Insulin sensitivity and brain reward activation in overweight Hispanic girls: a pilot study

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What is already known about this subject
• Obesity is associated with alterations in brain reward activity in response to food cues in adults.

What this study adds
• Obesity is associated with alterations in brain reward activity in response to food cues in children.
• Peripheral insulin sensitivity is associated with alterations in brain reward activity independent of obesity.
• Peripheral insulin sensitivity may co-occur with central insulin resistance even in children.

Summary

Background: Insulin resistance is a link between obesity and the associated disease risk. In addition to its role as an energy regulatory signal to the hypothalamus, insulin also modulates food reward.

Objective: To examine the relationship of insulin sensitivity (SI) and fasting insulin with cerebral activation in response to food and non-food cues in children.

Methods: Twelve overweight Hispanic girls (age: 8–11) participated in two study visits, a frequently sampled intravenous glucose tolerance test and a functional neuroimaging session (GE HDxt 3.0Tesla) with visual stimulation tasks. Blocks of images (high calorie [HC], low calorie [LC] and non-food [NF]) were presented in randomized order.

Results: Comparing HC with NF, SI was inversely associated with activation in the anterior cingulate ($r^2 = 0.65; P < 0.05$), the insula ($r^2 = 0.69; P < 0.05$), the orbitofrontal cortex ($r^2 = 0.74; P < 0.05$), and the frontal and rolandic operculum ($r^2 = 0.76; P < 0.001$). Associations remained significant after adjustment for body mass index. Association of fasting insulin and cerebral activation disappeared after adjustment for waist circumference.

Conclusion: In addition to weight loss, insulin sensitivity may pose an important target to regulate neural responses to food cues in the prevention of excessive weight gain.

Keywords: Brain reward, childhood obesity, functional imaging, insulin sensitivity.

Introduction

The alarming prevalence of childhood obesity is not only a significant health problem in the paediatric population, but it also poses a risk factor for adult morbidity and mortality as well (1). One of the most common complications associated with obesity is type 2 diabetes caused in part by the effects of adiposity on insulin resistance (2). Insulin resistance is thought to be a link between obesity and the
associated increased disease risk (3). While body adiposity and insulin resistance are strongly associated in the adult population, the paediatric population of overweight and obese children still present with a wide variety of insulin sensitivity (4).

Converging evidence shows that insulin as well as sensitivity to insulin is of major importance for homeostatic, but also for the hedonic, mesolimbic regulation of hunger and satiety (5). In addition to being an energy regulatory signal to the hypothalamus, insulin acts within the central nervous system (CNS) to decrease food reward possibly through a direct alteration of dopaminergic signalling (6). Insulin was shown to modulate activity of ventral tegmental area dopaminergic neurons (7,8).

While CNS insulin production cannot be ruled out, converging evidence suggests that the majority of insulin enters the brain through active transport from the periphery (9). It is therefore not surprising that manipulations of peripheral insulin concentrations were reflected in CNS insulin alterations (10). One hypothesis referred to as the ‘Central Resistance Model’ is that genetic or acquired resistance to adiposity-regulating hormones occurs quite commonly and undermines the ability of those hormones to biologically protect against the development of obesity (11).

In human research, the existence of brain insulin resistance along with peripheral insulin resistance was suggested after the observation of a reduction in insulin-induced changes in the global cerebral metabolic rate for glucose in insulin-resistant research participants compared to insulin-sensitive participants (12). Because insulin resistance is so closely intertwined with body fat, it has been difficult to tease apart the individual contribution of either one to alterations in brain reward activity. More detailed study designs comparing lean and obese individuals found specific associations of either body mass index (BMI) or insulin sensitivity with activity in food-related resting state networks (13). Results show that, especially for reward-related processing, insulin sensitivity (SI) might be of particular importance.

Considering the range of SI despite obesity, children appear to be well suited for a more specific investigation of the individual contribution of SI and body adiposity to altered food-related brain processing, specifically highly palatable foods.

Central insulin resistance may contribute to altered food related reward perception even in the paediatric population (14). An uninhibited, overactive reward circuit would then lead to overeating, setting children up for a chronic weight issue in the future. Purpose of the current study was to assess the importance of SI for brain reward activation in response to visual food cues in a paediatric population. It was hypothesized that lower SI in the periphery is associated with stronger activation of brain reward areas in response to high calorie food images compared to control images. The association was furthermore expected to be independent of body fat mass.

**Methods**

**Participants**

Twelve Hispanic, overweight girls were recruited through flyers and presentations at schools and boys and girls clubs in the greater Los Angeles area. Inclusion criteria for participants were female gender, Hispanic ethnicity, defined by all four grandparents being of Hispanic descent, Tanner stage 1 or 2, a BMI above the 85th percentile for age and gender (15) and right handedness. Participants were excluded from the study if they had diabetes, any major illness since birth, had a condition known to affect SI or had a neurological illness. Written informed consent was obtained from parents and youth assent from participants. The study was approved by the USC Internal Institutional Review Board (IRB) committee.

**Procedures**

Participants came to the laboratory twice. The first visit included anthropometric measurements followed by an overnight stay in the USC Clinical trials unit, followed by a frequently sampled intravenous glucose tolerance test (FSIVGTT). The second visit included functional imaging. Visits were scheduled 1 week apart. All imaging sessions took place at 3:00 p.m. with the participants being instructed to not eat anything after 12 noon.

**Anthropometric measures**

A paediatric healthcare provider conducted a medical history and physical examination and determined maturation using the criteria of Tanner (16). Height was measured to the nearest 0.1 cm by a wall-mounted stadiometer and weight was recorded to the nearest 0.1 kg by a balance beam medical scale. BMI and BMI percentiles for age and gender were determined based upon established normative curves of the Centers for Disease Control and Prevention (17).

**Body composition**

Body composition (total body fat and total lean mass) for all participants was determined with dual-energy X-ray absorptiometry using a Hologic Discovery A model (82702, Hologic, Bedford, MA, USA).
Measures of insulin sensitivity

Frequently sampled intravenous glucose tolerance test

All girls participated in a FSIVGTT, assessing SI. After a 10-h fasting period, two fasting blood samples (−15 and −5 min) were followed by a 0.3g kg⁻¹ body weight intravenous (iv) glucose administration at timepoint 0. At 20 min, insulin (0.02 U kg⁻¹ body weight, Humulin R; Eli Lilly, Indianapolis, IN, USA) was administered iv. Blood samples were taken at 2, 4, 8, 19, 22, 30, 40, 50, 70, 100 and 180 min. SI was calculated with minimal modelling (18). Blood samples from the FSIVGTT were centrifuged for 10 min at 2500 rpm and plasma aliquots were frozen at −80 C until further analysis.

Fasting insulin

In addition to the FSIVGTT, the relationship of fasting insulin with cerebral activation was assessed. Measurements of SI that are based on fasting insulin and glucose concentrations have been suggested to reflect hepatic SI rather than skeletal muscle glucose uptake and are therefore also considered in the current paper (19,20).

Analysis of blood parameters

Blood for glucose analysis was collected in fluoride tubes and blood for insulin analysis was collected in lithium heparin tubes. Plasma was assayed in duplicate for glucose using the glucose oxidase method and a Yellow Springs Instrument 2700 analyzer (YSI Inc., Yellow Springs, OH, USA). Insulin was assayed in duplicate using an ELISA kit from Linco (St. Charles, MO, USA).

Food picture exposure paradigm

A block design consisting of two visual activation blocks and control blocks was presented in randomized order. All images were chosen based on previous studies (21) or through the International Affective Picture System (22). Activation task blocks consisted of 10 pictures of either high calorie (i.e. pizza, cheeseburger) or low calorie food items (i.e. broccoli, carrots). Before, between and after activation task blocks, control blocks of 10 non-food items were presented in randomized order (i.e. book, bus, bicycle). Control items were selected based on similarity in object complexity, colour, texture and shape variety. All visual stimuli were presented for 2.5 s with a 0.5 s inter-stimulus interval. Pictures were projected from a Macintosh computer onto a screen placed near the foot of the scanner, where they were viewed through a mirror mounted on the head coil. Participants were instructed to view the series of pictures with the intention to recall them after the scanning procedure.

Imaging and statistical analysis

Magnetic resonance imaging was conducted on a 3.0 Tesla scanner (General Electrics, GE HDxt, Fairfield, CT) equipped with an eight-channel head coil. A functional echo-planar T2* (BOLD)-weighted sequence (Temporal Resolution (TR): 3000 ms, Echo time (TE): 25 ms, flip angle: 90°, Field of view (FOV): 192 mm, matrix: 64 x 64, voxel size: 3.4375 x 3.4375 x 5 mm) was used to acquire 50 volumes during the visual stimulus presentation. A T1-weighted anatomical volume (TR: 500 ms, TE: 9.88 ms, flip angle: 90°, FOV: 192 mm, matrix: 256 x 256, voxel size: 0.8594 x 0.8594 x 5 mm) was acquired at the same slice locations as the functional sequence to allow for functional-anatomical co-registration.

Visual stimuli were presented by PsychToolbox for Matlab (Mathworks, Natick, MA) (23). The functional images were corrected for motion in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) smoothed and co-registered to the standard Montreal Neurological Institute template for comparison. Through the co-registration process, images were sliced into 2 x 2 x 2 mm isotropic voxel space using sinc interpolation. A statistical parametric map was generated for each subject using the general linear model with the stimulus as the primary parameter of the model. To eliminate activation due to movement, six movement parameters were also included in the model. A box-car waveform was used as the reference paradigm.

For the statistical analysis, one participant was excluded due to the inability to finish the IVGTT. Another participant was excluded due to too much head motion during the scanning process, leaving a total of 10 participants for the final analysis. Contrast images of each subject were used for group statistics calculated as random effects analysis at the second level, which takes variances between subjects into account. Spatial Parametric Maps produced by either high or low calorie activation tasks vs. the non-food control condition were computed. For the next step, we performed a second level analysis by comparing maps produced by high and low calorie activation tasks. Regions of interest were established based on the relevant literature (24,25) and a cluster threshold of 10 voxels. Contrast maps were set at a standard threshold of P < 0.05, corrected for multiple comparisons.
Variables were tested for normality and log transformed if necessary. Separate regression analyses were utilized to assess the association between cerebral activation, fasting insulin, and SI. Regression data was analysed using SPSS (SPSS Inc., Chicago, IL, USA). A \( P \)-value of <0.05 was considered significant.

**Results**

Mean values (\( \pm \) standard deviation) of anthropometric measures and range are described in Table 1.

Three of the participating girls were in Tanner stage 1 and seven were in Tanner stage 2. All girls were of Hispanic descent (as defined by all four grandparents identifying as Hispanic) and overweight (BMI above the 85th percentile for age and gender). Mean fasting glucose concentrations were 5.07 ± 0.5 mmol L\(^{-1}\) (ranging from 4.38 to 6.07 mmol L\(^{-1}\)). Three participants had impaired fasting glucose according to the standards of the American Diabetes Association and therefore qualified as pre-diabetic (26). Fasting insulin concentrations were on average 26.69 ± 19.6 \( \mu \)U mL\(^{-1}\) (ranging from 3.52 to 50.90 \( \mu \)U mL\(^{-1}\)).

BMI was related to waist \( (r = 0.88; \ P < 0.05) \), percent body fat \( (r = 0.69; \ P < 0.05) \) and SI \( (r = -0.66; \ P < 0.05) \). BMI was not related to fasting insulin concentrations. Fasting insulin concentrations were related to waist circumference \( (r = 0.74; \ P < 0.05) \), but not to percent body fat, BMI or SI. SI was related to BMI, but not to waist circumference, percent body fat or fasting insulin concentrations.

Brain responses comparing high calorie with low calorie and non-food visual cues

Significant differences in BOLD activation in response to high calorie foods and non-food pictures (Table 2) were observed in the caudate \( (P < 0.05) \), orbitofrontal cortex \( (P < 0.05) \), the insula \( (P < 0.05) \), the frontal and rolandic operculum \( (P < 0.05) \), and the anterior cingulate \( (P < 0.05) \). Differences were mostly observed in the left hemisphere, except for difference in insula activation, which were observed bilaterally.

**Brain responses to visual stimulation and insulin sensitivity**

Contrasting high calorie images with non-food images, SI was inversely associated with activation in insula \( (r^2 = 0.69; \ P < 0.05, \text{Fig. 1a}) \), orbitofrontal cortex \( (r^2 = 0.74; \ P < 0.05) \), anterior cingulate \( (r^2 = 0.65; \ P < 0.05, \text{Fig. 1b}) \), frontal and rolandic operculum \( (r^2 = 0.76; \ P < 0.05) \). SI was also negatively correlated with activation in putamen \( (r^2 = 0.74; \ P < 0.05, \text{Fig. 2a}) \) and insula \( (r^2 = 0.66; \ P < 0.05, \text{Fig. 2b}) \).
Fig. 2b). Given the association of BMI with SI, BMI was considered as a covariate in the regression analysis in the next step. When BMI was considered in the regression, the relationship of SI stayed significantly associated with insula activation ($r^2 = 0.76$; $P < 0.05$), orbitofrontal cortex ($r^2 = 0.89$; $P < 0.05$), frontal and rolandic operculum ($r^2 = 0.79$; $P < 0.05$). The association between SI and activation in the anterior cingulate and putamen disappeared.

Contrasting high calorie food images with low calorie food images, significant differences in BOLD signal were observed for the putamen and the insula. More specifically, SI was negatively correlated with activation in putamen ($r^2 = 0.74$; $P < 0.005$, Fig. 2a) and insula ($r^2 = 0.66$; $P < 0.005$, Fig. 2b).

**Brain responses and fasting insulin**

Contrasting high calorie food images with non-food control images, fasting insulin concentrations were associated with activation in the caudate ($r^2 = 0.70$; $P < 0.005$; Fig. 3a) and the insula ($r^2 = 0.69$; $P < 0.005$; Fig. 3b). Given the association of fasting insulin with waist circumference, waist was considered as a covariate in the regression analysis in the next step. When waist was considered, the association of fasting insulin with caudate and insula activation disappeared.

**Discussion**

Results of the current study show that – even in children – peripheral SI appears to be associated with the cerebral response to food vs. non-food cues. Stronger cerebral activation was associated with lower SI and higher fasting insulin concentrations, particularly in brain areas associated with motivation and reward. SI was also inversely associated with activation of gustatory (frontal operculum) and somatosensory (rolandic operculum) regions encoding food taste and texture. Our results lend support to the idea of a co-occurrence of peripheral and central insulin resistance, which was previously suggested by other authors (12).

In rodent studies, environmental set-ups to test food reward demonstrated the ability of insulin to significantly decrease lever presses for a sweet solution (5). That ability, however, was abolished after a few weeks of high fat feeding. Strikingly, the high-fat fed animals did not gain weight different from the chow-fed control rats, excluding weight as the responsible variable for the results. In human research participants, glucose metabolism was shown to be significantly decreased in insulin resistant individuals, particularly in areas of the brain.
reward system (12). Both, animal and human studies suggest the presence of central insulin resistance, possibly in concert with peripheral insulin resistance.

It has been traditionally difficult to tease apart whether associations between obesity and brain reward activity are primarily due to obesity, or are secondary, a result of the insulin resistance accompanying obesity. SI is strongly related to body weight and insulin resistance is often thought of as the linking factor between obesity and disease risk (27). There are a few recent studies that support the idea of SI affecting the brain reward response to food cues, rather than obesity alone (13,28). Children present with a wide range of SI despite obesity, spared still from the long-term impact of excess body fat on tissues and receptors. Therefore, a paediatric group of research participants seems to be ideal to investigate the respective importance of SI, obesity or both, for brain reward signalling. Our results may support the importance of both insulin and obesity for cerebral activity, possibly independently different for specific networks, as has been shown in a study investigating resting state network activity in obese individuals (13).

A striking observation in the study population is that SI and fasting insulin are completely unrelated. It was hypothesized that fasting measures of SI are more reflective of hepatic SI, while SI, as a glucose stimulated measure, is more reflective of peripheral SI (20). This assumption is supported by the fact that fasting insulin was only related to waist circumference, but not to overall body adiposity. After adjustment for waist circumference, the relationship between fasting insulin and cerebral activation disappeared, suggesting that overall SI may be more relevant for cerebral activity. However, this result may also be due to the fact that Hispanic children have a higher risk for insulin resistance even independent of body adiposity (29).

Traditionally, insulin resistance has been approached as a consequence of obesity.

Functional imaging in children is often criticized for the difficulties of children to not move in the process. We had to exclude one participant due to too much head movement. Other studies support that large-scale functional magnetic resonance imaging (fMRI) studies are feasible in children as young as 5 years (30). Therefore, we believe that the advantage of young-aged participants investigating the cause and effect of health issues weighs out practical issues of the study execution.

Research studies in adults show that abnormal neural responses to food cues persist in participants after weight loss and that successful dieters are characterized by a strong prefrontal activation in response to food cues (31–33). The prefrontal cortex is involved in self-control and behavioural monitoring, emotion regulation and salience attribution. In children, the implementation of inhibitory choices regarding food intake is in part dependent on the morphological and physiological development of the brain during childhood and adolescence (34) and a progressively greater focal activation in the prefrontal cortex during adolescent development has been observed regarding tasks that require inhibition of behavioural responses (35). Results suggest that chronic activation of the brain reward system may develop before the prefrontal cortex is mature enough to inhibit detrimental behaviours such as overeating, establishing neurological patterns that predispose children for overweight and lifelong weight struggle. Given the current study design, it is warranted to point out several limitations and refrain from overstated conclusions. The current results have to be seen in the light of a small number of female participants of one particular ethnicity in Tanner stage 1 and 2. The number of participants allows only for preliminary conclusions. Further studies, including a control group are warranted to draw more solid conclusions regarding the relationship between SI and brain reward activation in children.

In conclusion, the results suggest SI as a target in addition to weight loss to regulate neural responses to food cues to prevent excessive weight gain through overeating and obesity.

Conflict of interest statement
The authors have no conflict to disclose.

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