Altered Hypothalamic Function in Response to Glucose Ingestion in Obese Humans

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The hypothalamus plays a central role in the regulation of energy intake and feeding behavior. However, the presence of a functional abnormality in the hypothalamus in humans that may be related to excess energy intake and obesity has yet to be demonstrated in vivo. We, therefore, used functional magnetic resonance imaging (fMRI) to monitor hypothalamic function after oral glucose intake. The 10 obese (34 ± 2 years of age, BMI 34.2 ± 1.3 kg/m²) and 10 lean (32 ± 4 years of age, BMI 22.0 ± 0.9 kg/m²) subjects with normal glucose tolerance ingested 75 g of glucose while a midsagittal slice through the hypothalamus was continuously imaged for 50 min using a conventional T₂*-weighted gradient-echo pulse sequence. After glucose ingestion, lean subjects demonstrated an inhibition of the fMRI signal in the areas corresponding to the paraventricular and ventromedial nuclei. In obese subjects, this inhibitory response was markedly attenuated (4.8 ± 1.3 vs. 7.0 ± 0.6% inhibition, P < 0.05) and delayed (9.4 ± 0.5 vs. 6.4 ± 0.5 min, P < 0.05) compared with that observed in lean subjects. The time taken to reach the maximum inhibitory response correlated with the fasting plasma glucose (r = 0.75, P < 0.001) and insulin (r = 0.47, P < 0.05) concentrations in both lean and obese subjects. These results demonstrate in vivo, for the first time, the existence of differential hypothalamic function in lean and obese humans that may be secondary to obesity. *Diabetes 48:1801–1806, 1999

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CV, coefficient of variation; fMRI, functional magnetic resonance imaging; FPG, fasting plasma glucose; I₉₅, averaged inhibition over time; I₉₅ₙₐₓ, maximum magnitude of inhibition; LAH, lower anterior hypothalamus; LPFH, lower posterior hypothalamus; MRI, magnetic resonance imaging; NGT, normal glucose tolerance; PVN, paraventricular nucleus; T₁₉₅, time lag from beginning of glucose or water intake to time at which the functional magnetic resonance imaging signal intensity reached its maximal inhibition; UAH, upper anterior hypothalamus; UPH, upper posterior hypothalamus; VMH, ventromedial hypothalamus.
(Haifa, Israel) at the Research Imaging Center at the University of Texas Health Science Center at San Antonio. During the fMRI scan, subjects were positioned supine inside the bore of the magnet and their heads were immobilized using a plastic facial mask. A polarized circular head coil was used for radio frequency transmission and reception of the MRI signal. For each subject, routine scout $T_1$-weighted three-dimensional MRI images were acquired for accurate anatomical localization of the hypothalamus. A midsagittal section of the brain was used to determine the location of the hypothalamus in the longitudinal orientation, was chosen for the functional scan. A conventional $T_2$-weighted gradient-echo MRI pulse sequence was used for the functional scan. The parameters of the sequence were as follows: echo time equals 40 ms, repetition time equals 60 ms, a flip angle of 20°, and an in-plane spatial resolution equals 1 mm by 1 mm. The temporal resolution was 1.2 s per image. A total of 250 images were acquired for each subject after the sagittal plane through the hypothalamus was continuously scanned for 50 min: an initial 10-min period of baseline preglucose ingestion acquisition (50 images) followed by 40 min of post-glucose ingestion acquisition (200 images).

**Oral glucose tolerance test.** Before beginning the study, a catheter was placed in an antecubital vein for blood withdrawal. Blood samples for determination of plasma glucose, insulin, and leptin concentrations were obtained at 15-min intervals starting 15 min before glucose ingestion. Subjects ingested 75 g of glucose dissolved in 296 ml of flavored water 10 min after the start of the fMRI scan. A peripheral rubber tube of 1.5 m length was placed in situ, before positioning the subject inside the bore, to prevent the tubing from obstructing the scan. A 20-g glucose (Human Leptin RIA kit; Linco, St. Louis, MO) concentration was analyzed in duplicate by the glucose oxidase method (Beckman Glucose Analyzer D, Fullerton, CA). Plasma insulin (Diagnostic, Los Angeles, CA) and leptin (Human Leptin RIA kit, Linco, St. Louis, MO) concentrations were analyzed in duplicate by radioimmunoassay. The intra-assay coefficient of variation (CV) for insulin was <5% and the interassay CV was <7% at insulin levels >10 pmol/l. Both the intra-assay and interassay CVs for leptin were <6%.

**fMRI data analysis.** fMRI data processing for both the oral glucose tolerance test (OGTT) and water tests was performed on a SUN Ultra workstation using a MEDX package (Sensor Systems, Sterling, VA) and in-house programs written in C or MATLAB (The Mathworks, Natick, MA). For each subject, all 250 images were realigned to a reference image and motion was corrected using an automated realignment-to-reference-image (24). The global MR signal intensities in different areas of the hypothalamus were calculated by dividing the change from baseline in the MRI signal during a time window of 8 min, centered at $I_{max}$. **Calculations and statistics.** All of the data represent the mean ± SE. Statistical significance between the observed fMRI responses in lean and obese groups in different areas of the hypothalamus was analyzed by the analysis of variance method. Multiple comparisons between the baseline and the 10 time frames after glucose ingestion were made with a Student’s t test. Correlation coefficients between the fMRI responses and other parameters were determined by simple regression analysis. The statistical analysis was performed by Statview (version 5.0; SAS Institute, Cary, NC).

**RESULTS**

**fMRI response observed after oral glucose ingestion.** The average duration taken to ingest glucose was 4.0 ± 0.8 min in lean subjects and 4.4 ± 0.8 min in obese subjects (NS). A transient inhibition of the fMRI signal was observed in all subjects within 4–10 min after glucose ingestion and was followed by a return of the fMRI signal to the baseline. Typical examples of the fMRI responses in an obese subject and a lean subject are illustrated in Fig. 1. After glucose ingestion, both lean and obese subjects showed a consistent and significant inhibition of the fMRI signal in the LPH and UAH areas of the hypothalamus corresponding to the VMH and PVN, respectively. The Talairach coordinates (means ± SE) of the inhibited areas were $Y = -3.33 ± 0.17$ mm and $Z = -1.71 ± 0.19$ mm (n = 19) for the UAH area, and $Y = -7.47 ± 0.19$ mm and $Z = -6.87 ± 0.27$ mm (n = 19) for the LPH area. Because the midsagittal slice thickness was 10 mm, the Talairach coordinates in the x-axis ranged from $X = -5$ mm to $X = 5$ mm. The LAH and UPH areas of the hypothalamus showed no significant response.

**Differential hypothalamic response in lean and obese subjects after oral glucose ingestion.** There was a significant difference in the nature of the fMRI inhibitory response between the lean and obese groups in the LPH area after glucose ingestion. The magnitude of the response was markedly attenuated in the obese group (4.8 ± 1.3%, $P < 0.05$) compared with the lean control group (7.9 ± 0.6%) (Fig. 2A). Moreover, the delay to reach the maximum inhibitory response was significantly delayed in obese subjects (9.4 ± 0.5 vs. 6.4 ± 0.5 min, $P < 0.01$) (Fig. 2B). In the UAH area, the magnitude of the inhibitory response after glucose ingestion was slightly diminished in obese subjects compared with lean control subjects, but this difference was not statistically significant (6.6 ± 1.6 vs. 9.9 ± 1.0%) (Fig. 2C). However, a significant delay in the time taken to reach the maximum inhibitory response was observed in the UAH area in the obese group (9.0 ± 0.6 vs. 6.4 ± 0.5 min, $P < 0.01$) (Fig. 2D).
FIG. 1. A: T₁-weighted sagittal anatomical MRI image including the hypothalamic region in an obese subject. B: fMRI (in color) overlaid on an anatomical MRI image (in gray) depicts the typical inhibitory response observed in the hypothalamus after glucose ingestion in the same obese subject. The color-coded areas represent the areas of statistically significant inhibition (\( z < -2.0; P < 0.05 \)) of brain activity after glucose ingestion. After glucose ingestion, two areas with consistent inhibition were observed: the UAH and the LPH, which correspond to the paraventricular and ventromedial nuclei, respectively. C: The time courses of the MRI signal intensities in the LPH area in an obese subject and a lean subject after glucose ingestion. The inhibitory response on fMRI was observed 4 min after the start of oral glucose ingestion and reached a maximum at ~10 min before returning to the baseline after 14 min.

FIG. 2. A summary of the inhibitory fMRI response in lean and obese subjects after oral glucose ingestion. A: In the LPH area, a significant attenuation in the magnitude of the inhibitory response was noted in the obese versus lean group (4.8 ± 1.3 vs. 7.9 ± 0.6%). B: In the LPH area, a significant delay in the time taken to reach this maximum inhibitory response was also observed in the obese versus lean group (9.4 ± 0.5 vs. 6.4 ± 0.5 min). C: In the UAH area, an attenuation in the magnitude of the inhibitory response was noted in the obese versus lean group (9.4 ± 0.5 vs. 1.0%). D: In the UAH area, a significant delay in the time taken to reach the maximum inhibitory response was noted in the obese versus lean group (9.4 ± 0.5 vs. 6.4 ± 0.5 min). *P < 0.05, **P < 0.01. Data represent the mean ± SE.
in obese subjects was related to either a greater blood flow response or decreased local neural activity in the hypothalamic region. The exact mechanism, however, is not fully understood at this time.

**Relationship between the plasma glucose and insulin concentrations and the observed fMRI response after oral glucose ingestion.** The fasting plasma glucose (FPG) concentrations in obese and lean subjects were 5.0 ± 0.1 and 4.8 ± 0.1 mmol/l, respectively, and reached values of 7.3 ± 0.5 and 7.2 ± 0.7 mmol/l, respectively, 2 h after glucose ingestion. The fasting plasma insulin concentration was significantly higher in obese versus lean subjects (72 ± 11 vs. 29 ± 5 pmol/l, *P* < 0.01). The mean plasma insulin concentration during the OGTT increased by 161% in obese versus lean subjects (359 ± 54 vs. 138 ± 16 pmol/l, *P* < 0.001). The insulin concentrations at each time point were significantly higher in obese subjects than in lean subjects (*P* < 0.01). Obese subjects demonstrated NGT, but at the expense of hyperinsulinemia. When lean and obese subjects were examined collectively, a strong correlation between the FPG concentration and the *T*\(_{m}\) in the LPH (*r* = 0.75, *P* < 0.001) and UAH (*r* = 0.68, *P* < 0.01) areas was observed (Fig. 3A and B). In both the LPH (*r* = 0.47, *P* < 0.05) and UAH (*r* = 0.43, *P* < 0.05) areas, a weaker but significant correlation between the fasting plasma insulin concentration and *T*\(_{m}\) was also demonstrated (Fig. 3C and D). There was no correlation of *I*\(_{m}\) with the FPG or fasting plasma insulin concentrations after oral glucose ingestion in lean and obese subjects in either the LPH or UAH areas of the hypothalamus.

**Correlation between the plasma leptin concentration and the observed fMRI response after oral glucose ingestion.** According to the “lipostatic hypothesis,” leptin plays a central role in energy intake and body fat content (36,37). In our study, the fasting plasma leptin concentration was significantly increased in obese versus lean subjects (25 ± 6 vs. 4 ± 1 µg/l, *P* < 0.01). When data obtained from all the subjects were analyzed, no relationship between the plasma leptin concentrations and the fMRI inhibitory response was observed (*r* = –0.26, NS). However, when the data obtained from seven lean subjects with a BMI of <25 was analyzed, the plasma leptin levels showed a significant correlation with the *I*\(_{m}\) in the LPH area (*r* = 0.82, *P* < 0.02).

**fMRI response observed after water ingestion.** To examine whether gastric distention and/or altered esophageal/gastric motility played any role in the fMRI response observed after glucose ingestion, eight of the 20 subjects underwent a repeat fMRI scan on a separate day after consuming an equivalent amount of water (296 ml) without glucose. In the UAH area, ingestion of water elicited an fMRI inhibitory response that was virtually identical to that of glucose. Seven of the eight subjects showed absolutely no fMRI response in the LPH area (Table 1). The LAH and UPH areas of the hypothalamus showed no significant response.

**DISCUSSION**

The thrifty gene hypothesis was first proposed by Neel (34) in 1962 and has been invoked to explain the increased incidence of obesity and type 2 diabetes in westernized societies. It is based on the assumption that humans, who survive prolonged periods of starvation, develop genetic mutations that lead to the development of insulin resistance and hyperinsulinemia, both of which favor the storage of energy as adipose tissue. Individuals with the insulin resistance syndrome (35), i.e., obesity, hyperlipidemia, hypertension, and glucose intolerance, may represent a consequence of the thrifty gene hypothesis. The development of obesity represents an imbalance between energy intake and energy expenditure. In humans it is likely that the major cause of obesity is excessive energy intake and not diminished energy expenditure (36,37), although both play some role in the increase in body weight. Therefore, the investigation of eating behavior and the hypothalamic response to nutrient ingestion is important in elucidating the pathogenesis of obesity.

fMRI represents a noninvasive method that can detect transient hemodynamic and functional changes in the brain in response to a variety of stimuli. The use of fMRI to study

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**FIG. 3. Correlation between the time taken to reach the maximum inhibitory response and the FPG and fasting plasma insulin concentrations after glucose ingestion in obese (○) and lean (▲) subjects. A and B: In both the LPH (*r* = 0.75, *P* < 0.001) and UAH (*r* = 0.68, *P* < 0.01) areas of the hypothalamus, the FPG concentration correlated strongly with the *T*\(_{m}\). C and D: In both the LPH (*r* = 0.47, *P* < 0.05) and UAH (*r* = 0.43, *P* < 0.05) areas of the hypothalamus, the fasting plasma insulin concentration was also correlated with the *T*\(_{m}\).**
hypothalamic function in an animal model has been reported previously (21,22). Torii et al. (22) demonstrated altered brain function after glucose loading in streptozotocin-induced diabetic rats compared with normal rats. A decrease in the fMRI signal intensity in the area between the hypothalamus and thalamus in control rats was observed 20–30 min after the intraperitoneal injection of isotonic saline. This inhibition was not observed in streptozotocin-induced diabetic rats. The inhibition of fMRI activity in the VMH after oral glucose ingestion in lean subjects in the present study is very similar to that reported in rats (21,22). The slightly longer time required to observe the inhibition of fMRI activity in rats compared with humans can be explained by a number of factors, including the use of anesthesia in the animal studies, differences in the concentration and route of administration of glucose, and/or species differences.

After oral glucose intake, an acute transient reduction in the MRI signal intensity in the LPH and UAH areas of the hypothalamus, corresponding to the ventromedial and paraventricular nuclei, respectively, was observed in the human brain. This inhibition was altered in its magnitude and time course in obese subjects with NGT. The delay in the inhibitory response in the obese group was correlated strongly with the FPG concentration. Previous studies have documented that the VMH plays a significant role in glucose homeostasis (6,20) and that its activation causes inhibition of the parasympathetic nervous system and stimulation of the sympathetic nervous system (38,39). Activation of the sympathetic nervous system by hypoglycemia (40,41) has been shown to be inhibited by local perfusion of the VMH by glucose (42). Based upon these observations, one would expect that an increase in plasma glucose concentration, or a local increase in glucose concentration within the VMH, should inhibit the sympathetic nervous system through direct inhibition of neural activity in the VMH. Thus, it is most likely that the inhibitory fMRI response observed in the LPH area in both lean and obese subjects in the present study was probably caused by changes related to glucose ingestion. Consistent with this, water intake did not cause any response in the LPH area in either lean or obese subjects, indicating the “specificity” of the inhibitory fMRI response to glucose. Although, fundamentally, this response appears to be specific to oral glucose ingestion, it is virtually impossible to exclude other factors like smell, carbonation, and toxicity (osmotic effect) that may have contributed to this response. In lean subjects, since the inhibitory response was observed within 4–10 min, before any changes in the plasma glucose concentration occur, it is likely that inhibition of fMRI activity is mediated via a neurohormonal pathway that links the gastrointestinal tract to the hypothalamus.

The arcuate-PVN complex has been implicated as a site involved in the integration of neuronal and hormonal signals regulating food intake (15–18). Although the PVN has been proposed as an important center for appetite regulation (13,14,43), the fMRI response was specific to the VMH area, since water intake did not cause an inhibition of fMRI activity. In the UAH area, ingestion of water elicited an fMRI inhibitory response that was virtually identical to that of glucose. This is consistent with the important role of this hypothalamic region in the regulation of water balance (44–46).

Table 1: Comparison of the fMRI responses obtained after glucose intake with that observed after water ingestion

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<tr>
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<th>UAH</th>
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<tr>
<td></td>
<td>$I_m$ (%)</td>
<td>$I_{max}$ (%)</td>
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<tr>
<td>Water</td>
<td>8.0 ± 1.9</td>
<td>15.4 ± 3.2</td>
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<tr>
<td>Glucose</td>
<td>10.8 ± 2.0</td>
<td>19.8 ± 2.5</td>
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Data are means ± SE and were pooled from eight of the 20 subjects who participated in the study (four lean and four obese subjects). *P < 0.01, significance of the difference of the mean fMRI response (glucose vs. water, n = 8).

Acknowledgments

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