Monounsaturated Fatty Acids Prevent the Aversive Effects of Obesity on Locomotion, Brain Activity, and Sleep Behavior

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Fat and physical inactivity are the most evident factors in the pathogenesis of obesity, and fat quality seems to play a crucial role for measures of glucose homeostasis. However, the impact of dietary fat quality on brain function, behavior, and sleep is basically unknown. In this study, mice were fed a diet supplemented with either monounsaturated fatty acids (MUFAs) or saturated fatty acids (SFAs) and their impact on glucose homeostasis, locomotion, brain activity, and sleep behavior was evaluated. MUFAs and SFAs led to a significant increase in fat mass but only feeding of SFAs was accompanied by glucose intolerance in mice. Radiotelemetry revealed a significant decrease in cortical activity in SFA-mice whereas MUFAs even improved activity. SFAs decreased wakefulness and increased non–rapid eye movement sleep. An intracerebroventricular application of insulin promoted locomotor activity in MUFA-fed mice, whereas SFA-mice were resistant. In humans, SFA-enriched diet led to a decrease in hippocampal and cortical activity determined by functional magnetic resonance imaging techniques. Together, dietary intake of MUFAs promoted insulin action in the brain with its beneficial effects for cortical activity, locomotion, and sleep, whereas a comparable intake of SFAs acted as a negative modulator of brain activity in mice and humans. ***Diabetes*** **61:1669–1679, 2012**

Besides a decrease in physical activity, the main factor in the progression toward insulin resistance, obesity, and type 2 diabetes is overeating and, in particular, dietary fat intake. Although the daily intake of dietary fat remained stable over the past decades, the number of patients with obesity and type 2 diabetes reached epidemic proportions worldwide. A large number of studies blamed both the quantity and quality of dietary fat as a major culprit impairing insulin sensitivity and insulin secretion, but recent studies suggested that fat quality more strongly correlated with weight gain and metabolic alterations than fat quantity (1–3). In particular, dietary monounsaturated free fatty acids (MUFAs) prevent or ameliorate measures of the metabolic syndrome. Moreover, MUFAs were shown to prevent the deleterious effects of palmitate and glucose on pancreatic β-cell turnover and function (4), and MUFAs completely prevented palmitate-induced apoptosis in β-cells (5).

Of note, an acute rise in saturated fatty acids (SFAs) is able to increase insulin secretion whereas unsaturated fatty acids were ineffective (6), and chronically elevated SFAs inhibited insulin secretion and led to an increased rate of apoptotic β-cells in the pancreas (7). In human myotubes, palmitate promoted interleukin-6 expression whereas unsaturated fatty acids even abolished inflammation (8). As an inverse correlation between free fatty acids and insulin sensitivity was demonstrated in offspring of patients with type 2 diabetes (9), elevated free fatty acids may represent an early step in the progression toward type 2 diabetes, and a fatty acid pattern associated with insulin resistance is characterized by elevated concentrations of SFAs and a low concentration of the unsaturated n-6 fatty acid linoleic acid (10).

By magnetoencephalography studies in humans, we showed that chronically elevated serum levels of SFAs are associated with impaired insulin action in the brain. SFA levels were negatively correlated with insulin-stimulated brain activity in certain frequency bands, and obese subjects that are characterized by elevated levels of SFAs displayed insulin resistance independent of body weight (11,12). In addition, insulin levels were shown to affect brain function during rest in networks that support reward and food regulation (13). Moreover, rodent data suggested that overnutrition with SFAs leads to insulin resistance in the brain and hyperphagia (14,15).

We lately reported that a high-fat diet based on lard mediates insulin resistance in the mouse brain and impairs cortical activity and locomotion, which might further promote glucose intolerance, physical inactivity, and obesity (16), however, the diet used in that study contained a large amount of fat. Although the aversive effects of SFA overload on glucose metabolism are well documented in a number of studies (17), the impacts of MUFAs on insulin sensitivity in the brain and brain activity are largely unknown. The current design therefore reflects variations of fat quality as present in Western societies and consisted of two moderate, isocaloric, fat-enriched diets that just differ in fat quality but not quantity. Canola oil is rich in MUFAs, with >63% oleic acid, and low in SFAs. By contrast, the fat

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content of milk fat is dominated by SFAs, with a high content of palmitic acid (for analysis of fat quality, please see Supplementary Fig. 1 and Supplementary Table 1).

In contrast to an isocaloric, SFA-enriched diet, we report that the intake of an MUFA-enriched diet protected from obesity-associated glucose intolerance and brain and physical inactivity. Further, an intervention study in humans demonstrated that SFA intake deteriorates brain activity in the hippocampus and the inferior parietal cortex whereas MUFAs did not display aversive effects.

RESEARCH DESIGN AND METHODS

Animals. Male C57BL/6J mice were purchased from Charles River Laboratories (Sulzfeld, Germany). Four-week-old animals were fed chow diet (5% total fat by weight) or chow diet supplemented with either 3.3% fat by weight from canola oil or milk fat (Altromin, Lage, Germany) for 8 weeks, and diet feeding continued during electrocorticography (ECoG) measurements. A detailed analysis of the fatty acid composition of the diets is given in Supplementary Fig. 1 and Supplementary Table 1. All animal procedures were approved by local government authorities for animal research according to the guidelines of laboratory animal care.

Glucose and insulin tolerance tests. Overnight-fasted mice were intraperitoneally injected with 2 g/kg body weight of α-D-glucose, and blood samples from tail bleeds were drawn for the determination of glucose using a Glucometer Elite (Bayer, Elkhart, IN) at basal and 15, 30, 60, and 120 min after injection. Insulin tolerance testing was performed with 1 unit/kg body weight of human insulin intraperitoneally in fed mice (Novo Nordisk, Bagsvaerd, Denmark) at 8:00 A.M., and serum insulin was measured at basal and blood glucose levels from tail bleeds at basal and 15, 30, and 60 min after injection.

ECoG measurements and locomotor activity. Each mouse received an implantable telemetry electrocorticography transmitter and was instrumented with a guide cannula for microinjection of substances into the lateral ventricles as described previously (16). Telemetry signals were recorded and stored digitally using the Dataquest A.R.T. 4.2 software (DSI). Data analysis for ECoG measurements was performed as previously described (18). Basal ECoG measurements were conducted for four consecutive days.

Intracerebroventricular injection of human insulin or palmitic acid. Human insulin was delivered in a volume of 2 μL over 1 min into fasted mice intracerebroventricularly. The mice received an injection of either human insulin (3.75 mU total, purchased from Novo Nordisk) or vehicle (0.9% NaCl) in random order 4 days apart. Telemetry signals were recorded continuously for 120 min postinjection. Chow-fed control mice were injected intracerebroventricularly with vehicle or palmitic acid (225 μM total) shortly before the darkness period started (7:00 P.M.).

Sleep recordings and analysis of sleep data. ECoG signals were acquired digitally by using Somnologica Science software (version 3.3.1; Medcare, Reykjavík, Iceland). The three vigilance states, wake, rapid-eye-movement (REM) sleep, or non-REM (NREM or slow-wave) sleep, were scored in 4-s epochs.

Clinical chemistry. Serum levels of insulin, adiponectin, and leptin were obtained by RIA (Millipore, Billerica, MA). Analysis of nonesterified fatty acid (NEFA), cholesterin, triglycerides, and lipoproteins was performed on the automated clinical chemistry analyzer (ADVIA 1800; Siemens Healthcare Diagnostics, Eschborn, Germany).

Western blot analysis. Overnight-fasted mice received a bolus of human insulin (1 unit/mouse i.v. for 10 min; Novo Nordisk). Brain and liver tissues were homogenized as previously described (19). Lysates containing equal amounts of protein were immunoprecipitated with antibodies against insulin receptor (IR) (20) or IR substrate 2 (IRS2) (Millipore, Schwalbach, Germany). Antibodies directed against phosphotyrosine PY-20 (Santa Cruz, La Jolla, CA) or P-AKT (Ser473) (Upstate, Charlottesville, VA) were used.

Magnetic resonance imaging. Magnetic resonance imaging (MRI) examinations of fat volume were performed on a 3T whole-body imager (Siemens Tim Trio, Erlangen, Germany), applying a T1-weighted, fast spin echo technique. MRI examinations were simultaneously performed in mice fed a MUFA, SFA, or chow diet. Images for abdominal fat quantification were recorded with an in-plane spatial resolution of 0.4 mm and a slice thickness of 2 mm (21).

Functional MRI in humans

Subjects. Twenty four lean, healthy subjects were recruited. Any volunteer treated for chronic disease or taking any kind of medication other than oral contraceptives was excluded. The study was approved by the local ethics committee and informed written consent was obtained from all subjects. Subjects were divided into three groups matched for age (years) and BMI (kg/m^2) in a double-blinded design and consumed 500 g yogurt every day for 12 weeks (SFA enriched: milk fat 8% by weight, male/female 5/3, age 36.0 ± 12.3 years, and BMI 21.8 ± 2.3; MUFA enriched: canola oil 8% by weight, male/female 4/4, age 31.6 ± 10.8 years, and BMI 22.4 ± 2.6; or control group: milk fat 0.2% by weight, male/female 2/6, age 34.1 ± 11.8 years, and BMI 23.1 ± 2.2).

Study design. All subjects underwent a 10-min resting-state functional MRI (fMRI) before and after the 3 months of yogurt consumption. Subjects were instructed not to eat anything 2 h before scanning. During the 12 weeks of intervention, dietary protocols were obtained from all 24 subjects, and 5 were excluded due to missing data. Fasting insulin concentrations in humans were determined at 8:00 A.M. by a microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan).

Data acquisition. Whole-brain fMRI data were obtained by using a 3.0 T scanner (Siemens Tim Trio). Functional data were collected by using echo-planar imaging sequence: repetition time, 3 s; echo time, 30 ms; field of view, 192 mm^2; matrix, 64 × 64; flip angle, 90°; voxel size, 3 × 3 × 3 mm^3; no gap; 47 axial slices; and acquired in interleaved order. Each functional run contained 200 image volumes (total scan time, 10.06 min). In addition, high-resolution, T1-weighted anatomical images (MPRage, 192 slices; matrix, 256 × 240 and 1 × 1 × 1 mm^3) of the brain were obtained. All subjects were instructed not to focus their thoughts on anything in particular and to keep eyes closed during the recording.

Data preprocessing. SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5) was used for functional image preprocessing and statistical analysis. Unwarping of echo-planar images was performed using the FieldMap Toolbox to account for susceptibility by movement artifacts. Functional images were coregistered to the T1 structural image. The anatomical image was normalized to the Montreal Neurologic Institute template, and the resulting parameter file was used to normalize the functional images (voxel size, 3 × 3 × 3 mm^3). Finally, the normalized images were smoothed with a Gaussian kernel (full width at half maximum, 6 mm). fMRI data were high-pass (cutoff period, 128 s) and low-pass filtered (autoregression model AR) (22).

Fractional amplitude of low-frequency fluctuation. Low-frequency (0.01–0.08 Hz) fluctuations (LFFs) of the blood oxygenation level–dependent signal in resting-state fMRI data are thought to reflect intrinsic neural activity in humans (22). We used fractional amplitude of LFFs, which is a reliable and consistent index (23) to study intrinsic neuronal activity before and after 3 months of SFA- or MUFA-enriched diet (http://resting-fmri.sourceforge.net). For statistical analysis, a one-way ANOVA was used to analyze the main effect of fat-enriched diet by using the difference between the normalized whole-brain fractional amplitude of LFF maps of the pre- and postmeasurement. A P value of <0.001 was considered statistically significant. To compare the effect of SFA- and MUFA-enriched diet with the control diet, one-way ANOVA with Tukey post hoc test for multiple comparison analysis was used. Significance was determined at $P < 0.05$.

Statistical analysis. Data are expressed as mean ± SEM of the indicated number of experiments. All data were analyzed using Origin 8.1, and significance was performed using Student t test and one-way ANOVA with Dunnett or Bonferroni post hoc test for multiple comparison analysis. Significance was set at $P < 0.05$. Western blot results are presented as direct comparison of bands in autoradiographs and quantified by optical densitometry (Herolab, Wiesloch, Germany).

RESULTS

SFA-enriched diet leads to more weight gain upon isocaloric food intake compared with MUFAs. To evaluate the metabolic consequences of fat quality, 4-week-old mice were weaned on MUFA- or SFA-enriched diets and compared with chow-fed animals. As early as 4 weeks on a fat-enriched diet, both groups displayed a significant increase in body weight (Fig. 1*A*), and the SFA-fed mice were significantly heavier than the MUFA-fed group (Fig. 1*B*). However, there was no difference in the total food intake in grams (data not shown), as well as in the daily calorie intake between the fat groups (Fig. 1*C*).

SFA-enriched diet promotes glucose intolerance by inhibiting insulin secretion and impairs insulin sensitivity. Intraperitoneal glucose tolerance tests (GTTs) revealed that SFA-fed mice were glucose intolerant (Fig. 1*D*) compared with the MUFA-enriched diet and chow group, and fasted blood glucose tended to be higher in the SFA group; however, it did not reach significance (P = 0.06). Serum insulin concentrations during the GTT showed that glucose-stimulated insulin secretion was impaired in the

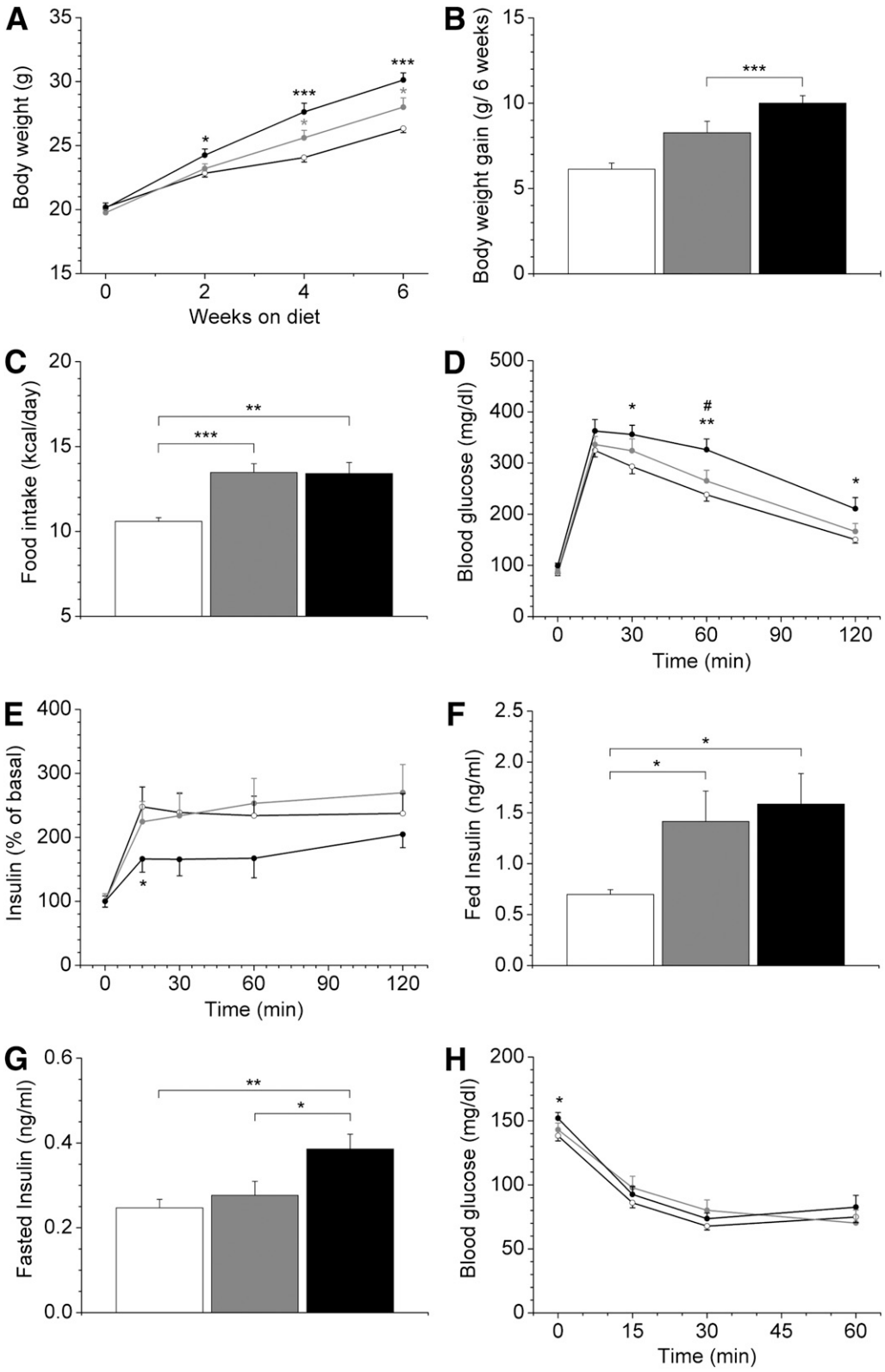

FIG. 1. Metabolic consequences of fat-enriched diets in C57BL/6 mice. C57BL/6 mice were weaned on MUFA-enriched (gray), SFA-enriched (black), or chow (white) diet for 8 weeks. *A* and *B*: SFA- and MUFA-fed mice displayed significantly increased body weight gain after 6 weeks compared with the control group (n = 15–30/group; *P < 0.05, ***P < 0.001). *C*: Food intake in kcal/day for control (white bars), MUFA (gray bars), and SFA (black bars) groups during the feeding period. Asterisks mark significance to controls (*P < 0.001 and **P < 0.005). *D*: Intraperitoneally injected glucose during a GTT was cleared slower and less effectively in SFA-fed mice (black circles) compared with controls (white circles) and MUFA-fed animals (gray circles) (n = 15–30/group; *P < 0.05 and **P < 0.005 to controls; #P < 0.05 to MUFA group). *E*: Attenuated serum insulin concentration during the GTT in SFA-fed mice (black circles). Increased fed (*F*) and fasted (*G*) serum insulin concentrations in the SFA-diet animal group (*P < 0.05 and **P < 0.005). *H:* Blood glucose concentrations from tail bleed during an intraperitoneal insulin tolerance test in control (white circles), MUFA (gray circles), and SFA (black circles) mice (n =15–30/group; *P < 0.05 SFA group to controls). Data are presented as mean ± SEM.**

SFA group whereas MUFA enrichment did not affect insulin secretion (Fig. 1*E*). The fed insulin concentrations were elevated in both fat groups (Fig. 1*F*) whereas fasted insulin was solely increased in the SFA group (Fig. 1*G*). Glucose concentrations in the fed state were significantly increased in the SFA group, and feeding with both diets did not reveal a significant change in whole-body insulin sensitivity, determined by an intraperitoneal insulin tolerance test (Fig. 1*H*).

NEFA, triglyceride, and leptin concentrations were increased by SFA-enriched diet, whereas lipoproteins, cholesterin, and adiponectin levels were not affected. A significant difference was observed for NEFA levels between SFA-fed and control mice (Fig. 2*A*), and triglyceride concentrations were significantly increased both in MUFA- and SFA-fed animals (Fig. 2*B*). Both fat-enriched diets did not alter total cholesterol and lipoproteins, such as HDL and LDL (Fig. 2*B*). Although there was a significant increase in serum leptin concentrations by SFA enrichment (Fig. 2*C*), adiponectin levels were not affected by either diet (Fig. 2*D*).

MUFA- and SFA-fed mice are characterized by increased total and visceral adipose tissue. Both fat-enriched diets led to a significant increase in visceral and total adipose tissue as determined by MRI measurements compared with the chow-fed animals, whereas the SFA-fed mice displayed the highest percentage of visceral and total fat mass (Fig. 2*E*).

Insulin-mediated phosphorylation of the IR and AKT is attenuated in liver tissue of SFA-fed mice. As increased fasting insulin levels in mice fed an SFA diet suggested hepatic insulin resistance, we determined insulin action in liver tissues after insulin injection. Thereby,

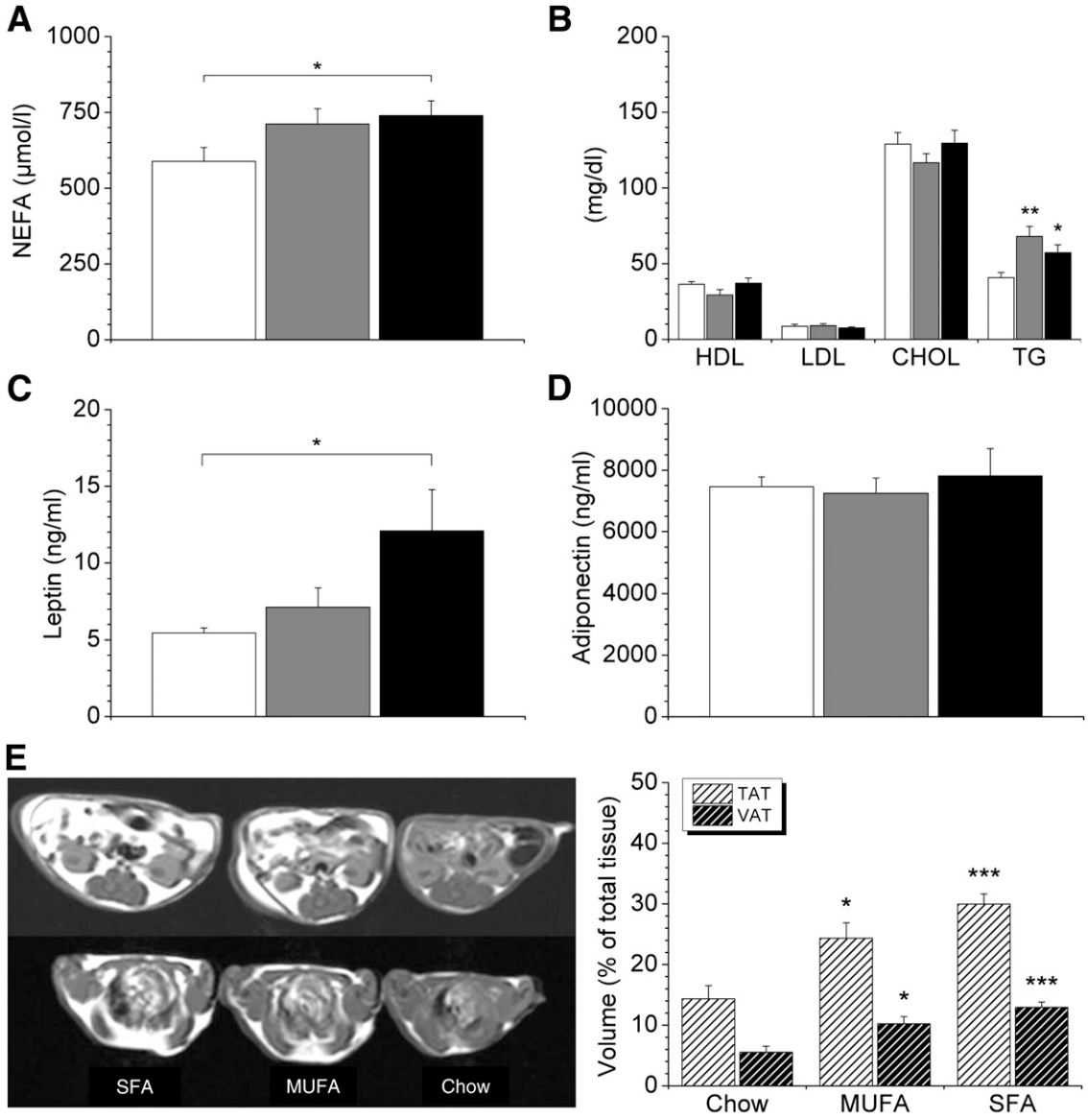

FIG. 2. Effect of fat enrichment with MUFAs and SFAs on serum parameters and visceral body fat mass. NEFA (*A*), HDL, LDL, cholesterol (CHOL), triglycerides (TG) (*B*), leptin (*C*), and adiponectin (*D*) of MUFA- (gray bars, $n = 14$), SFA- (black bars, $n = 14$), and chow-fed (white bars, $n = 24$) animals in the fasted state. Significance is indicated for fat-enriched diets to controls: *$P < 0.05$ and **$P < 0.005$. *E*: Magnetic resonance images of total (TAT) and visceral fat (VAT) deposits in mice fed an SFA-enriched, MUFA-enriched, or chow diet. Bright (hyperintense) areas represent fat tissue. Calculated volumes of TAT and VAT integrated over 24 such slices are quantified of $n = 9$ mice per diet group. Significance is indicated for fat-enriched diets to controls: *$P < 0.05$ and ***$P < 0.001$.

insulin significantly increased tyrosine phosphorylation of IR in chow-fed mice (Fig. 3*A*) and to a lesser degree in MUFA-fed animals, whereas SFA-fed mice displayed insulin resistance. At the level of AKT, insulin promoted phosphorylation of AKT in the chow- and MUFA-fed group, whereas insulin was not able to increase phosphorylation of AKT in SFA-fed mice (Fig. 3*B*).

SFA enrichment impairs insulin sensitivity in the brain and lowers cerebrocortical activity. Insulin was able to increase phosphorylation of IR in brain tissues in the chow group and to a lesser degree in the MUFA-fed group whereas the SFA-fed mice displayed insulin resistance (Fig. 4*A*). However, tyrosine phosphorylation of IR and IRS2 was enhanced in the basal state (Fig. 4*A*) in both fat-enriched diet groups compared with the control group. Insulin resistance in the brain of SFA-fed mice was further supported by the fact that insulin-mediated phosphorylation of AKT (S473) (Fig. 4*A*) was greatly diminished in this group. Interestingly, hyperinsulinemia in the SFA-fed mice led to an enhanced basal phosphorylation of AKT, which was absent in the MUFA- and chow-fed group. In addition, cortical activity was measured. As depicted in Fig. 4*B*, it became evident that mice fed an SFA-enriched diet were characterized by a decline in cortical activity in all of the frequency bands. Of note, MUFA-fed mice showed an even elevated cortical activity and suggested that MUFAs might even have a beneficial effect on the level of cortical activity.

Previously, we demonstrated that insulin given intracerebroventricularly promotes locomotor activity in lean mice whereas diet-induced obese mice were resistant. To translate insulin-mediated modulation of cortical activity to behavior, locomotion in freely moving mice after intracerebroventricular injection of insulin was monitored. An acute insulin application resulted in less pronounced locomotion in SFA-fed mice compared with MUFA- or chow-fed animals (Fig. 4*C*), suggesting that SFA enrichment negatively affects insulin sensitivity in the brain on the behavioral level.

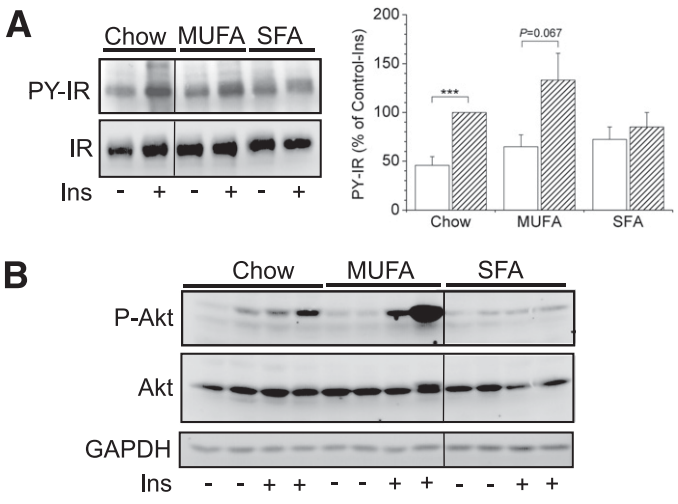

FIG. 3. Liver tissue analysis after food enrichment with MUFAs or SFAs. *A* and *B*: Attenuated phosphorylation of IR and AKT in liver tissue of SFA-fed animals. Liver lysates were separated by SDS-PAGE, and tyrosine phosphorylation (PY) of IR (*A*) and AKT (S473) (*B*) as well as protein expression of IR and AKT was analyzed after intravenous insulin injection (cross-hatched bars) in overnight-fasted MUFA-, SFA-, or chow-fed animals. Densitometric quantification of PY-IR (relative increase of PY-IR compared with insulin-stimulated control mice; n = 6–8/group; ***$P < 0.001$) is presented in histograms. Ins, insulin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Different sleep-wake patterns in MUFA- and SFA-fed mice and after intracerebroventricular application of palmitic acid. Based on recent data on metabolism and sleep and in the face of altered brain activity in mice fed a MUFA- or SFA-enriched diet, we tested whether the fat-enriched diets might lead to an altered or even disturbed sleep-wake pattern in mice. All mice exhibited a clear-cut diurnal sleep-wakefulness rhythm, and all mice spent more time awake in the 12-h darkness period than in the 12-h daylight period (Fig. 5*A*), but SFA-fed mice were significantly less awake during the dark period. Interestingly, SFA-enriched diet had a significantly stronger effect on NREM than on REM sleep in the dark period (Fig. 5*B* and *C*). Moreover, fat diet led to a significant increase in NREM sleep in the presence of decreased wakefulness compared with the control group (Fig. 5*B*). MUFA-fed mice spent 19% less time in REM sleep during the daylight period compared with controls and 24% less compared with SFA-fed mice (Fig. 5*C*). To prove the fact that SFAs indeed alter wakefulness and sleep behavior, we injected palmitic acid intracerebroventricularly into chow-fed mice shortly before the dark period. Thereby, palmitate significantly decreased the time awake during the night phase while it increased NREM sleep, as demonstrated for the SFA-fed mice (Fig. 5*D* and *E*). As expected, palmitic acid had a minor effect on REM sleep (Fig. 5*F*), as was demonstrated in the SFA-fed mice (Fig. 5*C*).

Body weight–matched SFA-fed mice are glucose intolerant and characterized by increased fasted serum insulin levels and greatly impaired cortical activity compared with MUFA-fed animals. To finally evaluate the impact of certain dietary fat on metabolic parameters and brain activity independent of body weight, body weight–matched mice (MUFA 27.2 ± 0.6 g, SFA 27.4 ± 0.8 g, control 27.0 ± 0.6 g; n = 4 per group) were compared. Mice that received the SFA-enriched diet were glucose intolerant (Fig. 6*A*) compared with the MUFA-enriched diet and mice of the control group, and this was accompanied by a slightly increased fasted blood glucose concentration (P = 0.05) (Fig. 6*B*). Further, serum insulin concentrations during the GTT were even enhanced by MUFA enrichment (Fig. 6*C*), whereas body weight–matched, SFA-fed animals tend to have increased fasted insulin concentrations (P = 0.13) (Fig. 6*D*). This was further supported by an enhanced homeostasis model assessment of insulin resistance in the SFA-fed group, demonstrating insulin resistance (Fig. 6*E*). Strikingly, basal ECoG analysis revealed a significant decrease in overall cortical activity in all of the frequency bands in the SFA-fed group, indicating that the higher content of SFAs has a negative impact on neuronal activity in the brain, whereas MUFA enrichment induced only a slight decline in the alpha and beta frequency band (Fig. 6*F*).

Body weight–matched SFA-fed animals displayed insulin resistance in the brain. To validate the impact of distinct dietary fatty acids on neuronal insulin sensitivity in vivo, ECoG was analyzed in the postinjection period after an acute intracerebroventricular insulin application. Clearly, intracerebroventricular insulin injection was accompanied by an increase in the delta, theta, alpha, and beta frequency bands as previously described (16), and this was also true for MUFA-fed animals (Fig. 7*A–D*), whereas SFA enrichment led to a significant decrease in cortical activity compared with body weight–matched controls, as

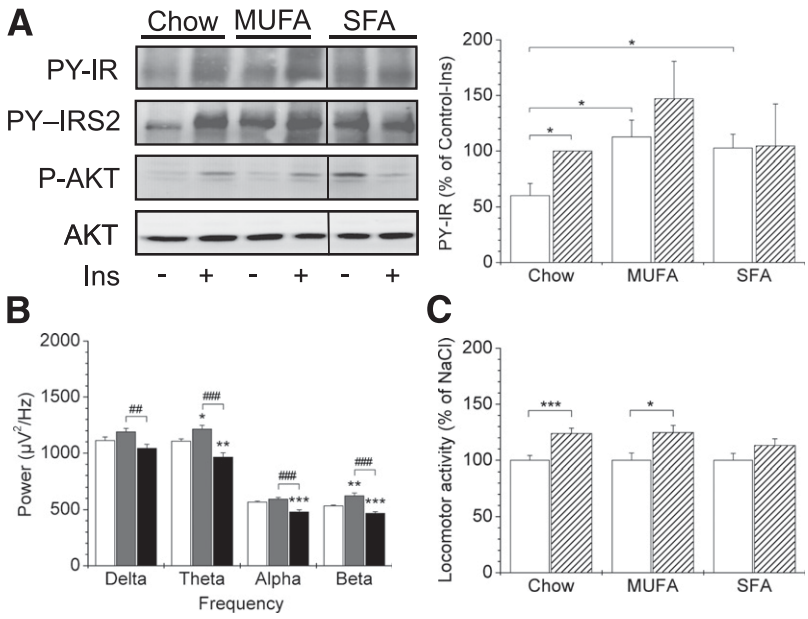

FIG. 4. Influence of SFA and MUFA enrichment on insulin sensitivity in the brain and the impact on cortical and locomotor activity. *A*: Attenuated tyrosine phosphorylation (PY) of IR, IRS2, and phosphorylation of AKT (S473) as well as protein expression of AKT was analyzed by SDS-PAGE after intravenous insulin injection in overnight-fasted MUFA-, SFA-, or chow-fed animals. Densitometric quantification of PY-IR (relative increase of PY-IR compared with insulin-stimulated control mice; n = 5–6/group; *$P < 0.05$) after intravenous insulin (cross-hatched bars) or vehicle (white bars) injection is presented in histograms. A representative blot is shown. *B*: Analysis of basal power spectral density (indicated for delta [0.5–4 Hz], theta [4–8 Hz], alpha [8–12 Hz], and beta [12–30 Hz] frequencies) using fast Fourier transformation and estimated by ECoG in MUFA- (gray bars), SFA- (black bars), and chow-fed (white bars) animals. Basal ECoG measurements were conducted during a 4-day period (n = 11–24/group; *$P < 0.05$, **$P < 0.005$, and ***$P < 0.001$ compared with controls; ##$P < 0.005$ and ###$P < 0.001$ between fat-enriched diet groups). *C*: An acute intracerebroventricular insulin application (cross-hatched bars) vs. vehicle (white bars) results in less pronounced locomotor activity in SFA-fed mice. ***$P < 0.001$ and *$P < 0.05$ to vehicle. Data are mean ± SEM. Ins, insulin.

well as MUFA-fed animals (Fig. 7*A–D*). Taken together, these data further demonstrate that insulin failed to stimulate neuronal activity in body weight–matched SFA-fed mice independent of body weight.

fMRI before and after SFA and MUFA enrichment in healthy human subjects. Our rodent data provide evidence for SFA-mediated insulin resistance in the brain; however, the relevance in humans is hard to predict. Recently, we demonstrated that brain function during rest is altered in obese subjects (13). Although fasting insulin levels were associated with brain activity in the cortex and the putamen, the impact of dietary fat on networks that represent object recognition and food regulation are unknown. To proceed in this respect, we made use of a well-established approach where we measured brain activity by fMRI after a moderate SFA or MUFA intervention in healthy human subjects. Before the intervention, there was no significant difference of fasting insulin (pmol/L) between the groups (one-way ANOVA, $P > 0.05$) (SFA enriched 38.6 ± 16.2, MUFA enriched 41.4 ± 17.7, or control group 51.1 ± 23.2). Upon fat intake, there was no significant increase in body weight, blood glucose, or lipid levels (data not shown). Dietary protocols revealed no significant difference in caloric intake (kcal/day) during the intervention between the three groups (one-way ANOVA, $P > 0.05$) (SFA enriched 2425 ± 665, MUFA enriched 2287 ± 571, or control group 2060 ± 309). All subjects underwent a resting-state fMRI before and after the 12 weeks of fat consumption. There was a significant main effect in whole-brain intrinsic activity in the hippocampus (Fig. 8*A* and *B*) and inferior parietal cortex (Fig. 8*A* and *C*) showing a decrease in intrinsic activity 3 months after the SFA-enriched diet, whereas the MUFA group displayed no difference.

DISCUSSION

The scope of this study was to characterize the effect of a moderate, isocaloric fat diet either supplemented by MUFAs (canola oil) or by SFAs (milk fat), which only differed in fat quality but not quantity. Measures of glucose homeostasis, lipid metabolism, brain activity, and sleep architecture were performed to judge the value of fat quality in the mouse brain, and this was verified in humans.

We previously showed that feeding of mice with high-fat diet resulted in impaired insulin action in the brain (16). Because of the extreme fat load rich in SFAs, the current study focused on the impact of a moderate, isocaloric fat enrichment with diets that differ in their MUFA and SFA content. Of note, both fat-enriched diets contained less polyunsaturated fatty acids compared with chow and further studies are needed to address this issue in more detail.

We show that slightly elevated, chronic SFA intake impaired peripheral glucose metabolism and led to insulin resistance, which was also verified in body weight–matched animals. Although MUFA-fed animals gained more body weight and fat mass compared with controls, they did not show signs of glucose intolerance. This is in agreement with a former study in humans where an increase in MUFAs augmented glucose-stimulated insulin secretion whereas SFAs were less effective (24). Moreover, the MUFA-enriched diet protected mice from insulin resistance, both detected in the liver and the brain. The diverse impact of MUFAs and SFAs on measures of glucose metabolism are subject to their differences in the metabolic fate, as MUFAs are oxidized more than SFAs (25), and is supported by data that showed that MUFAs are associated with a healthy lipid profile, improved insulin action, and lower blood glucose

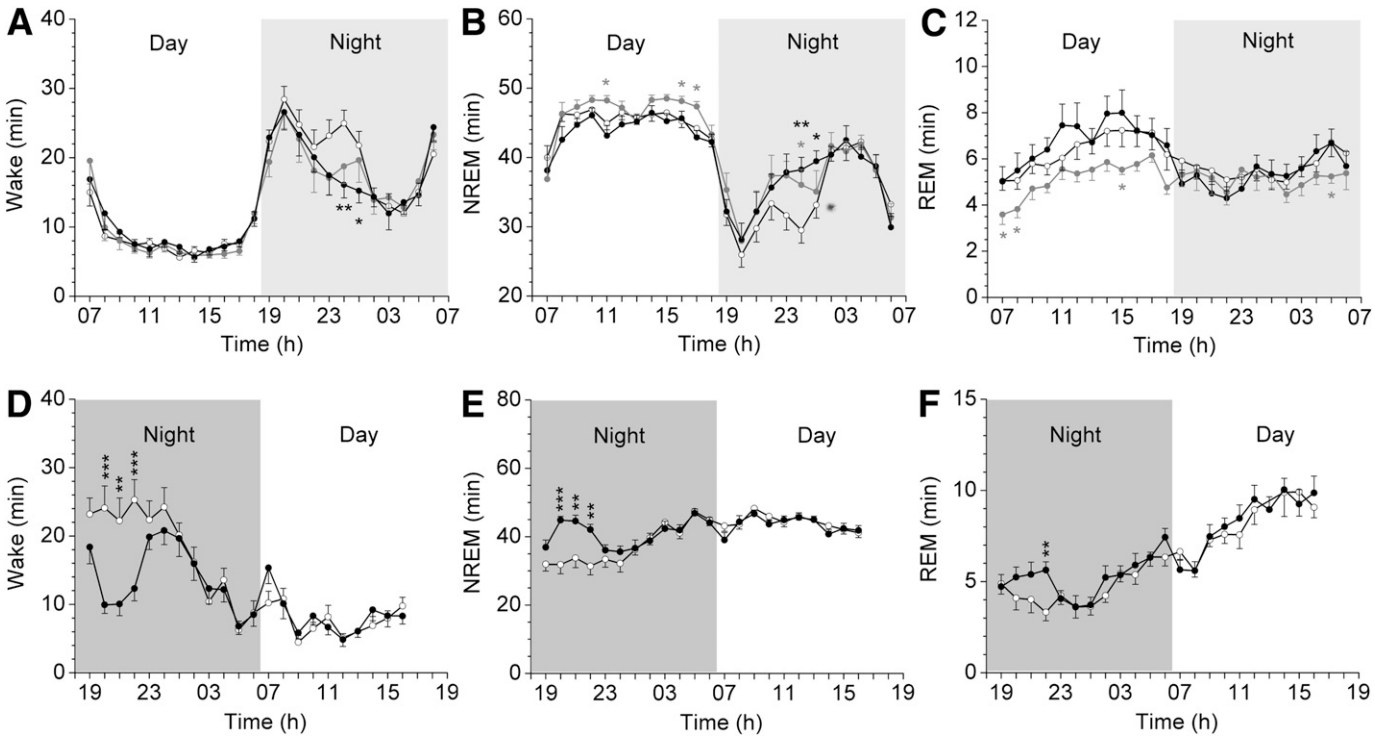

FIG. 5. Different sleep-wake patterns in MUFA- and SFA-fed mice and after intracerebroventricular application of palmitic acid. *A–C*: Diurnal variations of wakefulness (wake), NREM sleep, and REM sleep in MUFA- (gray circles), SFA- (black circles), or chow-fed (white circles) mice. Data (mean ± SEM of 11–24 animals/diet group) are expressed as minutes per hour for each hour over a 24-h episode (light on 7:00 A.M. to 7:00 P.M.). Statistically significant differences between fat-enriched diet groups and control group are depicted by asterisks (*$P < 0.05$ and **$P < 0.005$). Impact of intracerebroventricularly injected palmitic acid (225 μmol/L total, black circles) on wakefulness (wake) (*D*), NREM sleep (*E*), and REM sleep (*F*) vs. intracerebroventricularly applicated vehicle (white circles) in control mice. Data (mean ± SEM of 13–16 animals) are expressed as described above. Significant differences between intracerebroventricular palmitic acid and vehicle application are presented as follows: **$P < 0.005$ and ***$P < 0.001$.

levels (17). In this study, MUFAs were sufficient to neutralize the aversive effects of SFAs on measures of insulin sensitivity and secretion.

Further, we showed that body fat accumulation was less in mice fed the MUFA-enriched diet than in those fed the SFA-enriched diet. As both groups of mice were offered diets with the same energy intake, the difference in body fat accumulation between the two diet groups was ascribed to the value of fat quality. A study in rats demonstrated that animals fed safflower oil accumulated less body fat compared with beef tallow–fed rats due to an enhanced thermogenesis and fat oxidation (26), which might together with the increase in locomotor activity contribute to less fat mass in the MUFA-fed mice.

On the basis of our electrocorticogram recordings, we further propose that even a slight increase in SFAs was responsible for the reduction in cortical activity. Radiotelemetric analysis clearly showed that cortical activity was lower in mice fed the SFA-enriched diet compared with MUFA-enriched or chow diet. In this context, it should be noted that an intracerebroventricular insulin application into hyperinsulinemic SFA-mice even lowered brain activity, which might contribute to an impairment in activity and cognitive function as present in subjects with impaired glucose tolerance or even diabetes (27).

Electrocorticogram recordings are widely used to define sleep stages, and a large number of laboratory studies of humans as well as epidemiological data suggest that short sleep duration is associated with an increased risk of developing obesity, impaired glucose tolerance, and insulin sensitivity (28,29). Although most of these studies on food intake, sleep, and metabolic alterations are performed with nonprospective and nonobjective methods, we aimed to identify the impact of fat intake on sleep architecture in mice. Consumption of SFAs decreased wakefulness during the active night phase and increased NREM sleep. This is in agreement with other studies where an association was found for partial sleep loss and disturbance of glucose regulation that involves both reduced β-cell responsiveness and lower insulin sensitivity (30). It is known that the lateral and posterior hypothalamic areas contain neurons specifically active during wakefulness (31), and that the medial prefrontal cortex and basal forebrain are counted among wake-promoting regions (32). Although the effect of SFAs was demonstrated in these brain areas, alterations in REM sleep were only seen in the MUFA-fed animal group. The key brain structure for generating REM sleep is the brainstem and especially the pons and adjacent portions of the midbrain (33). These data suggest that the SFA-enriched diet may specifically act on brain areas controlling wakefulness and NREM sleep, whereas the MUFA-enriched diet preferentially alters mechanisms that act on REM sleep; however, the underlying mechanisms remain to be determined.

We showed that systemic insulin and insulin in the brain alters neuronal activity both in the resting state (13,34) and after stimulation with visual food stimuli in human subjects (35). In particular, the effect of insulin on neuronal activity in humans is impaired by obesity (12), genetic variances (36,37), aging (38), and increased levels of plasma fatty acids as demonstrated by MEG measurements, and

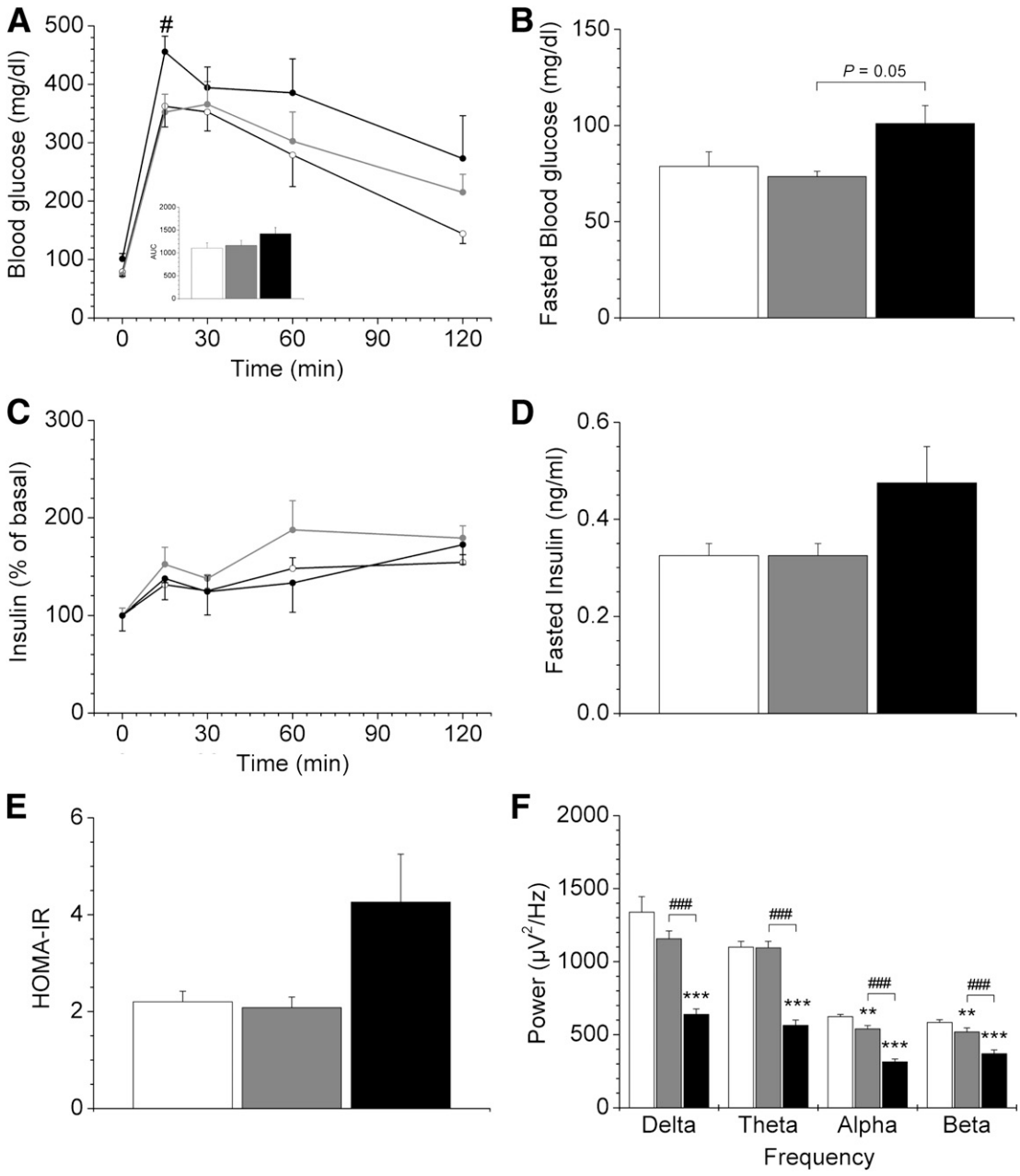

FIG. 6. Metabolic consequences of fat-enriched diets in body weight–matched C57BL/6 mice. C57BL/6 mice were weaned on MUFA-enriched (gray), SFA-enriched (black), or chow (white) diet for 8 weeks and body weight–matched animals of all diet groups were selected (n = 4/group). *A*: Intraperitoneally injected glucose during a GTT was cleared slower and less effectively in SFA-fed mice (black circles) compared with controls (white circles) and MUFA-fed animals (gray circles) (#P < 0.05 to MUFA group). *B*: Increased fasted blood glucose levels in SFA-fed mice compared with MUFA- (P = 0.05) and chow-fed animals. *C*: Slightly increased serum insulin concentration during the GTT in MUFA-fed mice (gray circles). *D*: Increased fasted serum insulin concentrations in the SFA-diet animal group (P = 0.137 to control and MUFA group). *E*: Increased homeostasis model assessment of insulin resistance (HOMA-IR) in SFA-fed mice (P = 0.1285 to control and P = 0.1137 to MUFA-fed group). *F*: Analysis of basal power spectral density (indicated for delta [0.5–4 Hz], theta [4–8 Hz], alpha [8–12 Hz], and beta [12–30 Hz] frequencies) using FFT and estimated by ECoG in MUFA- (gray bars), SFA- (black bars), and chow-fed (white bars) animals. Basal ECoG measurements were conducted during a 4-day period (P < 0.005 and ***P < 0.001 compared with controls; ###P < 0.001 between fat-enriched diet groups). Data are presented as mean ± SEM.**

further analysis using fMRI was able to identify specific brain areas related to object processing, memory, and locomotion in lean and obese subjects. By applying fMRI techniques to humans that received either SFAs or MUFAs, we determined that SFA-enriched diet led to a decrease in hippocampal and cortical activity whereas an isocaloric intake of MUFAs showed no such effect. The hippocampus is known to control brain activity in the theta frequency band (39,40). We were able to show that theta activity was correlated to locomotor activity during lifestyle intervention (41), and this was underlined by decreased theta activity in animal models of obesity and physical inactivity (16). Therefore, the human and animal data point in the same direction, where SFAs lead to specific changes in brain areas that are linked to locomotion, memory, and sleep. Of note, both enriched diets contained less polyunsaturated fatty acids compared with chow, and further studies are needed to address this issue in detail.

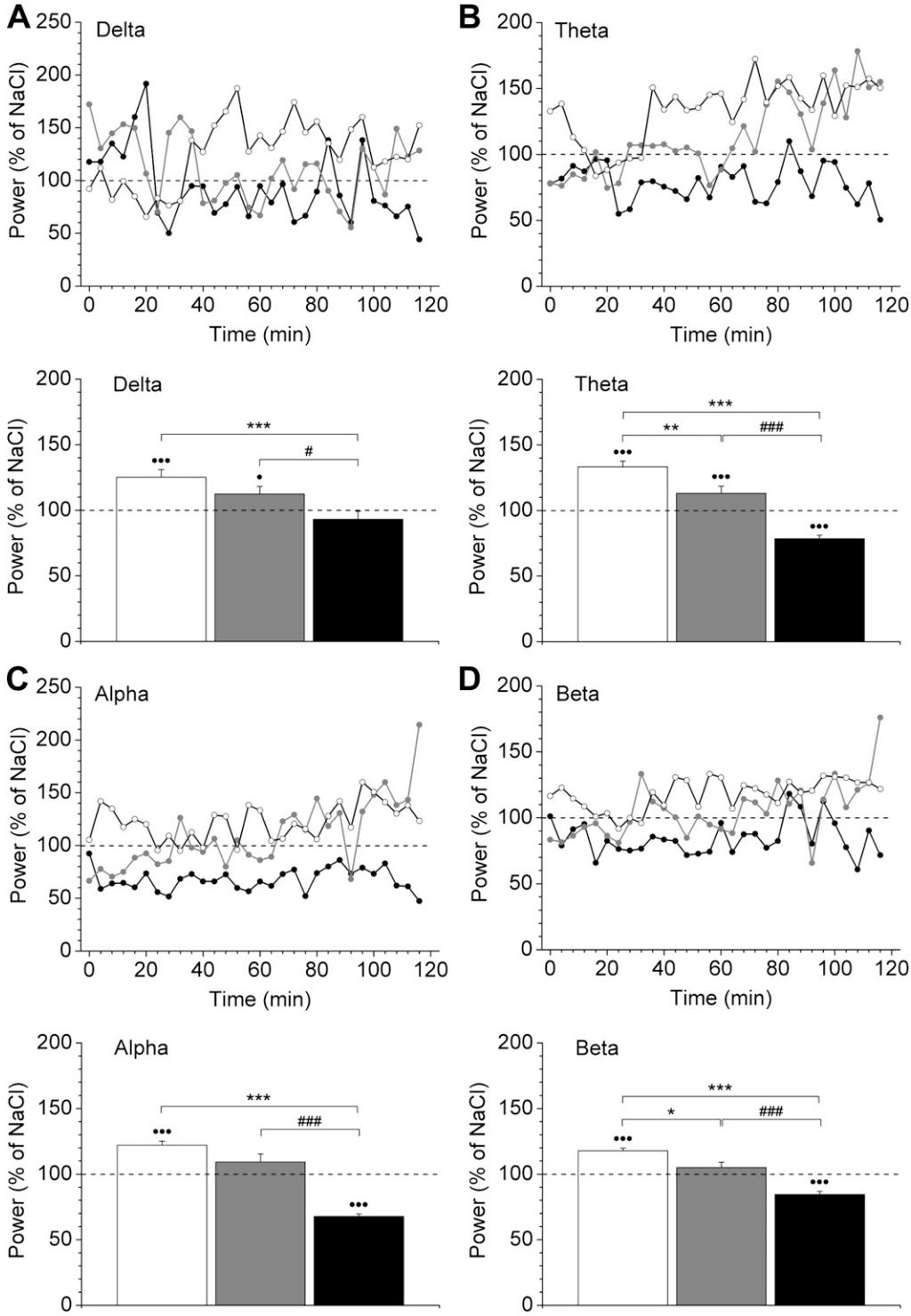

FIG. 7. Effect of SFA- and MUFA-enriched diet on intracerebroventricular insulin–stimulated cortical activity of body weight–matched mice. ECoG analysis in the 120-min postinjection period after an acute intracerebroventricular insulin application of delta (0.5–4 Hz) (*A*), theta (4–8 Hz) (*B*), alpha (8–12 Hz) (*C*), and beta (12–30 Hz) (*D*) frequencies of MUFA-fed (gray circles/bars, $n = 4$), SFA-fed (black circles/bars, $n = 3$), and control animals (white circles/bars, $n = 4$). Significance is indicated for fat-enriched diets to controls: *$P < 0.05$, **$P < 0.005$, and ***$P < 0.001$ between fat-enriched diet groups and #$P < 0.05$ and ###$P < 0.001$; and intracerebroventricular insulin to vehicle within diet group: $^{\bullet}P < 0.05$ and $^{\bullet\bullet\bullet}P < 0.001$. Data are mean ± SEM.

Together, our data strongly suggested that a MUFA-enriched diet displayed beneficial effects on glucose metabolism and locomotor and cortical activity, as well as sleep behavior even in the presence of obesity. By contrast, a comparable intake of SFAs acted as a mediator of insulin resistance in the brain, with its aversive effects on brain activity and locomotion, and was accompanied by altered sleep-wake architecture.

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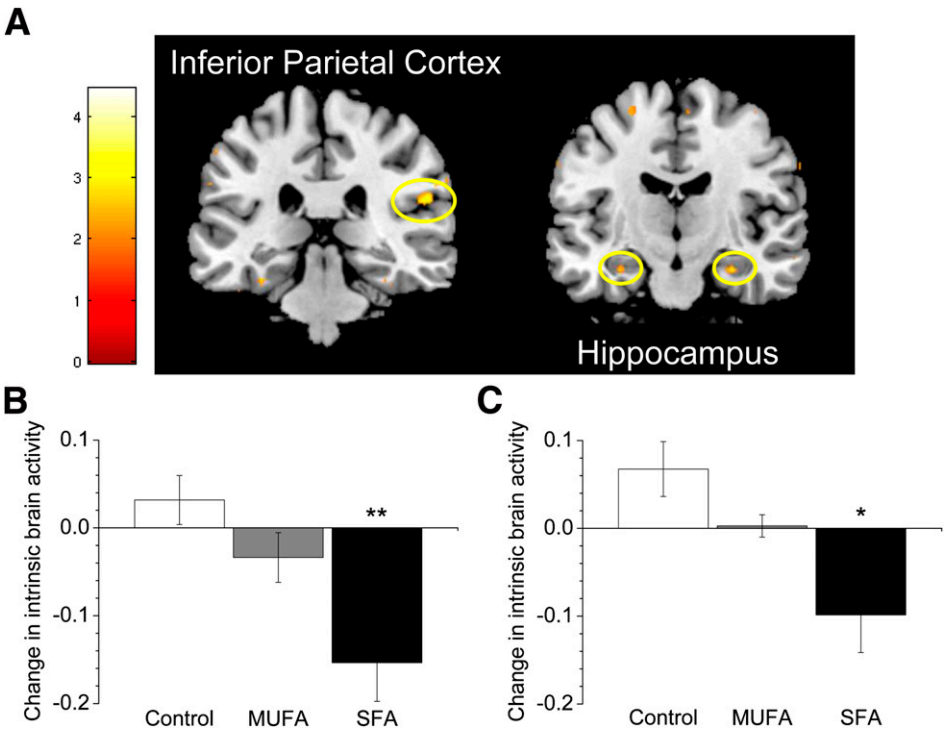

FIG. 8. Response of the human brain to MUFA- and SFA-enriched diet. *A*: Color-coded T-value map represents significant ($P < 0.05$ uncorrected, for display) voxels of decreased intrinsic brain activity 3 months after SFA-enriched yogurt consumption compared with the control group. $P < 0.001$, whole brain. Plots show change of intrinsic activity in the hippocampus (*B*) and inferior parietal cortex (*C*) 3 months after SFA-enriched, MUFA-enriched, and control diet. Only SFA-enriched diet revealed a significant decrease in hippocampal and cortical activity ($P < 0.005$ and *$P < 0.05$). Data are mean ± SEM; $n = 8$ for each group. (A high-quality color representation of this figure is available in the online issue.)**

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No potential conflicts of interest relevant to this article were reported.

T.S. and A.M.H. designed the study, researched data, and wrote the manuscript. C.K. researched data of the human intervention study. S.K. performed and analyzed the fMRI analysis in the human subjects. M.B. performed the fatty acid analysis of edible fats. C.R. accomplished in vivo studies. S.B. and M.H. contributed to the analysis and interpretation of the sleep parameters. J.M. arranged the MRI analysis and reviewed and edited the manuscript. F.S. and H.-U.H. reviewed and edited the manuscript. V.S. supervised the fatty acid analysis and contributed to the scientific approach and reviewed and edited the manuscript. H.P. designed the human intervention study and reviewed and edited the manuscript. A.F. researched data of the human intervention study and reviewed and edited the manuscript. T.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. Haag M, Dippenaar NG. Dietary fats, fatty acids and insulin resistance: short review of a multifaceted connection. Med Sci Monit 2005;11:RA359–RA367
2. Manco M, Bertuzzi A, Salinari S, et al. The ingestion of saturated fatty acid triacylglycerols acutely affects insulin secretion and insulin sensitivity in human subjects. Br J Nutr 2004;92:895–903
3. Field AE, Willett WC, Lissner L, Colditz GA. Dietary fat and weight gain among women in the Nurses' Health Study. Obesity (Silver Spring) 2007;15:967–976
4. Maedler K, Oberholzer J, Bucher P, Spinas GA, Donath MY. Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. Diabetes 2003;52:726–733
5. Eitel K, Staiger H, Brendel MD, et al. Different role of saturated and unsaturated fatty acids in beta-cell apoptosis. Biochem Biophys Res Commun 2002;299:853–856
6. Dobbins RL, Szczepaniak LS, Myhill J, et al. The composition of dietary fat directly influences glucose-stimulated insulin secretion in rats. Diabetes 2002;51:1825–1833
7. Eitel K, Staiger H, Rieger J, et al. Protein kinase C delta activation and translocation to the nucleus are required for fatty acid-induced apoptosis of insulin-secreting cells. Diabetes 2003;52:991–997
8. Weigert C, Brodbeck K, Staiger H, et al. Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes through proteasome-dependent activation of nuclear factor-kappaB. J Biol Chem 2004;279:23942–23952
9. Perseghin G, Ghosh S, Gerow K, Shulman GI. Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study. Diabetes 1997;46:1001–1009
10. Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of fatty acids and insulin action. Ann N Y Acad Sci 2002;967:183–195
11. Tschritter O, Preissl H, Hennige AM, et al. The insulin effect on cerebrocortical theta activity is associated with serum concentrations of saturated nonesterified fatty acids. J Clin Endocrinol Metab 2009;94:4600–4607
12. Tschritter O, Preissl H, Hennige AM, et al. The cerebrocortical response to hyperinsulinemia is reduced in overweight humans: a magnetoencephalographic study. Proc Natl Acad Sci USA 2006;103:12103–12108

13. Kullmann S, Heni M, Veit R, et al. The obese brain: Association of body mass index and insulin sensitivity with resting state network functional connectivity. Hum Brain Mapp. 21 April 2011 [Epub ahead of print]
14. Ropelle ER, Flores MB, Cintra DE, et al. IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS Biol 2010;8:e1000465
15. Milanski M, Degasperi G, Coope A, et al. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J Neurosci 2009;29:359–370
16. Hennige AM, Sartorius T, Lutz SZ, et al. Insulin-mediated cortical activity in the slow frequency range is diminished in obese mice and promotes physical inactivity. Diabetologia 2009;52:2416–2424
17. Gillingham LG, Harris-Janz S, Jones PJ. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids 2011;46:209–228
18. Hennige AM, Sartorius T, Tschritter O, et al. Tissue selectivity of insulin detemir action in vivo. Diabetologia 2006;49:1274–1282
19. Hennige AM, Burks DJ, Ozcan U, et al. Upregulation of insulin receptor substrate-2 in pancreatic beta cells prevents diabetes. J Clin Invest 2003; 112:1521–1532
20. Hennige AM, Lehmann R, Weigert C, et al. Insulin glulisine: insulin receptor signaling characteristics in vivo. Diabetes 2005;54:361–366
21. Machann J, Thamer C, Schnoedt B, et al. Standardized assessment of whole body adipose tissue topography by MRI. J Magn Reson Imaging 2005;21:455–462
22. Laufs H, Krakow K, Sterzer P, et al. Electroencephalographic signatures of attentional and cognitive default modes in spontaneous brain activity fluctuations at rest. Proc Natl Acad Sci USA 2003;100:11053–11058
23. Zuo XN, Di Martino A, Kelly C, et al. The oscillating brain: complex and reliable. Neuroimage 2010;49:1432–1445
24. Beysen C, Karpe F, Fielding BA, Clark A, Levy JC, Frayn KN. Interaction between specific fatty acids, GLP-1 and insulin secretion in humans. Diabetologia 2002;45:1533–1541
25. Bergouignan A, Momken I, Schoeller DA, Simon C, Blanc S. Metabolic fate of saturated and monounsaturated dietary fats: the Mediterranean diet revisited from epidemiological evidence to cellular mechanisms. Prog Lipid Res 2009;48:128–147
26. Shimomura Y, Tamura T, Suzuki M. Less body fat accumulation in rats fed a safflower oil diet than in rats fed a beef tallow diet. J Nutr 1990;120:1291–1296
27. Gatto NM, Henderson VW, St John JA, McCleary C, Hodis HN, Mack WJ. Metabolic syndrome and cognitive function in healthy middle-aged and older adults without diabetes. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn 2008;15:627–641
28. Knutson KL, Van Cauter E, Zee P, Liu K, Lauderdale DS. Cross-sectional associations between measures of sleep and markers of glucose metabolism among subjects with and without diabetes: the Coronary Artery Risk Development in Young Adults (CARDIA) sleep study. Diabetes Care 2011; 34:1171–1176
29. Chaput JP, Després JP, Bouchard C, Tremblay A. Association of sleep duration with type 2 diabetes and impaired glucose tolerance. Diabetologia 2007;50:2298–2304
30. Van Cauter E, Holmback U, Knutson K, et al. Impact of sleep and sleep loss on neuroendocrine and metabolic function. Horm Res 2007;67(Suppl. 1): 2–9
31. Vanni-Mercier G, Sakai K, Jouvet M. Specific neurons for wakefulness in the posterior hypothalamus in the cat. C R Acad Sci III 1984;298:195–200 [in French]
32. Datta S, Maclean RR. Neurobiological mechanisms for the regulation of mammalian sleep-wake behavior: reinterpretation of historical evidence and inclusion of contemporary cellular and molecular evidence. Neurosci Biobehav Rev 2007;31:775–824
33. Crochet S, Sakai K. Dopaminergic modulation of behavioral states in mesopontine tegmentum: a reverse microdialysis study in freely moving cats. Sleep 2003;26:801–806
34. Stingl KT, Kullmann S, Guthoff M, Heni M, Fritsche A, Preissl H. Insulin modulation of magnetoencephalographic resting state dynamics in lean and obese subjects. Front Syst Neurosci 2010;4:157
35. Guthoff M, Stingl KT, Tschritter O, et al. The insulin-mediated modulation of visually evoked magnetic fields is reduced in obese subjects. PLoS ONE 2011;6:e19482
36. Tschritter O, Preissl H, Yokoyama Y, Machicao F, Häring HU, Fritsche A. Variation in the FTO gene locus is associated with cerebrocortical insulin resistance in humans. Diabetologia 2007;50:2602–2603
37. Tschritter O, Haupt A, Preissl H, et al. An obesity risk SNP (rs17782313) near the MC4R gene is associated with cerebrocortical insulin resistance in humans. J Obes. 3 June 2011 [Epub ahead of print]
38. Tschritter O, Hennige AM, Preissl H, et al. Insulin effects on beta and theta activity in the human brain are differentially affected by ageing. Diabetologia 2009;52:169–171
39. Sławińska U, Kasicki S. The frequency of rat's hippocampal theta rhythm is related to the speed of locomotion. Brain Res 1998;796:327–331
40. Ekstrom AD, Caplan JB, Ho E, Shattuck K, Fried I, Kahana MJ. Human hippocampal theta activity during virtual navigation. Hippocampus 2005; 15:881–889
41. Tschritter O, Preissl H, Hennige AM, et al. High cerebral insulin sensitivity is associated with loss of body fat during lifestyle intervention. Diabetologia 2012;55:175–182