Hypothalamic damage is associated with inflammatory markers and worse cognitive performance in obese subjects

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Context: Growing evidence implicates hypothalamic inflammation in the pathogenesis of diet-induced obesity and cognitive dysfunction in rodent models. Few studies have addressed the association between obesity and hypothalamic damage in humans and its relevance.

Objective: To determine markers of obesity-associated hypothalamic damage on diffusion tensor imaging (DTI) and to determine whether DTI-metrics are associated with performance on cognitive testing.

Design and Participants: This cross-sectional study analyzed DTI-metrics (primary (λ_1), secondary (λ_2), and tertiary (λ_3) eigenvalues; fractional anisotropy (FA); and mean diffusivity (MD)) in the hypothalamus of 24 consecutive middle-aged obese subjects (13 women; 49.8 $\pm$ 8.1 years; body mass index [BMI] 43.9 $\pm$ 0.92 Kg/m^2) and 20 healthy volunteers (10 women; 48.8 $\pm$ 9.5 years; BMI 24.3 $\pm$ 0.79 Kg/m^2).

Outcome: measures: Hypothalamic damage assessed by DTI-metrics and cognitive performance evaluated by neuropsychological test-battery.

Results: λ_1 values in the hypothalamus were significantly lower in obese subjects ($P<0.0001$). The sensitivity, specificity, and positive and negative predictive values for obesity-associated hypothalamic damage by $\lambda_1<1.072$ were 75%, 87.5%, 83.3%, and 80.7%, respectively. Patients with hypothalamic $\lambda_1<1.072$ had higher values of BMI, fat mass, inflammatory markers, carotid-intima media thickness, and hepatic steatosis and lower scores on cognitive tests. Combined BMI and alanine aminotransferase had the strongest association with hypothalamic damage reflected by $\lambda_1<1.072$ (AUC=0.89).

Conclusions: DTI detects obesity-associated hypothalamic damage associated with inflammatory markers and worse cognitive performance. This study highlights the potential utility of λ_1 as a surrogate marker of obesity-associated hypothalamic damage.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received June 17, 2014. Accepted November 20, 2014.

Abbreviations:

Little is known regarding how brain systems that promote weight stability are altered in obesity (1). Growing evidence implicates immune-cell-mediated tissue inflammation as a mechanism linking obesity to insulin resistance and comorbidities such as cardiovascular disease (2). In animals, high-fat diets lead to increased inflammatory signaling in body-weight-regulating areas of the hypothalamus critical for energy homeostasis, contributing to leptin resistance and weight gain, (3–5) with reactive gliosis in the hypothalamic arcuate nucleus and median eminence (6).

Magnetic resonance imaging (MRI) can help study hypothalamic cytoarchitecture. Lee et al (7) found high-fat diets increased T2 relaxation times in rats' mediobasal hypothalamus, reflecting increased astrocytosis and microglial accumulation. Conventional MRI sequences, such as T2-weighted sequences, show signal changes in brain tissue related to pathophysiological phenomena such as astrocytosis or microglial accumulation.

Diffusion tensor imaging (DTI), an MRI technique that uses information on the predominant direction and degree of water diffusion, reveals microstructural brain-tissue damage after ischemia, infection, or inflammation (8). The degree that diffusion is direction-dependent (anisotropy) depends on the level of tissue organization and integrity and on the degree of freedom of water diffusion along axons, across cell membranes, and in extracellular space. The magnitude of diffusion characteristics can be calculated as eigenvectors/eigenvalues. Three eigenvalues are used to calculate fractional anisotropy (FA), a DTI-metric of the relative difference in water diffusivities along multiple axes: λ_1 (principal axis or axial diffusivity), representing water motion along axons, and λ_2 and λ_3 (shorter perpendicular axes or radial diffusivity), representing diffusion across the axon (8). Gray-matter density and microstructure can be assessed by mean diffusivity (MD) or other DTI-metrics (9). DTI can provide new and more precise information about possible changes in the microstructure of the hypothalamus.

Few studies have evaluated brain-tissue diffusion in obesity. Hypothalamic apparent diffusion coefficients (ADC) are higher in obese than nonobese subjects, (10) suggesting microstructural damage. Body mass index (BMI) correlates with FA in cerebral white matter (11–13). Hypothalamic gray-matter loss correlates with cognitive impairment in multiple sclerosis (MS); (14) however, obesity-associated hypothalamic damage's impact on cognitive function is poorly understood. We assessed obesity-associated hypothalamic damage by DTI and examined its impact on cognitive performance.

Subjects and Methods

Subjects

From January to September 2012, we recruited 24 consecutive obese subjects undergoing MRI in a study evaluating the role of intestinal microflora in nonalcoholic fatty liver disease. We included subjects aged 30 to 65 years with BMI > 30 kg/m^2 and excluded those with systemic disease, infection in the previous month, serious chronic illness, ethanol intake > 20g per day, or taking medications that might interfere with insulin action. We enrolled 20 healthy subjects (BMI < 25 kg/m^2) as controls. Control subjects were normotensive and selected on the basis of similarity in age and sex compared to obese subjects and the absence of a personal history of inflammatory diseases or current drug treatment. The institutional review board (IRB) approved the study, and all subjects provided informed written consent.

Study Protocol

Patients underwent anthropometric measurements, abdominal (15) and vascular (16) ultrasonography, and, after 8 hours fasting, provided blood for measurement of plasma lipids, glucose, and insulin. Glucose and lipid levels were determined by standard laboratory methods. Subjects underwent standard 75 g oral glucose tolerance tests, with determination of glucose and insulin at 0, 30, 60, 90, and 120 minutes. The area under the curve (AUC) for glucose and insulin was then calculated using the trapezoidal method. Serum insulin was measured by radioimmunoassay (RIA). Insulin resistance was determined by the homeostasis model assessment of insulin resistance (HOMA-IR). Serum alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) levels were determined using enzymatic methods. Serum lipopolysaccharide-binding protein (LBP) levels were measured by human-LBP enzyme-linked immunosorbent assay (ELISA) (HyCult Biotechnology, Huden, the Netherlands).

Body Composition

Fat mass was determined via dual energy x-ray absorptiometry (GE Lunar iDXA; Milwaukee, WI).

Magnetic resonance imaging

All patients underwent MRI on a 1.5T Intera scanner (Philips Healthcare, Best, the Netherlands). The protocol included axial fluid-attenuated inversion recovery (FLAIR) and DTI sequences. For FLAIR, the parameters were repetition time (TR), 7569msec; echo time (TE), 115msec; inversion delay, 2200msec; flip angle,90°; matrix,256 × 192; section thickness, 3 mm; interslice gap, 1 mm; and FOV, 230 × 180 mm. DTI data were acquired using single-shot EPI sequences with the SENSE parallel imaging scheme to reduce scanning time and minimize artifacts. Diffusion-sensitized gradients were applied along 15 noncollinear directions with a b-value of 1000 seconds/mm^2. Other acquisition parameters were TR/TE,6795/72ms; FOV,23 × 23cm; and matrix size,112 × 112. Forty-five contiguous 3-mm axial sections covering the brain were acquired parallel to the anteroposterior line. DTI scanning took 3 minutes.

Data Processing

DTI images were coregistered; a neuroradiologist blinded to clinical information used dedicated software (Olea SphereV.2.0,La Ciotat,France) to place free-hand regions of in-

terest (ROIs) in the right and left sides of the hypothalamus using validated landmarks (17). Primary(λ_1), secondary(λ_2), and tertiary(λ_3) eigenvalues, FA, and MD were calculated. DTI measurements were repeated 1 month later to assess intraobserver agreement. Values of DTI-metrics were obtained by averaging all voxels within the ROI (Supplemental Figure 1). Mean values from the each side were averaged for statistical analyses. Mean ROI area was 5 ± 1.5 mm^2. The hypothalamus was visually inspected for abnormalities on FLAIR.

Neuropsychological assessment

Cognitive performance was assessed with the Wechsler Adult Intelligence Scale (WAIS) third edition (vocabulary, digit span, picture completion, and picture arrangement subsets). Attention and executive functions were assessed with the Trail Making Test (parts A and B) and modified Wisconsin Card Sorting test. Processing speed and selective attention were assessed with the Stroop Neuropsychological Screening Test. Risk-taking and impulsive behaviors were assessed with the Iowa Gambling Task.

Statistical analysis

Results are expressed as means±standard deviation for continuous variables and as frequencies for categorical variables. To determine differences with respect to the presence of obesity, we used Student's *t* test for quantitative variables and the χ^2 test for qualitative variables. To calculate the λ_1 cutoff to predict hypothalamic damage, we used receiver operator characteristic curves. For each variable, differences according to the prespecified hypothalamic λ_1 cutoff were tested using Student's *t* test or the χ^2 test. Multivariate logistic regression and stepwise regression analyses were used to identify independent predictors of hypothalamic damage. The intraclass correlation coefficient (ICC) was used to classify intraobserver reliability as fair (ICC = 0.5–0.7), good (0.7–0.9), or almost perfect (>0.90). Statistical significance was set at $P < .05$. Statistical analyses were performed with Minitab, Version 16.2.1 (Minitab, State College, PA).

Results

Supplemental Table 1 reports subjects' characteristics. The intraobserver consistency for DTI measurements in the hypothalamus was good (ICC = 0.809). Mean λ_1 hypothalamic values were lower in obese subjects ($P < .001$). The mean hypothalamic λ_1 cutoff that best discriminated obese from control subjects was 1.072 (Figure 1). The sensitivity, specificity, and positive predictive value (PPV) and negative predictive value (NPV) for hypothalamic damage by mean $\lambda_1 < 1.072$ were 75%, 87.5%, 83.3%, and 80.7%, respectively (AUC:0.854; 95%CI:0.742–0.96). No significant differences were found for λ_2, λ_3, FA, or MD. After adjustment for age and sex, subjects with hypothalamic $\lambda_1 < 1.072$ had significantly increased values of BMI, fat mass, ALT, GGT, C-reactive protein (CRP), LBP, carotid intima-media thickness, and prevalence of hepatic steatosis (Table 1). Interestingly, when studying obese and control subjects separately, the hypothalamic λ_1 correlated negatively with fat mass within obese subjects (r=-0.51, $P = .01$) but not in nonobese subjects (r = 0.05, $P = .8$). We also found significant differences with respect to total fat mass according to a hypothalamic λ_1 cutpoint of 1.072 (Table 1). In a stepwise linear regression analysis, fat mass ($P < .0001$) and HOMA-IR value ($P = .037$), but not age, independently contributed to 22.4% of λ_1 hypothalamic variance. Finally, while fat mass ($P < .0001$) and HOMA-IR ($P = .011$) contributed to 56% of hypothalamic λ_1 variance among women, this was not observed in men.

The BMI cutoff that best discriminated hypothalamic damage was 34 kg/m^2, yielding 80.7% sensitivity, 83.3% specificity, 87.5% PPV, and 75% NPV (AUC:0.829; $P <$

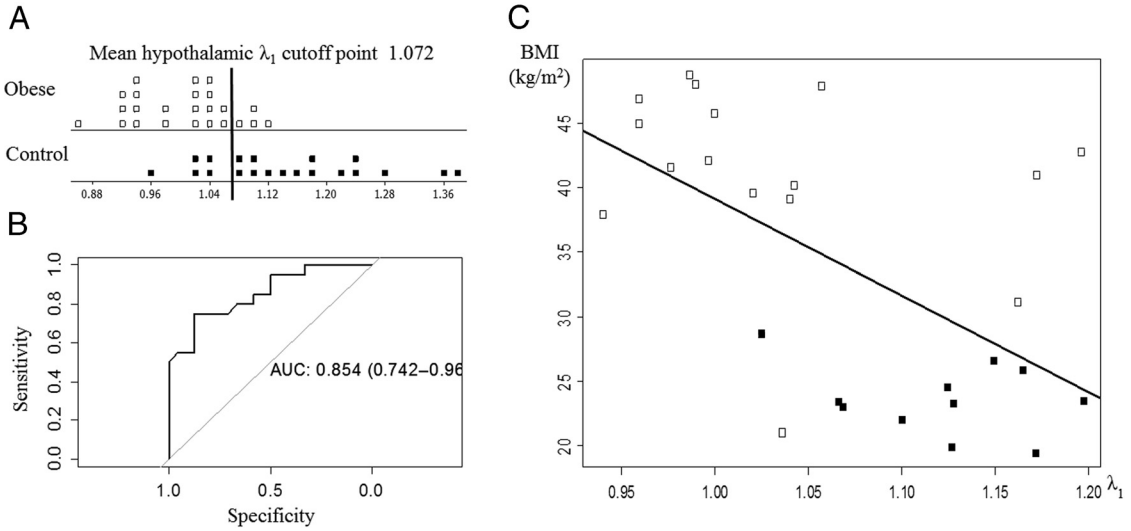

Figure 1. A, Obese subjects had lower hypothalamic primary eigenvalues (λ1) ($P < .001$). B, To calculate the λ1 cutoff to predict hypothalamic damage, we used receiver operator characteristic curves. C, Patients with hypothalamic $\lambda_1 < 1.072$ had higher BMI. The equation of the linear regression analysis was BMI = 114.32–75.13(λ_1), r = 0.58, $P = .001$.

Table 1. Univariate associations of mean hypothalamic λ_1 DTI-metric and clinical, laboratory, ultrasonographic data and neuropsychological tests in all 44 individuals

	Hypothalamic mean $\lambda_1 < 1.072$ ($n = 26$)	Hypothalamic mean $\lambda_1 > 1.072$ ($n = 18$)	p-value
Gender(male/female)	13/13	8/10	0.717
Age(years)	49.385 (8.668)	49.444 (9.018)	0.983
BMI(kg/m^2)	40.385 (9.212)	27.322 (7.687)	$<0.001^{**}$
Waist circumference(cm)	108.712 (38.198)	91.222 (18.252)	0.050
Fat mass (kg)	42.82 (19.8)	27.52 (13.9)	0.005^{**}
Systolic blood pressure(mmHg)	135.5 (20.506)	128.778 (19.368)	0.276
Diastolic blood pressure(mmHg)	74.923 (12.579)	73.778 (14.132)	0.784
Current smoking(no/former/yes)	12/6/8	9/7/2	0.254
Total cholesterol(mg/dl)	187.462 (42.296)	209 (34.626)	0.071
HDL--cholesterol(mg/dl)	51.231 (14.949)	58.889 (15.733)	0.114
Triglyceride(mg/dl)*	4.442 (0.577)	4.401 (0.479)	0.800
Glucose AUC(mg/dl/min)	17 062.462 (5255.036)	14 964.167 (4223.845)	0.151
Insulin AUC(mU/liter/min)	7650.358 (6131.49)	8791.389 (8591.502)	0.635
HOMA-IR(mean ± SD)	3.473 (2.747)	2.512 (3.502)	0.337
Aspartate aminotransferase(U/liter)	18.077 (12.977)	7.765 (12.794)	0.015^{**}
Alanine aminotransferase(U/liter)	30.577 (13.822)	18.722 (3.77)	$<0.001^{**}$
γ-glutamyltransferase(U/liter)	32.077 (23.394)	24.722 (23.649)	0.315
Ultrasensitive CRP(mg/dl)	0.715 (0.859)	0.289 (0.373)	0.031^{**}
LBP(ng/liter)	29.003 (12.417)	20.502 (10.559)	0.019^{**}
Hepatic steatosis(no/yes)	7/19	15/3	0.001^{**}
cIMT(mm)	0.78 (0.165)	0.664 (0.197)	0.049^{**}
WAIS-III vocabulary subtest	38.533 (12.27)	46.182 (4.895)	0.041^{**}
WAIS-III digit span subtest	14.2 (3.59)	16.333 (3.676)	0.143
WAIS-III picture arrangement subtest	34.267 (2.52)	33.583 (3.63)	0.586
WAIS-III picture completion subtest 1	18.533 (8.943)	17.792 (7.344)	0.815
WAIS-III picture completion subtest 2	18.233 (8.664)	18.542 (6.72)	0.918
Trail making test(part A)	49.267 (18.591)	29.909 (11.167)	0.003^{**}
Trail making test(part B)	107.533 (28.2)	107.909 (68.12)	0.987
Stroop Neuropsychological Screening Test	56.2 (8.385)	55.909 (9.268)	0.935
Iowa Gambling Task	47.4 (6.243)	44.818 (8.658)	0.412
Wisconsin Card Sorting Test	54 (5.774)	11.6 (1.265)	0.001^{**}

Abbreviations: AUC, area under curve; BMI, body mass index; cIMT, carotid intima-media thickness; CRP, C-reactive protein; HDL, high density lipoprotein; HOMA-IR, homeostasis model of assessment-insulin resistance; LBP, lipopolysaccharide-binding protein; WAIS-III, Wechsler Adult Intelligence Scale–Third Edition. *this variable was log transformed. **Parameters with $P < 0.05$.

.001). The ALT cutoff that best discriminated hypothalamic damage was 23.5 U/l, yielding 69.2% sensitivity,

94.4% specificity, 94.7% PPV, and 68% NPV (AUC: 0.829;$P = .005$). The LBP cutoff that best discriminated hypothalamic damage was 24.1ng/l, yielding 73% sensitivity, 83.3% specificity, 86.3% PPV, and 68.1% NPV (AUC:0.720;$P = .031$)(Supplemental Table 2).

Hypothalamic gray-matter loss defined as $\lambda_1 < 1.072$ significantly correlated with worse cognitive performance (Table 1).

Discussion

Obesity is known to be associated with gray and white matter changes, (10–13) and with hypothalamic inflammation in experimental models (3–5, 7). Obesity is also well known to affect cognitive performance (18, 19). To our knowledge, this would be the first study exploring cognitive function in association with hypothalamic inflammation in obese subjects. In this cross-sectional study, lower mean hypothalamic λ_1 in obese subjects suggests neuronal damage. Interestingly, the strongest predictor of hypothalamic damage in the multivariate analysis was combined BMI and ALT. Fat mass and insulin resistance contributed independently to hypothalamic λ_1 variance. Of note, even within obese subjects, fat mass was significantly associated with hypothalamic λ_1, and this was especially remarkable among women. Mueller et al had previously reported sex-dependent influences of obesity on cerebral white matter (11). Other factors associated with altered hypothalamic microstructure on DTI were the inflammatory markers CRP, LBP, and carotid intima-media thickness. These findings support the idea that liver and brain damage are interrelated with vascular status in obesity-associated inflammation, contributing to comorbidities such as insulin resistance and cardiovascular disease.

Rapid-onset inflammation and reactive gliosis in the hypothalamus of rodents consuming high-fat diets represents a response to neuronal injury, probably induced by ischemia or excitotoxicity (3–5, 7). Detecting and quantifying obesity-related hypothalamic damage in vivo represents a challenge. Longer hypothalamic T2 relaxation times in mice on high-fat diets probably reflect increased numbers of glial cells, reactive astrocytosis, and/or decreased neuronal populations (7). When visual assessment detected no signal-intensity abnormalities in a study of 34 human subjects, Thaler et al (6) devised a quantitative approach to compare mean signal intensity within ROIs in the mediobasal hypothalamus using ratios with ROIs in adjacent amygdalar tissue to show that obese individuals had higher T2 hyperintensity in the mediobasal hypothalamus suggestive of gliosis.

DTI is promising for in vivo brain-damage assessment. DTI-metrics express the degree of diffusion anisotropy from obstacles limiting molecular movement in some directions in brain tissue. We found that λ_1, rather than FA, accurately discriminated obese subjects based on hypothalamic microstructural changes. DTI-metrics probably differ with the stage of disease. As FA is obtained from eigenvalues representing relative differences in water diffusivities along multiple axes, subtle alterations in microarchitecture could be expressed first as a decrease in λ_1, because the largest eigenvalue will point in the direction of the largest diffusion coefficient; thus, λ_1 could detect early changes in hypothalamic glia better than FA, which would decrease later. Altered DTI-metrics do not prove gliosis, because edema or tumor would yield similar results. However, we excluded subjects with evidence of neurological abnormalities, making these alternative explanations unlikely. Furthermore, as in animal models, the signal suggestive of gliosis correlated positively with BMI but not with age or sex. The association with obesity was strong, lending translational relevance to preclinical studies by suggesting that hypothalamic neuronal injury and associated gliosis are also present in human obesity; therefore, DTI is a promising technique to detect and follow up these endpoints.

Our correlations between hypothalamic structural changes and cognitive tests agree with studies showing worse executive function after hypothalamus-pituitary-adrenal axis dysfunction (20). The hypothalamus is crucial for emotional regulation and vital functions, but it also has numerous bidirectional connections with other subcortical and cortical areas; (20, 21) moreover, animal and human studies demonstrate projections from the hypothalamus to all prefrontal regions (22). The hypothalamus is also linked to the hippocampus, amygdala, and insular cortex—regions usually associated with cognitive and executive functions (21). This complex system enables close integration of cognitive and emotional information and might explain the hypothalamus's role in cognitive performance.

Our study has limitations, including the relative small sample size. Future studies with larger samples will allow regression analyses to assess associations in greater detail. The hypothalamus' size and location make it susceptible to motion distortion and partial-volume effects. However, the good intraobserver reliability for DTI-measurements and their correlation with cognitive performance suggest DTI detects obesity-induced hypothalamic damage, although this must be confirmed histologically in experimental models. Monitoring hypothalamic changes over time might help define the course and potential reversibility of hypothalamic damage. A longitudinal study, following primary hypothalamic eigenvalues and metabolic

parameters over time in patients at high risk for obesity is required to substantiate these findings. We cannot say whether hypothalamic damage is involved in the pathogenesis of obesity or is simply a marker of obesity or associated conditions like HOMA-IR or obstructive sleep apnea (OSA). We collected no data on years' education, an important predictor of cognitive performance.

In conclusion, our preliminary data indicate that λ_1 could be useful for assessing hypothalamic damage in obese individuals. Given that the hypothalamus plays a pivotal role in the control of food intake and energy expenditure, this approach may offer new perspectives in further studies aimed at detecting obesity-induced hypothalamic damage and in attempts to predict the response to different treatments.

Acknowledgments

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This work was supported by .

Disclosure Summary: The authors have nothing to disclosure

References

1. **Myers MG Jr., Leibel RL, Seeley RJ, Schwartz MW.** Obesity and leptin resistance: distinguishing cause from effect. *Trends Endocrinol Metab*. 2010;21(11):643–651.
2. **Lumeng CN, Saltiel AR.** Inflammatory links between obesity and metabolic disease. *J Clin Invest*. 2011;121:2111–2117.
3. **De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ, Velloso LA.** Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology*. 2005;146:4192–4199.
4. **Thaler JP, Schwartz MW.** Minireview: Inflammation and obesity pathogenesis: the hypothalamus heats up. *Endocrinology*. 2010; 151:4109–4115.
5. **Posey KA, Clegg DJ, Printz RL, Byun J, Morton GJ, Vivekanandan-Giri A, Pennathur S, Baskin DG, Heinecke JW, Woods SC, Schwartz MW, Niswender KD.** Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. *Am J Physiol Endocrinol Metab*. 2009;296:E1003–E1012.
6. **Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschöp MH, Schwartz MW.** Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest*. 2012;122:153–162.
7. **Lee D, Thaler JP, Berkseth KE, Melhorn SJ, Schwartz MW, Schur EA.** Longer T(2) relaxation time is a marker of hypothalamic gliosis in mice with diet-induced obesity. *Am J Physiol Endocrinol Metab*. 2013;304:E1245–1250.
8. **Rovaris M, Agosta F, Pagani E, Filippi M.** Diffusion tensor MR imaging. *Neuroimaging Clin N Am*. 2009;19:37–43.
9. **Kantarci K, Senjem ML, Avula R, Zhang B, Samikoglu AR, Weigand SD, Przybelski SA, Edmonson HA, Vemuri P, Knopman DS, Boeve BF, Ivnik RJ, Smith GE, Petersen RC, Jack CR Jr.** Diffusion tensor imaging and cognitive function in older adults with no dementia. *Neurology*. 2011;77:26–34.
10. **Alkan A, Sahin I, Keskin L, Cikim AS, Karakas HM, Sigirci A, Erdem G.** Diffusion-weighted imaging features of brain in obesity. *Magn Reson Imaging*. 2008;26:446–450.
11. **Mueller K, Anwander A, Möller HE, Horstmann A, Lepsien J, Busse F, Mohammadi S, Schroeter ML, Stumvoll M, Villringer A, Pleger B.** Sex-dependent influences of obesity on cerebral white matter investigated by diffusion-tensor imaging. *PLoS One*. 2011;6: e18544.
12. **Mueller K, Sacher J, Arelin K, Holiga S, Kratzsch J, Villringer A, Schroeter ML.** Overweight and obesity are associated with neuronal injury in the human cerebellum and hippocampus in young adults: a combined MRI, serum marker and gene expression study. *Transl Psychiatry*. 2012;2:e200.
13. **Marks BL, Katz LM, Styner M, Smith JK.** Aerobic fitness and obesity: relationship to cerebral white matter integrity in the brain of active and sedentary older adults. *Br J Sports Med*. 2011;45:1208–1215.
14. **Riccitelli G, Rocca MA, Pagani E, Rodegher ME, Rossi P, Falini A, Comi G, Filippi M.** Cognitive impairment in multiple sclerosis is associated to different patterns of gray matter atrophy according to clinical phenotype. *Hum Brain Mapp*. 2011;32:1535–1543.
15. **Strauss S, Gavish E, Gottlieb P, Katsnelson L.** Interobserver and intraobserver variability in the sonographic assessment of fatty liver. *AJR Am J Roentgenol*. 2007;189:320–323.
16. **Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Fatar M, Hernandez Hernandez R, Jaff M, Kownator S, Prati P, Rundek T, Sitzer M, Schminke U, Tardif JC, Taylor A, Vicaut E, Woo KS, Zannad F, Zureik M.** Mannheim carotid intima-media thickness consensus (2004–2006). *An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc Dis*. 2007;23:75–80.
17. **Baroncini M, Jissendi P, Balland E, Besson P, Pruvo JP, Francke JP, Dewailly D, Blond S, Prevot V.** MRI atlas of the human hypothalamus. *Neuroimage*. 2012;59:168–180.
18. **Marques EL, Halpern A, Corrêa Mancini M, de Melo ME, Horie NC, Buchpiguel CA, Martins Novaes Coutinho A, Ono CR, Prando S, Santo MA, Cunha-Neto E, Fuentes D, Cercato C.** Changes in Neuropsychological Tests and Brain Metabolism After Bariatric Surgery. *J Clin Endocrinol Metab*. 2014 [Epub ahead of print] *PubMed*. PMID: 25157409.
19. **Fergenbaum JH, Bruce S, Lou W, Hanley AJ, Greenwood C, Young TK.** Obesity and lowered cognitive performance in a Canadian First Nations population. *Obesity (Silver Spring)*. 2009;17:1957–63.
20. **Swanson LW.** Cerebral hemisphere regulation of motivated behavior. *Brain Res*. 2000;886:113–164.
21. **Rempel-Clower NL, Barbas H.** Topographic organization of connections between the hypothalamus and prefrontal cortex in the rhesus monkey. *J Comp Neurol*. 1998;398:393–419.
22. **Plessow F, Schade S, Kirschbaum C, Fischer R.** Better not to deal with two tasks at the same time when stressed? Acute psychosocial stress reduces task shielding in dual-task performance. *Cogn Affect Behav Neurosci*. 2012;12:557–570.