Blunted brain energy consumption relates to insula atrophy and impaired glucose tolerance in obesity

Running title: Brain energy and obesity

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ABSTRACT
Brain energy consumption induced by electrical stimulation increases systemic glucose tolerance in normal weight men. In obesity, fundamental reductions in brain energy levels, gray matter density, and cortical metabolism, as well as chronically impaired glucose tolerance suggest that disturbed neuroenergetic regulation may be involved in the development of overweight and obesity. Here, we induced neuronal excitation by anodal direct current stimulation (tDCS) versus sham, examined cerebral energy consumption with $^{31}$phosphorus magnetic resonance spectroscopy, and determined systemic glucose uptake by euglycemic-hyperinsulinemic glucose clamp in 15 normal weight and 15 obese participants. We demonstrate blunted brain energy consumption and impaired systemic glucose uptake in obese compared with normal weight volunteers, indicating neuroenergetic dysregulation in obese humans. Broadening our understanding of reduced multifocal gray matter volumes in obesity, our findings show that reduced appetite- and taste-processing area morphometry is associated with decreased brain energy levels. Specifically, gray matter volumes of the insula relate to brain energy content in obese participants. Overall, our results imply that a diminished cerebral energy supply may underlie the decline in brain areas assigned to food intake regulation and therefore the development of obesity.
INTRODUCTION

The incidence and prevalence of obesity has been escalating worldwide and obesity has already reached epidemic proportions (1;2). This frightening development, in conjunction with the urgent need to replace more or less inefficient treatment strategies, has resulted in the development of new pathophysiological concepts of obesity. There is currently a growing consensus that this disease involves dysregulation within brain areas assigned to control food intake behavior and systemic energy homeostasis (3;4). Data show that complex neuronal pathways with reciprocal connections between the hypothalamus, brainstem, and higher cortical centers control appetite and food intake behavior (5), while afferent inputs from the periphery as well as efferent signals to peripheral organs regulate energy homeostasis (6). At large, appetite perception, food intake behavior, and energy homeostasis are synchronized in the hypothalamus as the cerebral ‘appetite center’ (3;4). In this context, lower levels of high-energy phosphates, i.e., adenosine triphosphate (ATP) and phosphocreatine (PCr), have been detected in obese compared with normal weight humans (7), which suggests a relationship between brain energy supply and body weight regulation. This view is supported by the finding that cerebral ATP and PCr levels predict the amount of calories subsequently consumed (8). Moreover, brain energy consumption by electrical stimulation increases glucose tolerance in normal weight men (9). On this basis, we aimed to test whether transcranial direct current stimulation (tDCS), i.e., a form of neurostimulation, which uses constant low current delivered directly to the brain via small electrodes, would improve the characteristic glucose intolerant state in obese volunteers. We further assumed that tDCS-induced energy consumption alters brain energy levels. Because obese individuals not only display reduced cerebral ATP and PCr levels but also lower gray matter density (10-12) and cortical metabolism (8) compared with normal weight individuals, we likewise hypothesized that the morphometric decline could be due to the observed neuroenergetic deficit.
To test these hypotheses, we quantified brain energy levels and systemic glucose tolerance in obese vs. normal weight volunteers by $^{31}$P phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS) before and after tDCS under the conditions of a standard hyperinsulinemic-euglycemic clamp procedure, which is considered the experimental ‘gold standard’ to determine glucose tolerance in vivo (13). A glucose clamp places plasma glucose concentrations under the investigator’s control and therefore breaks the endogenous glucose-insulin feedback loop. This technique consists of an insulin infusion at a predetermined fixed dosage and a variable glucose infusion rate, which is continuously adapted to the target blood glucose. Under steady-state conditions of euglycemia, the glucose infusion rate gives information about the systemic glucose tolerance of an individual. Additionally, we examined the voxel-based morphometry (VBM) measures of key brain areas assigned to appetite and taste processing in relation to systemic glucose uptake and brain energy content. VBM involves a voxel-wise comparison of the local volumes of gray matter between two groups of individuals (15). The measure of structural differences between populations derives from a comparison of the local composition of different brain tissue types (i.e., grey matter, white matter) (16). Overall, VBM has been assigned to be sensitive to these differences, while discounting positional and other large-scale volumetric differences in gross anatomy.

**RESEARCH DESIGN AND METHODS**

**Participants**

Fifteen normal weight (BMI: $23.2 \pm 0.38$ kg/m$^2$) and 15 obese right-handed men (BMI: $36.3 \pm 1.04$ kg/m$^2$) matched for age ($24.6 \pm 0.69$ vs. $24.7 \pm 0.66$ years) participated in the experiments. Metabolic characteristics of the participants are summarized in Table 1. All subjects had a regular sleep-wake cycle during the week before testing. Before participation, all volunteers completed the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-1). Volunteers with a psychiatric disease were excluded from the study. Further
exclusion criteria were: acute and chronic internal or neurological diseases, alcohol and drug
abuse, smoking, shift work, competitive sports, exceptional physical or mental stress, and any
kind of current medication. On the days before experimental testing, participants were
instructed to go to bed no later than 23:00 h and to avoid exhausting physical effort. The study
was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical
Association and was approved by the ethics committee of the University of Luebeck. Each
participant gave written informed consent.

Study design
The study was performed in a randomized sham-controlled crossover design. Each subject
was tested in two experimental conditions spaced at least 2 weeks apart. On the days of
experimental testing, subjects reported to the Department of Neuroradiology at 3:30 p.m. after
fasting for at least 6 hours. Subsequently, one cannula was inserted into a peripheral vein on
the back of the hand and a second cannula into an antecubital vein of the contralateral arm.
Thereafter, baseline blood samples were collected for glucose and insulin measurements. The
euglycemic-hyperglycemic clamp was started at 4:30 p.m. by administration of an insulin
bolus (H-insulin, Hoechst, Frankfurt, Germany) of 5 mU/ kg body weight/min over 2 min.
Afterwards, insulin was infused at a constant rate of 1.5 mU/ kg body weight/min until the end
of the clamp. Simultaneously, a 20% dextrose solution was infused at a variable rate to
maintain plasma glucose values at approximately 5.5 mmol/l for 310 minutes. After reaching
euglycemic steady-state conditions, structural magnetic resonance imaging (MRI) for VBM
and baseline $^{31}$P-MR spectra were recorded (more details below). Subsequently, tDSC
stimulation occurred and a second sequence of $^{31}$P-MR spectroscopy measurements was
performed. In accordance with previous studies (7), a series of five continuous $^{31}$P-MR
spectroscopy sequences started 65 minutes and ended 105 minutes after tDSC. The last
spectroscopy sequence was recorded after another 2.5 hours in order to detect persistent
tDCS-induced effects. Thereafter, insulin infusion was stopped and plasma glucose concentration was normalized by increasing the glucose infusion rate.

**Transcranial direct current stimulation protocol**

For tDCS two electrodes were applied. The anodal electrode was placed over the primary motor cortex representation of the left first interdigital muscle (Id1). Prior to placement, the “hot spot” of Id1 was identified using suprathreshold local transcranial magnetic stimulation (TMS) in a stepwise manner by shifting the coil in 1-cm steps over the right vertex. The cathodal electrode was positioned over the left forehead. Stimulation electrodes were surrounded by a flat sponge soaked in an isotonic NaCl solution and fixed by elastic bands. Transcranial DCS was performed using a DC-stimulator plus (neuroConn, GmbH, Illmenau, Germany), which delivered 20 minutes of anodal stimulation (1 mA, fade in/out 8 s). During sham stimulation, the procedure was identical, except for the stimulator being turned off.

**MRI scanning, and $^{31}$P-magnetic resonance spectroscopy measurements**

Structural MRI as well as cortical $^{31}$P-MR spectra were acquired in a 3.0 Tesla MR scanner (Achieva 3T, Philips Medical Systems, Best, The Netherlands). Structural MRI was performed using a T1-weighted FLASH-3D MR sequence (echo time [TE] = 5 ms; repetition time [TR] = 15 ms; flip angle = 30°; isotropic voxel size = 1x1x1 mm$^3$). Data were processed and examined using SPM8 software (Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL, London) implemented in Matlab Version 7.6 (Mathworks, Sherborn, MA, USA), and the VBM8 toolbox. Applying a probabilistic framework, images were registered using linear (12-parameter affine) and non-linear transformations (warping). Subsequently, images were tissue-classified, and bias-corrected within the same generative model (14) as well as tissue-classified into gray matter (GM), white matter (WM), and CSF.
Finally, modulated gray matter images were smoothed with a Gaussian kernel of 8 mm full width at half maximum.

$^{31}$P-MR spectra were acquired using a double-tuned $^1$H/$^{31}$P-head coil (Advanced Imaging Research Inc., Cleveland, USA). Before acquisition, standardized volumes of interest were localized by taking scout images (please see Fig. 1 in the Online Appendix illustrating the localization of the voxels). Overall, eight $^{31}$P-MR spectroscopy sequences were measured as described in the study design. In order to allow satisfactory relaxation of the phosphorus metabolites, a repetition time of 4500 ms combined with a three-dimensional chemical shift imaging (3D-CSI) sequence (6 x 5 x 3 voxel, 6 kHz bandwidth, 1024 data points, 8:51 min measuring time; for a representative spectrum please see Fig. 2 in the Online Appendix) was chosen. For better spectral resolution during excitation and receiving, we applied $^1$H-decoupling and Nuclear-Overhauser-Effect (NOE) (15) with a broadband proton decoupling (10 rectangular RF pulses at a proton resonance frequency of 10 ms duration and 10 ms delay between each other to generate a 90° flip angle on the 1H nuclei) as well as $^1$H-decoupling (wideband alternating-phase technique for zero-residual splitting: WALTZ-4) (16) using the 2$^{nd}$ channel of the head coil for transmitting on the $^1$H-resonance frequency. For evaluation of the spectral data, a Magnetic Resonance User Interface (MRUI) was used. Zero-filling to 4096 datapoints and apodizing by a 20 Hz-Lorentzian-filter were applied. Peak positions and intensities were calculated using the AMARES algorithm (17). Before statistical analyzes, spectral data were corrected for transmit power and receiver gains.

We examined the high-energy phosphate compounds ATP and PCr, which directly reflect the overall high-energy phosphate turnover (18). ATP was calculated as the sum of alpha-, beta-, and gamma-ATP. In addition to PCr and ATP, the ratios of PCr/inorganic phosphate (Pi) and ATP/Pi were evaluated as an indicator of intracellular energy status (7). High-energy compounds are presented as single values at each time point. These values depict an arithmetic mean of all data points measured over all voxels at a given time point.
**Statistical analyses**

Data are presented as mean values ± standard error of mean (SEM). Statistical analysis was based on analysis of variance for repeated measurements (ANOVA) including the factors ‘time’ (time points of data collection), ‘treatment’ (tDCS vs. sham), and ‘group’ (for differences between normal weight and obese participants), as well as the interaction effect between these factors. The unpaired Student’s t-test was used for pairwise comparisons between groups and the paired Student’s t-test was used to compare single time points between the conditions within one group. To account for baseline differences in the cerebral high-energy phosphate content between groups, data were transformed to relative changes from baseline and then included in the respective ANOVA models. Correlation analyses were performed by bivariate correlation analysis according to Pearson. Voxel-wise gray matter differences between obese individuals and normal weight controls were examined using independent-sample t-tests. To avoid possible edge effects around the border between gray and white matter or cerebrospinal fluid and absolute gray matter, the threshold of 0.01 (absolute threshold masking) was used. For statistical analyses, in the first step, we employed an uncorrected threshold of $P < 0.001$ across the whole brain. In a second step, we performed multiple regression analyses (threshold of $P < 0.001$) to explore the association between regional brain volumes and spectroscopy values. Coordinates were reported in the standard anatomical space developed at the Montreal Neurological Institute (MNI). All tests comprised $n = 15$ in each group. A $P$-value $< 0.05$ was considered significant.

**RESULTS**

**Plasma glucose, serum insulin, and systemic glucose uptake**

Plasma glucose concentrations were comparable between groups both at baseline and throughout the euglycemic-hyperinsulinemic clamp (baseline plasma glucose: $P = 0.460$;
plasma glucose during clamp: \( P = 0.520 \) for group main effect; \( P = 0.707 \) for group by time interaction). Insulin concentrations were significantly higher in obese than in normal weight volunteers (baseline serum insulin: \( P = 0.037 \); serum insulin during clamp: \( P < 0.001 \) for group main effect; \( P = 0.003 \) for group by time interaction, Fig. 1). Additional analyses on a per-subject basis showed no differences in the insulin concentrations between single time points throughout the experiments in both groups (all \( P > 0.190 \)).

Consistent with previous data, during the entire experiments obese volunteers displayed distinctly reduced systemic glucose tolerance compared with the normal weight group (\( P < 0.001 \) for time main effect, \( P < 0.001 \) for group main effect; \( P < 0.001 \) for group by time interaction, Fig. 2). Accordingly, the overall glucose infusion rates required to maintain target blood glucose values during the clamp were significantly lower in obese compared with normal weight participants (66.21 ± 3.95 g vs. 85.27 ± 5.66 g; \( P = 0.010 \), Fig. 2, small insert).

**Cerebral high-energy phosphate content**

Baseline cerebral PCr, ATP, and Pi content and PCr/Pi and ATP/Pi ratios in the two groups are presented in Fig. 3. Confirming previous data, one-way ANOVA showed significantly different PCr and ATP levels as well as PCr/Pi and ATP/Pi ratios, but no differences in the Pi values, between the two groups. Overall, obese men displayed lower cerebral PCr and ATP contents than normal weight controls (both \( P < 0.001 \) for group effect; Fig. 3a and d, respectively). PCr/Pi and ATP/Pi ratios were likewise reduced in obese compared with normal weight participants (PCr/Pi: \( P = 0.014 \); ATP/Pi: \( P = 0.003 \); Fig. 3b and e, respectively), while Pi content was similar in the two groups (\( P = 0.143 \) for group effect; Fig. 3c).

In obese subjects, comparison of baseline-adjusted neuroenergetic changes revealed no significant differences between tDCS and sham stimulation (PCr: \( P = 0.375 \); ATP: \( P = 0.113 \); Pi: \( P = 0.347 \); PCr/Pi: \( P = 0.317 \) and ATP/Pi: \( P = 0.213 \), respectively for treatment by time
interaction; Fig. 4a-e). In this group, tDCS showed a tendency to cause a drop in PCr/Pi ratios 105 minutes after the end of the stimulation period relative to the sham condition ($P = 0.088$ for treatment by time interaction; Fig. 4b), whereas PCr, ATP, and Pi values as well as ATP/Pi ratios did not differ between the tDCS and the sham condition in obese participants (all $P > 0.213$).

Analyses in normal weight volunteers revealed significantly different PCr and ATP levels as well as PCr/Pi and ATP/Pi ratios after tDCS vs. sham stimulation (PCr: $P = 0.023$, ATP: $P = 0.048$, PCr/Pi: $P < 0.001$, and ATP/Pi: $P < 0.001$ for treatment by time interaction, respectively; Fig. 4a,b,d,e), without any differences in Pi content ($P = 0.101$; Fig. 4c). Compared with sham stimulation, tDCS caused an early decline in PCr and PCr/Pi ($P = 0.021$ and $P < 0.001$ for treatment by time interaction) followed by an increase above the level of the sham condition ($P = 0.010$ and $P < 0.001$ for treatment by time interaction) and return to baseline levels at the end of the clamp experiment ($P = 0.756$ and $P = 0.627$ for treatment by time interaction, respectively; Fig. 4a,b). In addition, there was an initial fall in ATP values and ATP/Pi ratios after tDCS vs. sham stimulation ($P = 0.007$ and $P < 0.001$ for treatment by time interaction) followed by a rapid rise ($P = 0.004$ and $P < 0.001$ for treatment by time interaction, respectively) and return to baseline values thereafter ($P = 0.348$ and $P = 0.781$ for treatment by time interaction; Fig. 4d,e).

Interindividual comparisons of tDCS-induced effects between the two participant groups revealed that, in contrast to normal weight controls, obese men did not display the initial drop in PCr and ATP values as well as PCr/Pi and ATP/Pi ratios, which occurred 65 minutes after ending the stimulation period in normal weight men (PCr: $P = 0.007$, ATP: $P = 0.004$, PCr/Pi: $P = 0.014$, ATP/Pi: $P = 0.002$ for time by group interaction, respectively; Fig. 4a, b, d, e). ATP levels as well as ATP/Pi ratios did not change at all in obese participants during the entire experiments ($P = 0.711$ and $P = 0.899$ for time main effect, respectively; Fig. 4d,e). However, we found a delayed and blunted drop in PCr values and PCr/Pi ratios 105 minutes
after the stimulation period ($P = 0.027$ and $P = 0.003$ for time by group interaction, Fig. 4a,b), which was followed by a return to baseline levels by the end of the clamp ($P = 0.995$ and $P = 0.885$ for time by group interaction).

Correlation analyses between body weight-adapted glucose infusion rates and high-energy phosphate ratios involving both groups resulted in a significant negative relationship ($r = -0.461, P = 0.010$ and $r = -0.421, P = 0.021$, respectively, Fig. 5), i.e., the higher the cerebral energy content, the lower the overall glucose infusion rates in both groups.

**Voxel-based morphometry**

Obese participants showed smaller gray matter volumes in fronto-temporal brain structures, anterior cingulum, putamen, insula and cerebellum compared with normal weight volunteers (Table 2; Fig. 6a). Regression analyses of VBM measures with BMI revealed a strikingly similar pattern in the above-described structural changes, i.e., a negative relation between fronto-temporal brain structures, anterior cingulum, putamen, insula as well as cerebellum and BMI, in both groups (Table 3; Fig. 6b), confirming the previous observation of gray matter atrophy in obesity.

Multiple regression analyses between gray matter volumes and the cerebral high-energy phosphate content revealed a relationship between the neuroenergetic content and volumes in the right insula, putamen, and cerebellum ($P = 0.007$; Fig. 6c). Post hoc analyses in obese individuals including gray matter volumes of the right insula and high-energy phosphates revealed significant correlations in terms of ATP ($r = 0.39, P < 0.001$), PCr ($r = 0.27, P = 0.021$), ATP/Pi ($r = 0.58, P = 0.022$), and PCr/Pi ($r = 0.34, P = 0.007$; Fig. 7).

**DISCUSSION**

Our data demonstrate blunted neuroenergetic reactivity and diminished systemic glucose uptake in response to electrical brain stimulation in obese compared with normal weight
volunteers. Moreover, analyses revealed reduced grey matter volumes in the fronto-temporal brain areas, anterior cingulum, putamen, insula, and cerebellum of obese men. In turn, insular gray matter volumes in obese participants correlated with the cerebral high-energy phosphate content, which suggests that decreased ATP and PCr levels may underline the reduced appetite- and taste-processing area morphometry in these individuals.

This is the first study showing a reduced response of brain energy variability and systemic glucose uptake to tDCS in obesity. While normal weight participants display a biphasic course of high-energy phosphate levels with an initial drop followed by a rise above baseline values upon tDCS, obese volunteers did not show any significant stimulation-induced variance in cerebral energy metabolism. It was only after 105 minutes, i.e., strikingly delayed, that we found a tendency for a drop in the PCr/Pi ratio in the obese, which returned to baseline values by the end of the experiment. As our observations preclude any conclusion in terms of underlying mechanisms, we can only speculate about the reason for this neuroenergetic rigidity in obese individuals. In view of the pre-existing lowered ATP and PCr levels in obese compared with normal weight individuals (7), the blunted neuroenergetic response to tDCS could be interpreted as a counter-regulatory provision to prevent any further decline in the cerebral ATP content. In this regard, the reduced response to tDCS in obesity would represent a neuroprotective mechanism.

However, the neuroenergetic rigidity in obese individuals was accompanied by reduced gray matter volumes within fronto-temporal brain structures, anterior cingulum, putamen, insula, and cerebellum compared with normal weight controls, which is consistent with previous data providing evidence of obesity-related structural changes within the prefrontal cortex and the ventral striatum (19;20). Again, the question arises, which mechanism might be behind the obesity-related brain morphology changes. At this point, it is conceivable that the observed morphometric alterations may relate to the reduced ATP and PCr levels in obesity. We found that the volume of the right insula was correlated with the
cerebral high-energy phosphate content, i.e., the morphometric decline is apparently accompanied by a neuroenergetic undersupply, which is at least true for this specific region. Therefore, one could hypothesize that the disturbed energy homeostasis may have caused the morphometric shrinking in obesity. This seems likely as, strikingly, all of the volume-reduced brain areas are involved in food intake regulation via control of food reward (21), taste processing (22), and motivational goal-directed behavior (23), which is relevant in terms of food preferences. In this context, the right anterior dorsal insula as well as the dorsal mid-insula fulfill an integrative function in the olfacto-gustatory system (24) and play an important role in flavor identification (25). The orbitofrontal cortex (OFC), in turn, is involved in the regulation of impulse control, eating behavior, and meal termination (21;22). Thus, it may be speculated that morphometric alterations in these brain areas due to a neuroenergetic decline are associated with abnormal sensory perception (26) and, in consequence, enhanced consumption of highly palatable foods, i.e., sweet or high-fat nutrients. In the long term, the subsequent increase in caloric intake would result in body weight gain and obesity. This reasoning is consistent with previous data showing a negative relationship between gray matter volumes, particularly in fronto-temporal brain areas and the insula, and BMI (27). However, we cannot draw definite conclusions about the cause-effect relationship between body weight gain and obesity-related brain atrophy. On the one hand, one could speculate that high caloric intake may induce a modification of the brain, which, in turn, potentiates caloric intake and further alters the brain in a self-reinforcing cycle. On the other hand, a part of the human population remains normal-weight despite the spread of high caloric food round the world suggesting that the cause-effect relationship between obesity and brain atrophy is much more complex. For example, there is evidence that chronic stress enhances food intake. Increasing stress in daily life and chronic HPA axis hyperactivity are linked to enhanced appetite, consumption of palatable high calorie foods, and obesity (28). On the other hand, stress per se causes damage to brain structures (29).
However, in our study, obese participants not only showed suppressed neuroenergetic reactivity upon tDCS but also lower systemic glucose uptake. We have previously demonstrated that tDCS increases systemic glucose tolerance in normal weight volunteers and that glucose infusion rates relate to respective changes in the high-energy phosphate content (9). Despite the obvious relationship between cerebral energy metabolism and systemic glucose uptake, it is not entirely clear how the two are linked. One explanation is provided by the so-called ‘energy on demand’ mechanism, which proclaims that the brain, as the only organ that is able to supply itself with energy, can allocate glucose from the peripheral blood circulation to satisfy its own needs. (30). In case of a neuroenergetic drop, e.g., as occurs upon tDCS, the brain would therefore request higher amounts of glucose from the systemic circulation, which becomes manifest in increased glucose infusion rates (31). In line with this, we found a negative correlation between ATP and PCr levels and glucose infusion rates during the hyperinsulinemic-euglycemic clamp in our study, i.e., the higher the brain energy levels, the lower the glucose uptake, which indicates that individuals with already high cerebral energy content need lower glucose infusion rates to meet their cerebral energy requirements. Given that this indeed is a physiological process, this fundamental mechanism seems to be disturbed in obesity and may be part of the observed neuroenergetic undersupply.

In conclusion, our data show an overall reduction in cerebral energy content with blunted reactivity of cerebral ATP and PCr levels and reduced systemic glucose uptake in response to tDCS in obese compared with normal weight volunteers. As well as expanding our knowledge of lowered multifocal gray matter volumes in obesity, our study reveals that these changes are associated with reduced brain energy levels. Overall, our data suggest that a diminished brain energy supply may underlie the volume reduction of brain areas assigned to taste and appetite regulation, and therefore to body weight gain. More generally, our study provides further support for the assumption that alterations in cerebral energy homeostasis may lead to enhanced food intake behavior and, in the long term, to obesity.
Author Contributions. KJC is the guarantor of this work and, as such, had full access to all data in the study, and takes responsibility for the integrity and the accuracy of the data analysis. KMO and FB conceived and designed the study. ML, GJ, and UHM collected the data. KJC, KMO, KR and UHM analyzed the data. KJC, FB, KR, US and KMO interpreted the data and wrote the manuscript. All authors edited and approved the final version for submission.

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Duality of Interests. There are no potential conflicts of interests relevant to this article.
Figure legends

**Fig. 1:** Plasma glucose and insulin concentrations. Mean ± SEM concentrations of plasma glucose and serum insulin (small insert) during hyperinsulinemic-euglycemic clamp sessions in normal weight (open circles) and obese (black circles) men (n = 15 per group). Gray area: the tDCS stimulation interval vs. sham.

**Fig. 2:** Systemic glucose uptake. Changes (mean values ± SEM) in glucose infusion rates in ml/kg/min during the hyperinsulinemic-euglycemic clamps in normal weight (open circles) and obese (black circles) men (n = 15 per group). Gray area: the tDCS stimulation interval vs. sham. Small panel: overall glucose administration in g.

**Fig. 3:** Baseline cerebral high-energy phosphates. Mean ± SEM levels of PCr (a), PCr/Pi (b), Pi (c), ATP (d) and ATP/Pi (e) during the hyperinsulinemic-euglycemic clamps in groups of normal weight (open plots) and obese (black plots) men (n = 15 per group). Because phosphate values were determined by area under the spectral peak, no units are indicated for high-energy phosphate measurements. * P < 0.050; ** P < 0.010.

**Fig. 4:** Effects of tDCS on cerebral high-energy phosphates. Relative changes in PCr (a), PCr/Pi (b), Pi (c), ATP (d), and ATP/Pi (e) during hyperinsulinemic-euglycemic clamp sessions in normal weight (open circles) and obese (black circles) men (n = 15 per group) after tDCS as well as in both groups (gray circles) after placebo stimulation (n = 30). Because phosphate values were determined by area under the spectral peak, no units are indicated for high-energy phosphate measurements. Gray areas: stimulation intervals vs. sham.
**Fig. 5:** Relationship between the cerebral energy content and glucose uptake. Correlation analyses between mean values of ATP/Pi (a) and PCr/Pi (b) ratio during the $^{31}\text{P}$-MR spectroscopy measurements and body weight-adapted glucose infusion rates in both participant groups (n = 30). Because phosphate values were determined by area under the spectral peak, no units are indicated for high-energy phosphate measurements. Projected slope and 95% confidence intervals shown by black and gray lines, respectively.

**Fig. 6:** Gray matter volumes, body mass index, and cerebral high-energy phosphate content.  
a) Categorical comparison between obese individuals and healthy participants by voxel-based morphometry demonstrating gray matter atrophy predominantly in the frontal cortex, putamen, insula, and cerebellum. b) Regression analysis with gray matter volumes and body mass index (BMI) of obese and healthy controls revealing a remarkably similar pattern as shown in the categorical comparison. c) Correlation analyses between gray matter volumes and cerebral energy levels. Multiple regression analysis between gray matter volumes and high-energy phosphate content in obese individuals (n = 15) and normal weight control participants (n = 15). $P$ uncorrected 0.001.
Table 1 – Metabolic characteristics of the participants

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Table 2 – Categorical comparisons between obese and normal weight participants

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Table 3 – Regression analyses between gray matter volumes and body mass index (BMI) in obese and normal weight participants

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Fig. 1: Plasma glucose and insulin concentrations. Mean ± SEM concentrations of plasma glucose and serum insulin (small insert) during hyperinsulinemic-euglycemic clamp sessions in normal weight (open circles) and obese (black circles) men (n = 15 per group). Gray area: the tDCS stimulation interval vs. sham.

180x119mm (300 x 300 DPI)
Fig. 2: Systemic glucose uptake. Changes (mean values ± SEM) in glucose infusion rates in ml/kg/min during the hyperinsulinemic-euglycemic clamps in normal weight (open circles) and obese (black circles) men (n = 15 per group). Gray area: the tDCS stimulation interval vs. sham. Small panel: overall glucose administration in g.
Baseline cerebral high-energy phosphates. Mean ± SEM levels of PCr (a), PCr/Pi (b), Pi (c), ATP (d) and ATP/Pi (e) during the hyperinsulinemic-euglycemic clamps in groups of normal weight (open plots) and obese (black plots) men (n = 15 per group). Because phosphate values were determined by area under the spectral peak, no units are indicated for high-energy phosphate measurements. * P < 0.050; ** P < 0.010.

119x189mm (300 x 300 DPI)
Effects of tDCS on cerebral high-energy phosphates. Relative changes in PCr (a), PCr/Pi (b), Pi (c), ATP (d), and ATP/Pi (e) during hyperinsulinemic-euglycemic clamp sessions in normal weight (open circles) and obese (black circles) men (n = 15 per group) after tDCS as well as in both groups (gray circles) after placebo stimulation (n = 30). Because phosphate values were determined by area under the spectral peak, no units are indicated for high-energy phosphate measurements. Gray areas: stimulation intervals vs. sham.

Figure 4
Fig. 5: Relationship between the cerebral energy content and glucose uptake. Correlation analyses between mean values of ATP/Pi (a) and PCr/Pi (b) ratio during the 31P-MR spectroscopy measurements and body weight-adapted glucose infusion rates in both participant groups (n = 30). Because phosphate values were determined by area under the spectral peak, no units are indicated for high-energy phosphate measurements. Projected slope and 95% confidence intervals shown by black and gray lines, respectively.
Fig. 6: Gray matter volumes, body mass index, and cerebral high-energy phosphate content. a) Categorical comparison between obese individuals and healthy participants by voxel-based morphometry demonstrating gray matter atrophy predominantly in the bilateral frontal structures, putamen, insula, and cerebellum. b) Regression analysis with gray matter volumes and body mass index (BMI) of obese and healthy controls revealing a remarkably similar pattern as shown in the categorical comparison. c) Correlation analyses between gray matter volumes and cerebral energy levels. Multiple regression analysis between gray matter volumes and high-energy phosphate content in obese individuals (n = 15) and normal weight control participants (n = 15). P uncorrected 0.001.
Fig. 1: Localization of the standardized volumes of interest for $^{31}$P-magnetic resonance spectroscopy measurements. Respective voxels are marked by continuous lines.
Fig. 2: A representative $^{31}$P-MR spectrum from a normal weight participant.