high-energy meals were lower compared to those of low-energy meals (p<0.001). In the sweet vs. savoury snack foods comparison, the wanting ratings of savoury snack foods were greater compared to sweet snack foods in the fasted state (p<0.001). In the fed state, the mean wanting ratings of sweet snack foods were greater compared to savoury snack foods (p<0.001).

When comparing the mean wanting ratings between the fasted and the fed states, the wanting ratings of high-energy meals, low-energy meals and savoury snack foods were higher in the fasted compared to the fed state (p<0.001; Figure 2). The wanting ratings for the sweet snack foods did not differ between the metabolic states.

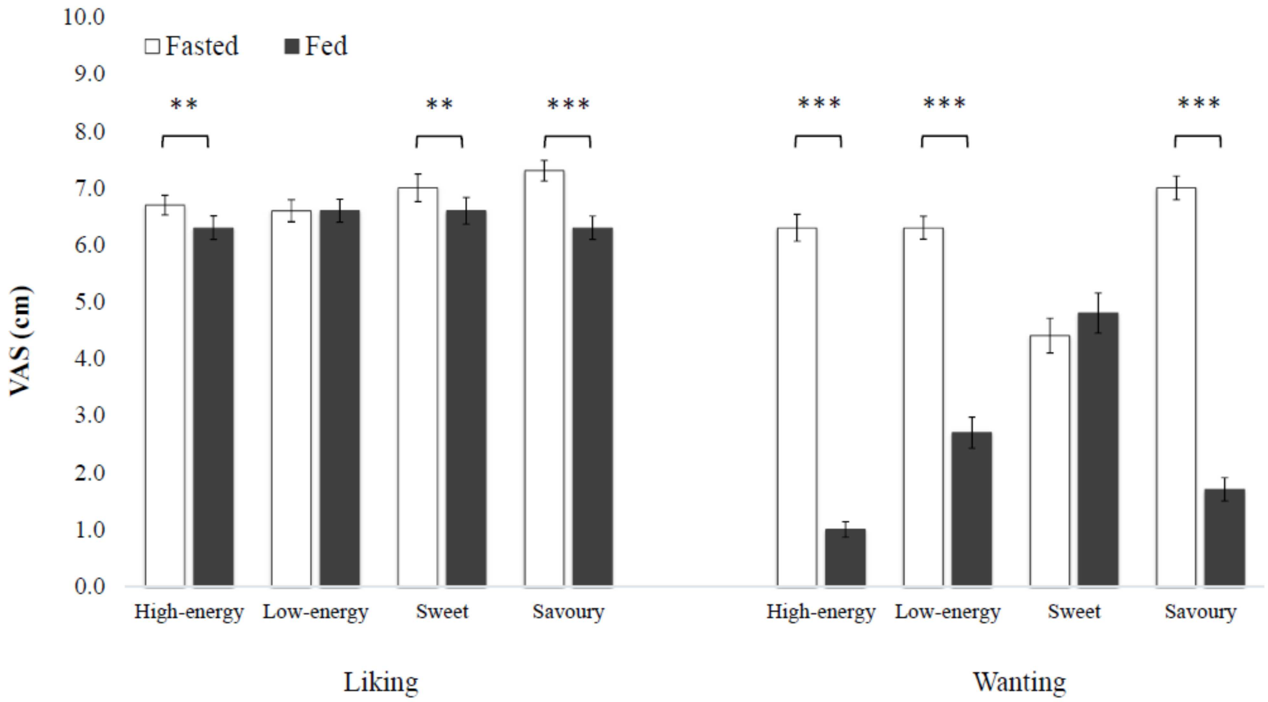

Figure 2. Mean explicit liking and wanting ratings (with standard errors, SEM) of the images in the high-energy and low-energy meals and sweet and savoury snack foods categories in fasted and fed state; Wilcoxon Signed-Ranks Test; **p<0.01, ***p<0.001 (two-tailed); n=60; Measured with an electronic visual analogue scale (VAS).

Correlations between implicit and explicit responses

We examined the correlations of hunger and satiety ratings (i.e., subjective indicators of the metabolic state) with explicit liking and wanting ratings and implicit IAT scores in fasted and fed states (Table 3). Correlations between implicit IAT scores and explicit liking and wanting ratings were also assessed in both metabolic states.

Fasted state. Hunger or satiety ratings did not correlate with explicit liking ratings, except for a negative correlation between satiety and liking for sweet snack foods, i.e., the stronger the satiety, the lower the liking of sweet snack foods (Table 3). Instead, hunger ratings correlated positively with all the wanting ratings; the stronger the hunger, the higher the explicit wanting of food. Satiety ratings, in turn, correlated negatively with all the explicit wanting ratings except for low-energy meals; the stronger the satiety, the lower the wanting ratings of high-energy, sweet and savoury foods. In addition, desire to eat ratings correlated positively with liking ratings of sweet snacks ($r=0.26$, $p<0.05$) and high-energy ($r=0.29$, $p<0.05$) foods and wanting ratings of all food categories ($r=0.37$–0.51, $p<0.01$), whereas fullness ratings correlated negatively with wanting ratings of low-energy meals ($r=-0.31$, $p=0.015$) and savoury snack foods ($r=-0.27$, $p=0.04$). The correlations of desire to eat with wanting ratings of high-energy and low-energy meals remained significant also after the Bonferroni correction for multiple comparisons.

No significant correlations were found between hunger or satiety ratings and any of the mean IAT scores of the used IAT tests (Table 3), or between desire to eat or fullness ratings and IAT scores. When the IAT scores of Sweet – Savoury and High-energy – Low-energy tests were correlated with corresponding explicit liking and wanting ratings, Sweet – Savoury IAT test scores correlated positively with mean liking ($r=0.26$, $p=0.05$) and wanting ($r=0.26$, $p<0.05$) ratings of sweet snack foods and the High-energy – Low-energy test scores correlated negatively with the mean liking scores of low-energy meals ($r=-0.32$, $p<0.05$). None of these correlations remained significant after the Bonferroni correction for multiple comparisons.

Fed state. Neither hunger nor satiety ratings correlated with the explicit liking ratings measured in fed state (Table 3). Instead, hunger ratings correlated positively and satiety ratings negatively with explicit wanting ratings of high- and low-energy meals and savoury snack foods, i.e., the stronger the hunger, the higher the wanting scores and the stronger the satiety, the lower the wanting scores, respectively. Desire to eat ratings correlated positively with liking ratings of sweet ($r=0.26$, $p<0.05$) foods and wanting ratings of all food categories ($r=0.39$–0.54, $p<0.01$). Fullness ratings correlated negatively with wanting ratings of high-energy meals ($r=-0.33$, $p=0.009$) and savoury snack foods ($r=-0.28$, $p=0.03$). The positive correlations of hunger and desire to eat with wanting ratings of high-energy meals and savoury snack foods, and of desire to eat with wanting ratings of sweet snack foods, as well as the negative correlation of satiety with wanting ratings of savoury snack foods remained significant also after the Bonferroni correction.

No significant correlations were found between hunger or satiety ratings and any of the mean IAT scores of the used IAT tests ~~Hunger or satiety ratings did not correlate with any of the mean IAT scores of the used IAT tests~~ (Table 3), or between desire to eat or fullness ratings and IAT scores. When the IAT scores of Sweet – Savoury and High-energy – Low-energy tests were correlated with corresponding explicit liking and wanting ratings, Sweet – Savoury IAT test scores correlated positively with mean liking ratings of savoury snack foods ($r=0.26$, $p<0.05$). This correlation did not remain significant after the Bonferroni correction. No correlations were found between High-energy – Low-energy IAT test scores and corresponding explicit liking or wanting ratings.

Table 3. Correlations[1] of mean hunger and satiety ratings with mean explicit liking and wanting ratings of images used in each IAT category and IAT scores assessed with Visual Analogue Scales and Implicit Association Test (IAT), respectively, in fasted and fed state.

Variables	**Fasted state**		**Fed state**	
	Hunger	Satiety	Hunger	Satiety

Explicit ratings of images used in IAT tests[2]				
Liking, high-energy meals	.15	-.19	.20	-.25
Liking, low-energy meals	-.16	-.01	-.25	.20
Liking, sweet snack foods	.19	-.26*	.02	-.15
Liking, savoury snack foods	.05	-.17	.15	-.22
Wanting, high-energy meals	.41**	-.26*	.53***[4]	-.44**
Wanting, low-energy meals	.32*	-.21	.37**	-.30*
Wanting, sweet snack foods	.42**	-.30*	.12	-.21
Wanting, savoury snack foods	.43**	-.30*	.58***[4]	-.49***[4]
IAT score[3]				
Food – Non-food	.19	-.05	-.05	.12
High-energy – Low-energy	.21	-.08	.23	-.04
Sweet – Savoury	.17	.02	-.05	.04

[1]Spearman correlation, *p<0.05, **p<0.01, ***p<0.001 (two-tailed); [2]n=60; [3]Food – Non-food IAT (n=59), High-energy – Low-energy IAT (n=56) Sweet – Savoury IAT (n=59); [4]remained significant after the Bonferroni correction for multiple comparisons

Discussion

There has been a growing interest in delineating the role of implicit and explicit components of food reward in appetite control and human eating behaviour. Previous research suggests that a shift in the metabolic state affects explicit judgments of wanting and liking but the effect on implicit responses is unclear, partly due to various implicit measures and designs used in different contexts. Thus, to control the influence of participant-related factors we examined the responses within the same study population in a pre-post design. Furthermore, a widely used implicit measure, IAT, was applied to assess the effect of metabolic state on implicit responses measured in three separate food contexts, in which visually closely-matched food – non-food stimuli and sole food stimuli, i.e., sweet – savoury taste and high-energy – low-energy pairs, were contrasted. Finally, correlations between implicit and explicit responses were assessed to evaluate the relationship between these variables in our study design. ~~Thus, in the present study we investigated the effect of metabolic state on implicit associations and explicit ratings and their relationship in food context.~~ Our results showed that in the implicit food – non-food context, food was preferred over non-food items both in fasted and fed states, though the strength of implicit associations declined significantly from fasted to fed state. However, the direction or strength of implicit associations was not significantly different between the metabolic states when concepts within a sole food context differing either in energy content (high-energy vs. low-energy) or taste (sweet vs. savoury) were compared. As expected, explicit responses reflected the change in the metabolic state in a manner consistent with alliesthesia and/or sensory-specific satiety; liking and wanting ratings decreased in most of the food categories in fed compared to fasted state, most markedly among the wanting ratings.

Implicit responses

The results from the implicit measures used in our study showed that the effect of the metabolic state on implicit responses varies among the contexts of the measurements; the shift in the physiological state was reflected in the food–non-food context, but not in the contexts based on sole food concepts. Previous research also reveals mixed findings on the effect of motivational or physiological state on implicit responses; in some studies implicit responses varied according to the prevailing state (e.g., Mogg et al., 1998; Seibt et al., 2007; Stafford & Scheffer 2008; Zogmaister et al., 2016) while in others not (Finlayson et al., 2008). Also, the temporal stability of implicit measures tends to show considerable fluctuation over time (e.g., Cunningham et al., 2001; Gawronski et al., 2017) unless contextual constraints are strong and consistent across measurements (Gawronski, 2019). In addition, while implicit responses, including those measured with the IAT, can shift substantially due to various factors (e.g., Gregg et al., 2006; for a review, see Blair, 2002; Gawronski & Sritharan, 2010), it has been argued that contextual factors determine practically every finding with implicit measures, including overall scores and temporal stability (Gawronski, 2019). In our study, the contextual factors remained stable, except for the intentional shift in the metabolic state, which likely explains the response difference between states in the food-non-food IAT context.

The implicit bias towards food versus non-food items likely reflects the pronounced significance of food in our environment, in which it must be among top priorities of an individual to survive. The stronger implicit preference for foods is therefore rational; the importance of energy sources is translated behaviourally into a response, where food is preferred over non-edible items, especially in an energy depleted state, but also when energy stores were replenished. Moreover, the stronger bias for food compared to non-food items was apparent despite a marked visual resemblance between the stimuli used in the Food–Non-food IAT test. Furthermore, our results suggest that hunger sharpens attention and discrimination between food and non-food items shown as faster responses to food stimuli in fasted versus fed state, emphasising the impact of energy deprivation in directing human behaviour.

Contrary to our expectations, low-energy meals were preferred over high-energy meals both in fasted and fed states. Additionally, implicit associations were not significantly affected by the change in the metabolic state when the context of the IAT was built solely on food-related target categories; low-energy meals and sweet snack foods were similarly preferred despite prevailing metabolic state. One possible explanation for this finding is that the shift in the metabolic state was not a factor strong enough to modulate the strength (or the direction) of the implicit associations in a context including only food-related items compared to food–non-food context. Moreover, it appears that in the modern sociocultural environment young healthy females are commonly body and health-conscious and have presumably assimilated, also implicitly, a preference for low-energy foods over more energy-dense foods, an attitude which prevails despite the current metabolic state. Hence, the temporal stability and the direction of the implicit measure in high–low-energy context likely reflects shared environmental and individual trait-like characteristics rather than less effective impact of transient situational factors (i.e., metabolic state). Previously, Maison et al., (2001) have also shown that young females had more positive implicit attitudes to low-calorie products than high calorie products. This health-related perspective is also supported by Trendel and Werle (2016) who stated that overall implicit attitudes to food are not driven merely by automatic perceived tastiness (i.e., a measure of the affective basis of implicit attitudes) but also by automatic perceived healthiness (i.e., a measure of the cognitive basis of implicit attitudes).

Finally, the stronger implicit association for sweet over savoury snack foods, which dominated unchangeably regardless of the metabolic state, was interesting. The implicit preference for sweet foods likely reflects our learned and inherent preference for sweet taste (Drewnowski et al., 2012), therefore emphasising the special role of certain energy sources over others in human diet. Moreover, the results suggest that implicit motivation (wanting)-related measure can disclose innate human preferences, which are not readily modulated by the variations in the metabolic state like explicit evaluations.

Taken together, the results from the implicit measures of our study support the view that implicit responses are probably better understood in terms of complex person-by-situation interactions rather than sole reflections of person- or situation-related factors (Gawronski, 2019).

Explicit responses

As hypothesised, explicit ratings were more sensitive to acute change in the metabolic state than implicit responses. Overall, our results show that most of the explicit liking and wanting ratings were significantly higher in fasted than in the fed state. The metabolic shift from energy depletion to energy repletion was reflected especially in the post-meal wanting ratings that decreased more markedly than the post-meal liking ratings; the decrease in post-meal wanting ratings was evident in each savoury food category (high- and low-energy meals, savoury snack foods) compared to pre-meal ratings. A less pronounced, yet significant, reduction was also observed in the pre- to post-meal liking ratings in high-energy meals and sweet and savoury snack foods categories. Moreover, it is noteworthy that when food categories (high- vs. low-energy meals, sweet vs. savoury snack foods) were contrasted within fasted or fed states, no significant differences in liking ratings were found, while most of the wanting ratings differed significantly.

Our results are in line with previous findings showing that a shift, acute or after a more prolonged fasting period / caloric restriction, between metabolic states affects explicit liking and wanting responses; not only the wanting (desire to eat, appetite) ratings are reduced after a meal, but also the pre- to post-meal liking (palatability, pleasantness) ratings are reduced accordingly, albeit to a lesser extent compared to wanting responses (e.g., Finlayson et al., 2007, 2008; Havermans et al., 2009; Cameron et al., 2014, Attuquayefio et al., 2016; Stevenson et al., 2017; Pender et al., 2019). The phenomenon ~~also~~ observed in this study, in which the hedonic and motivational value of food changes in a state dependent manner, represents two underlying concepts of consummatory reward. First, sensory-specific satiety (Rolls et al., 1981), which describes transient declines in reactions to food already consumed in relation to unconsumed food, and second, alliesthesia (Cabanac, 1971), which refers to a relationship between person's internal state (e.g., fasted vs. fed) and perceived sensation of a given (food) stimulus. As demonstrated in our study, together with other evidence (e.g., Finlayson et al., 2007, 2008; Cameron et al., 2014), the post-meal reduction in liking and especially in wanting ratings is also observed when *visual food stimuli* (as compared to exposure to orosensory stimuli) are used and in addition to the finding that the wanting responses of all savoury food categories, but not the sweet category were reduced after a savoury pizza meal, observations that both support the concept of alliesthesia. Moreover, as was proposed previously (Stevenson et al., 2018) there are plausible explanations for the unequal change in the liking and wanting responses across a meal; a change in the metabolic state leading to a more pronounced reduction or even termination in wanting postprandially allows a shift in goals to other relevant and/or rewarding targets, whereas equally dramatic post-meal change in liking or hedonic responses would be maladaptive and could lead to adverse effects on appropriate food choice and consumption later on. Hence, the unequal across meal changes in liking and wanting likely directs human eating behaviour to support versatile (food) choices and better nutritional status in the long term. Furthermore, as Berridge and Kringelbach (2008) theorised wanting is a *motivational* process, a motivation for (food) reward, whereas liking is a *hedonic* reaction of (food) reward, which also suggests that wanting should be more sensitive to change in metabolic state than liking. Thus, our results corroborate earlier findings that explicit components of food reward – liking and wanting (responses) – are susceptible to the changes in the 'internal milieu' and can be differentiated by the changes in the physiological state corresponding to the concept of alliesthesia.

Correlation measures

When interpreting the results, it appears that appetite is reflected through explicit wanting rather than explicit liking ratings. Both in fasted and fed states hunger and desire to eat ratings correlated positively and satiety ratings negatively with the explicit wanting ratings, while only weak correlations were observed between explicit liking and appetite measures. Certain correlations, especially the positive correlations of hunger and desire to eat and a negative correlation of satiety with wanting ratings of high-energy meals and savoury snack foods, as well as correlations of desire to eat with wanting of sweet snack foods tended to get stronger when shifted from fasted to fed state. These correlations were also the ones that remained significant in the fed state after the correction for multiple comparisons. Moreover, significant correlations between desire to eat and wanting ratings highlights the finding that desire to eat ratings appears to be equally potential measure in reflecting the prevailing appetitive or motivational state as hunger ratings are (see e.g., Stevenson et al., 2017). Taken together, these results are consistent with the reward concept of Berridge and Robinson (2003) postulating that the explicit appetite related wanting or cognitive desires can be thought to have a motivational basis, a goal-directed mechanism to a desired object, whereas explicit liking refer to the hedonic impact of the target object.

We found no significant correlations between implicit measures and explicit appetite (hunger, satiety) ratings, which implies that implicit responses, as measured with the IAT, are not systematically regulated by the fluctuations in metabolic state ~~in a comparable manner than~~ as opposed to explicit responses. This suggests that implicit measures are largely unaffected by the physiological processes related to homeostatic control, as noted earlier by Finlayson et al. (2008). ~~Interestingly,~~ Instead, when the relationship between implicit responses and corresponding explicit ratings was assessed, implicit responses were associated with some of the explicit wanting and liking ratings, more so in the fasted state. However, these correlations did not remain significant after correction for multiple comparisons. In the present study, we chose to use approach–avoidance-based IAT tests, because it has been argued that this IAT variant assesses especially motivational wanting-related implicit components in comparison to variants measuring evaluative (liking)-related implicit components (Tibboel et al., 2011). While some studies have reported that both motivational and evaluative-based implicit measures are dissociative with significant correlations with corresponding explicit measures (e.g., Kraus & Piqueras-Fiszman, 2016), others have reported low discriminant validity (e.g., Tibboel et al., 2011) and no correlations with explicit responses (e.g., Finlayson et al., 2008). Hence, our results together with previous findings indicate that the implicit–explicit-relationship is not straightforward, and although metabolic state may affect implicit responses and their relationship with explicit ratings, several other factors, including correspondence of the measured contents, can modify these interactions (e.g., Hofmann et al., 2005; Nosek, 2005, Gawronski, 2019).

Limitations

The limitations of our study warrant consideration when interpreting the results. The participants of our study were young healthy Caucasian females mostly within normal weight range. Given the importance of generalisation of the research findings, future studies should include other populations, representing a wider range of age, ethnicity, and weight in both sexes, in order to define whether the findings are consistent across different populations or concern only those with distinctive physiological and/or psychosocial characteristics.

Some potential limitations may be related to the stimuli used in the IAT tests and in the explicit ratings. Especially the results concerning High-energy – Low-energy IAT should be interpreted with caution, because the images of low-energy meals (i.e., colourful salad-based portions) were more colourful and potentially more appealing compared to the images of the high-energy meals (i.e., main meals without salad portions). This may have facilitated the categorisation task and biased the IAT score towards the low-energy category. Instead, in the Food – Non-food IAT all the images were carefully matched and thus the results are unlikely visually biased towards either of the categories. However, the explicit ratings were not available for these stimulus images and thus the result of the Food – Non-food IAT could not be compared with the explicit ratings. Furthermore, the IAT test seems to be sensitive to both the specific stimuli and categories used in the test (e.g., Govan & Williams, 2004; Mitchell et al., 2003). We assessed only the explicit ratings to the stimuli used in the IAT tests, although the explicit evaluation of both the stimuli and categories could have been more beneficial to clarify the authentic association between implicit and explicit bias.

Conclusions

The results of the present study show that implicit associations are relatively resistant to acute change in metabolic state compared to explicit ratings, which shift more readily according to the fasted –fed continuum. The change in the prevailing metabolic state, however, was reflected in the strength of implicit responses towards food in relation to non-food items, yet in the sole food contexts implicit associations were comparable between the metabolic states.

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