Weight Loss After Bariatric Surgery Reverses Insulin-Induced Increases in Brain Glucose Metabolism of the Morbidly Obese

Jetro J. Tuulari,[1] Henry K. Karlsson,[1] Jussi Hirvonen,[1,2] Jarna C. Hannukainen,[1] Marco Bucci,[1] Mika Helmiö,[3] Jari Ovaska,[3] Minna Soinio,[4] Paulina Salminen,[3] Nina Savisto,[1] Lauri Nummenmaa,[1,5,6] and Pirjo Nuutila[1,4]

Obesity and insulin resistance are associated with altered brain glucose metabolism. Here, we studied brain glucose metabolism in 22 morbidly obese patients before and 6 months after bariatric surgery. Seven healthy subjects served as control subjects. Brain glucose metabolism was measured twice per imaging session: with and without insulin stimulation (hyperinsulinemic-euglycemic clamp) using [18F] fluorodeoxyglucose scanning. We found that during fasting, brain glucose metabolism was not different between groups. However, the hyperinsulinemic clamp increased brain glucose metabolism in a widespread manner in the obese but not control subjects, and brain glucose metabolism was significantly higher during clamp in obese than in control subjects. After follow-up, 6 months postoperatively, the increase in glucose metabolism was no longer observed, and this attenuation was coupled with improved peripheral insulin sensitivity after weight loss. We conclude that obesity is associated with increased insulin-stimulated glucose metabolism in the brain and that this abnormality can be reversed by bariatric surgery. *Diabetes* 62:1–5, 2013

I nsulin increases glucose uptake, storage, and usage in tissues, such as skeletal muscle and liver (1). Evidence from animal studies shows that insulin enters brain tissue via a saturable receptor-mediated transport (2), and receptors for insulin are widely expressed in the central nervous system (CNS) (1). Thus, insulin has the potential to act in the CNS, but in humans insulin-mediated effects on glucose metabolism in the CNS are thought to be minimal (3,4). Correspondingly, hyperinsulinemia does not increase brain glucose metabolism in healthy subjects (3,5–7).

We previously found that brain glucose metabolism increases during hyperinsulinemia in subjects with obesity-related impaired glucose tolerance and peripheral insulin resistance compared with healthy lean subjects (6).

Weight gain causes insulin resistance in peripheral tissues (8), and brain insulin resistance may develop parallel to peripheral insulin resistance and plasma hyperinsulinemia (9). However, altered insulin responses seem to differ from those observed in other tissues (6,7). Weight loss decreases peripheral insulin resistance (8,10), but the effect of weight loss on brain glucose metabolism is unknown.

In the current study, we sought to determine whether rapid weight loss after bariatric surgery and a subsequent decrease in peripheral insulin resistance would result in decreased brain glucose metabolism during insulin stimulation. We used direct measurements of brain glucose uptake with [18F] fluorodeoxyglucose (FDG)–positron emission tomography (PET) during euglycemic-hyperinsulinemic clamp and during fasting condition. Subjects were morbidly obese patients who underwent bariatric surgery and healthy control subjects. Preoperatively, we aimed to replicate the previous findings of increased brain glucose metabolism in morbidly obese patients in contrast to healthy control subjects.

RESEARCH DESIGN AND METHODS

For the current study, 22 obese patients were recruited as a part of a larger randomized, controlled clinical study evaluating the effect of sleeve gastrectomy and Roux-en-Y gastric bypass (RYGB) (Sleevepass study) (11). Four had type 2 diabetes, 4 had impaired glucose tolerance (IGT) or IFT, and 14 had normal results in oral glucose tolerance test (OGTT). The four diabetic subjects used metformin (from 1 to 3 g daily). Subjects with insulin treatment or fasting glucose >7 mmol/L were excluded. Other inclusion criteria have previously been described (11). Ten healthy subjects were recruited via an advertisement in local newspapers. Inclusion criteria were normal glucose tolerance test values, fasting plasma glucose <6.1 mmol/L, and chronic disease (Table 1 and 2). Demographic information for the groups is provided in Tables 1–2.

In this prospective study, obese patients were studied at two time points: before and 6 months after bariatric surgery. Healthy subjects were studied once. The baseline studies were carried out before the patient started a 4-week very-low-energy diet before the surgery. The PET studies were performed both in a fasting state and during euglycemic-hyperinsulinemic clamp (12), on separate days, <2 weeks apart. Strenuous physical activity was prohibited from the preceding evening. Antidiabetes treatment was withheld 24 h before the metabolic studies. Prior to the inclusion, each participant gave written consent. The study protocol was approved by the ethics committee of the Hospital District of the Southwestern Finland and conducted in accordance with the Declaration of Helsinki.

PET study protocol and data acquisition. The studies were performed after a 12-h fast using the GE Advance PET camera (General Electric Medical Systems, Milwaukee, WI). The euglycemic-hyperinsulinemic clamp technique was used as previously described (6,12). [18F]FDG-PET (187 ± 9 MBq) was injected intravenously over 15 s, and radioactivity in brain was followed thereafter for 40 min (4 × 30, 3 × 60, and 7 × 300 s frames).

[18F]FDG was synthesized with a computer-controlled apparatus (18). All data were corrected for dead time, decay, and measured photon attenuation and reconstructed using a Hann filter with a cutoff frequency of 0.5 and an mannosylrentinyl phosphate reconstruction method (13). Arterialized blood

TABLE 1
Characteristics of the subjects in preoperative comparison of patients and control subjects

	Morbidly obese subjects (n = 22: 2 male and 20 female)		Healthy control subjects (n = 7: 2 male and 5 female)		
	Mean	SD	Mean	SD	P
General characteristics					
Age (years)	45.73	9.21	47.88	5.64	0.452
BMI (kg/m^2)	**43.72**	**3.34**	**23.80**	**2.07**	**0.000**
Metabolic characteristics					
Cholesterol (mmol/L)	**4.35**	**0.76**	**5.25**	**0.74**	**0.007**
Triglycerides (mmol/L)	**1.31**	**0.43**	**0.81**	**0.31**	**0.006**
HDL cholesterol (mmol/L)	**1.32**	**0.30**	**1.95**	**0.60**	**0.001**
LDL cholesterol (mmol/L)	2.43	0.64	2.93	0.51	0.059
HDL cholesterol (%)	31.00	7.03	36.75	8.65	0.073
ALAT (units/L)	**30.05**	**12.53**	**18.88**	**8.04**	**0.027**
GT (units/L)	**32.14**	**13.52**	**20.25**	**10.77**	**0.034**
HbA$_{1c}$ (%)	5.78	0.47	5.74	0.24	0.823
Leptin (ng/mL)	**56.3**	**19.2**	**12.3**	**8.6**	**0.000**
OGTT					
OGTT 0-min glucose (mmol/L)	**6.19**	**0.77**	**5.54**	**0.29**	**0.027**
Fasting insulin (mU/L)	14.64	16.06	5.13	1.55	0.109
OGTT 120-min glucose (mmol/L)	**8.61**	**2.58**	**5.91**	**1.15**	**0.009**
M value (whole-body glucose uptake)	**12.6**	**5.8**	**37.2**	**7.6**	**0.000**
PET acquisition					
Fasting glucose values 0 min	5.00	0.34	4.94	0.62	0.08
Minutes from clamp initiation to scan	82.3	32.9	97.9	16.9	0.24
Mean plasma glucose during clamp	5.10	0.65	4.82	0.58	0.23
Clamp 0-min insulin (mU/L)	14.64	16.05	5.13	1.55	0.74
Clamp 60-min insulin (mU/L)	74.43	20.60	64.00	9.53	0.11
Clamp120-min insulin (mU/L)	81.10	38.35	67.42	9.54	0.18

Last column indicates significant between-groups differences in independent-samples t test. Significant differences are shown in boldface.

samples were drawn during the scan and analyzed for radioactivity concentration in plasma using an automatic γ counter (6,12). In the clamp study, plasma glucose and serum insulin concentrations were taken at baseline and every 5, 30, and 60 min, respectively.

Q:3   In the preoperative setting, scans of morbidly obese subjects were compared with the paired scans of the control group. In the preoperative versus postoperative comparison, only subjects who participated in all four scans were included in the analysis (n = 17). We lost two control scans as a result of unsuccessful preprocessing, and one control subject was excluded because of an elevated BMI. In the postoperative setting, four subjects did not undergo bariatric surgery and one subject missed one scan.

Quantification of brain glucose metabolism. The influx constant (K_i) was calculated for each voxel separately using the linear Gjedde-Patlak plot with arterial plasma input function, with a linear phase start time of 20 min. Glu-
Q:4 cose uptake (CMR$_{glu}$ [μmol $\times$ 100 g $-$ 1 $\times$ min^{-1}]) was then calculated at voxel level as follows: CMR$_{glu}$ = K_i $\times$ Cp/LC, where Cp is the plasma glucose concentration and LC is the lumped constant (which was set at 0.80).

    Summed PET images were normalized spatially to a ligand-specific template in to the Montreal Neurological Institute (MNI) space (MNI International Consortium for Brain Mapping) using SPM8 (www.fil.ion.ucl.ac.uk/spm/) running on Matlab for Windows (version 7.7.0; Math Works, Natick, MA). Normalization parameters were subsequently applied to corresponding parametric glucose metabolism images. Parametric images were smoothed at 10 mm full width at half-maximum.

Statistical analysis. Preoperative data were analyzed with a 2 (fast, clamp) $\times$ 2 (patient, control) ANOVA and the follow-up data for patients with a 2 (fast, clamp) $\times$ 2 (preoperative, postoperative) ANOVA. The statistical threshold in SPM analysis was set at voxel level false discovery rate (FDR) corrected $P <$ 0.05, uncorrected at cluster level. Lowering of the statistical threshold did not yield statistically significant results. Further statistical analyses were done using SPSS, version 18.0, for Windows (SPSS, Chicago, IL). A more detailed complementary analysis included quantification of brain glucose metabolism by defining the LC in a region-specific manner instead of using a fixed lumped constant as in the analysis mentioned above and region of interest–based analysis (see Supplementary Data).

RESULTS

Effects of insulin on brain glucose metabolism in control subjects and patients prior to the surgery. In the morbidly obese patients, we found a global increase in brain [18F]FDG-PET uptake during clamping compared with fasting (Fig. 1B). This difference was not statistically significant in the control group. The regional maximum of the increased glucose metabolism was the region of the right caudate nucleus (Fig. 1A). There were no statistically significant differences between the groups in the fasting condition. Thus, the euglycemic-hyperinsulinemic clamp increased brain glucose metabolism only in the morbidly obese subjects.
Q:5

Effects of bariatric surgery on brain glucose metabolism. Elevation of glucose metabolism during clamp was markedly diminished after bariatric surgery (Fig. 1D). The postoperative regional CMR$_{glu}$ values in the morbidly obese patients were similar to the values of control subjects in a preoperative setting (Fig. 1E). Bariatric surgery resulted in rapid weight loss and improvement in glycemic control and whole-body insulin sensitivity (M values in Table 2). One of four in both diabetic and IGT/IFG patients was in diabetic remission postoperatively, and for the rest the diabetes status, defined by OGTT and American Diabetes Association criteria, was unchanged.

DISCUSSION

Our results indicate that insulin-induced stimulation during euglycemic-hyperinsulinemic clamp scans of the morbidly

TABLE 2
Comparison of patients in preoperative and postoperative states

	Morbidly obese subjects (n = 17 preoperative females)		Morbidly obese subjects (n = 17 postoperative females)		
	Mean	SD	Mean	SD	P
General characteristics					
Age (years)	45.41	9.28	45.53	8.71	0.88
Weight (kg)	**117.87**	**9.89**	**90.45**	**10.71**	**0.00**
BMI (kg/m^2)	**43.06**	**3.00**	**33.15**	**3.77**	**0.00**
Metabolic characteristics					
Cholesterol (mmol/L)	4.33	0.86	4.26	0.61	0.72
Triglycerides (mmol/L)	**1.24**	**0.42**	**0.89**	**0.22**	**0.00**
HDL (mmol/L)	**1.36**	**0.30**	**1.52**	**0.26**	**0.03**
LDL (mmol/L)	2.41	0.70	2.34	0.53	0.65
HDL cholesterol (%)	**32.29**	**6.51**	**35.94**	**6.19**	**0.01**
ALAT (units/L)	**27.94**	**10.41**	**18.76**	**9.93**	**0.01**
GT (units/L)	31.88	13.67	21.65	29.19	0.18
HbA$_{1c}$ %	**5.78**	**0.53**	**5.52**	**0.33**	**0.01**
Leptin (ng/mL)	**59.6**	**19.1**	**31.4**	**15.3**	**0.00**
OGTT					
OGTT 0-min glucose (mmol/L)	**6.24**	**0.86**	**5.33**	**0.56**	**0.00**
Fasting insulin (mU/L)	**14.82**	**18.26**	**5.12**	**2.20**	**0.04**
OGTT 120-min glucose (mmol/L)	**8.26**	**2.64**	**5.92**	**2.88**	**0.01**
M value (whole-body glucose uptake)	**14.0**	**5.94**	**26.2**	**7.6**	**0.000**
PET acquisition					
Fasting glucose values	**5.81**	**0.81**	**5.41**	**0.47**	**0.01**
Minutes from clamp initiation to scan	80.42	35.17	96.6471	19.49	016
Mean plasma glucose during clamp	5.02	0.38	5.22	0.52	023
Clamp 0-min insulin (mU/L)	**14.82**	**18.26**	**5.12**	**2.20**	**0.04**
Clamp 60-min insulin (mU/L)	74.87	21.62	68.50	12.63	032
Clamp120-min insulin (mU/L)	78.73	41.96	67.40	13.94	032

Last column indicates significant within-group differences in paired-samples t test. Significant differences are shown in boldface.

obese is reversible through weight loss and consequent reduced peripheral insulin resistance 6 months after bariatric surgery (Fig. 1*C* and *D*). Preoperatively, we detected elevations in glucose metabolism in morbidly obese subjects compared with normal-weight subjects in striatum and cerebellum during euglycemic-hyperinsulinemic clamp but not in the fasting condition (see Supplementary Data), which partly replicates our previous findings (6). These observations are not explained merely by increases in plasma levels of insulin during PET scans (10) (Tables 1 and 2).

Insulin effects on brain glucose metabolism. In individuals with normal weight, insulin has little effect on brain glucose metabolism (3,5–7). Nevertheless, normal-weight individuals may also have minor insulin-induced elevations in brain glucose metabolism that might not be detected as statistically significant in limited sample sizes. Obesity, however, seems to be associated with increased sensitivity to insulin in brain tissue (6).

How do we explain the increased metabolic sensitivity of brain to insulin? The ratio of insulin in cerebrospinal fluid to that in plasma is decreased in obesity (14), and obese individuals also show diminished catabolic responses after intranasal insulin administration (15). These findings could be a sign of brain insulin resistance (9,15,16), which could be caused by impeded insulin transport through the blood-brain barrier (BBB) or a weakened neuronal responses to insulin. On the other hand, in the fasting state glucose metabolism is not decreased in overweight subjects in comparison with

a control group (6). Chronically elevated plasma levels of insulin could also increase the delivery of insulin across the BBB. If the cells in the CNS adapt to this change by increasing the rate of insulin clearance or internalization, then decreased cerebrospinal fluid–to–plasma insulin ratio of the obese individuals may reflect greater insulin usage. This would also explain the diminished catabolic responses to intranasal insulin, as the given amounts of insulin would presumably be too small for cells accustomed to increased insulin levels.

Taken together, by increasing peripheral insulin resistance the brain tissue's glucose metabolism is increasingly stimulated independent of changes in plasma glucose levels. Molecular mechanisms for the changes are currently unclear. Clinically, intensifying the pharmacological therapy for glycemic control keeps the plasma glucose levels at bay and prevents glucose neurotoxicity in peripheral organs (4). The opposite might be true for the brain tissue (17).

Limitations in the study. The radioactive decay of the [^{18}F]FDG-PET tracer can occur at any level between BBB and intracellular space of neurons and glial cells. The clamp as an experimental tool also has some limitations (18). The subjects of this study are heterogeneous in terms of glucose metabolism and differ in terms of endogenous peripheral insulin resistance. Excluding diabetic/IGT subjects from SPM analysis or grouping subjects according to diabetes status did not affect the results, and the effects of medication and diabetes status are minimal. Hyperglycemia during fasting condition might diminish the [^{18}F]FDG
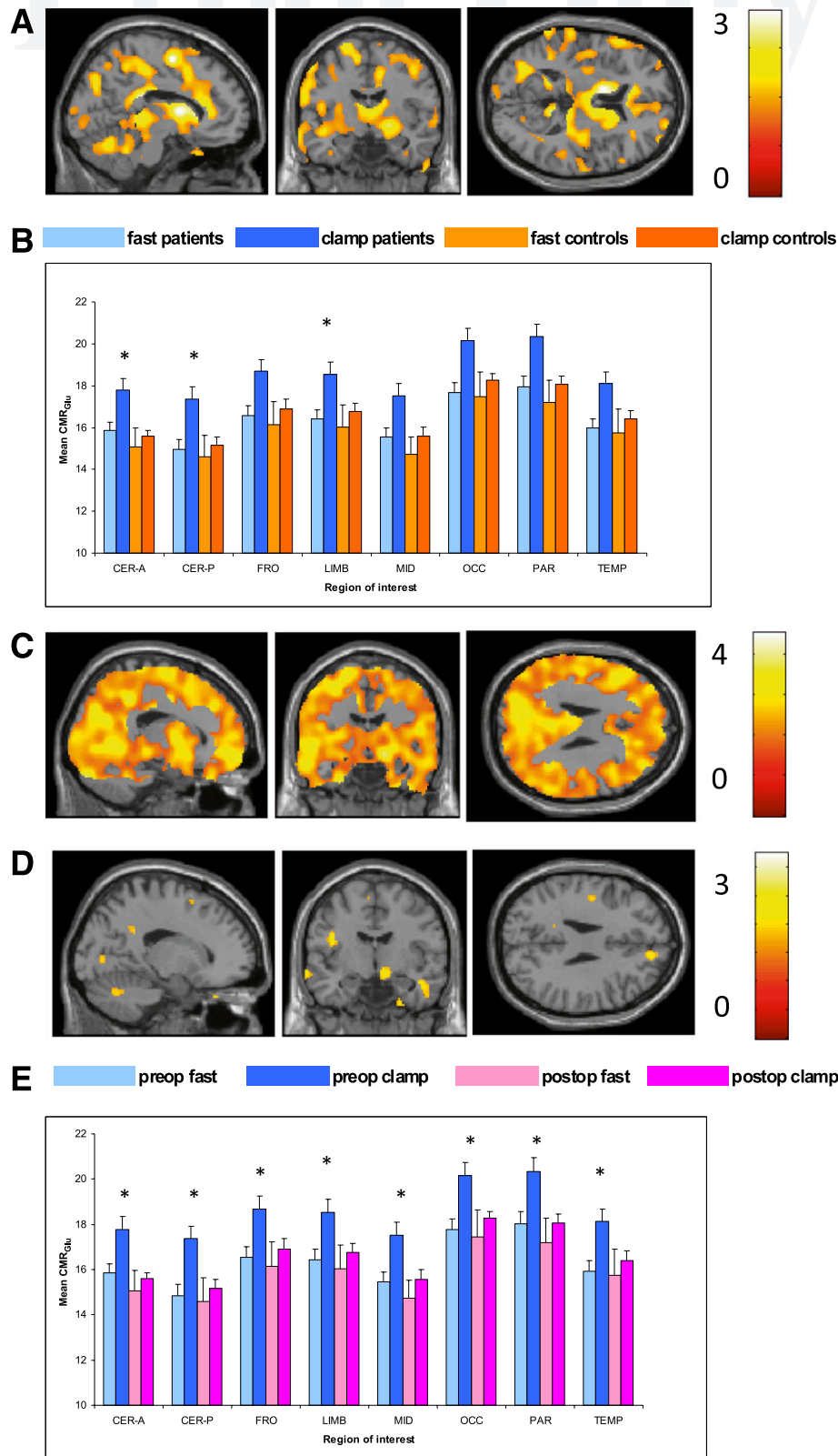
FIG. 1. Insulin-induced changes of brain glucose metabolism. *A*: Preoperative (Preop) clamp scan comparison of morbidly obese patients vs. control group. (*P* value 0.05 voxel level uncorrected; *P* < 0.05 cluster level FDR corrected). The bars represent *T* values. MNI coordinates −9, 2, and 10 are chosen so that regional maximum in right caudate nucleus can be seen. The data were thresholded at *P* < 0.05 FDR corrected *T* > 3.02. *B*: Preoperative CMR$_{glu}$ values of control subjects (*n* = 7) and morbidly obese patients (*n* = 22). Error bars represent the SEM. *Significantly increased CMR$_{glu}$ values during clamp scans in comparison with corresponding fasting scans. *C*: Preoperative comparison of CMR$_{glu}$ of morbidly obese patients for clamp vs. fast. *P* value 0.05 voxel level uncorrected; *P* < 0.05 cluster level FDR corrected. The bars represent *T* values. MNI coordinates −16, 8, and 28. The data were thresholded at *P* < 0.05 FDR corrected *T* > 3.52. *D*: Postoperative (Postop) comparison of CMR$_{glu}$ of morbidly obese patients for clamp vs. fast. *P* value 0.05 voxel level uncorrected; *P* < 0.05 cluster level FDR corrected. The bars represent *T* values. MNI coordinates −16, 8, and 28. *E*: Pre- and postoperative CMR$_{glu}$ values of morbidly obese patients (*n* = 17). Error bars represent the standard SEM. *Significantly increased CMR$_{glu}$ values during clamp scans in comparison with corresponding fasting scans.

uptake to brain tissue. However, obese subjects were not hyperglycemic and the scans and clamping conditions were similar in both groups (Tables 1 and 2). Postoperative comparison necessitated participation to four scans, and because of this the study had a relatively large drop-out rate.

Conclusions. The results of our study support the evidence showing that insulin stimulates brain glucose metabolism of the morbidly obese. We report, for the first time, that weight loss after bariatric surgery reverses increased insulin stimulation. This supports that bariatric surgery not only results in weight loss but also improves overall metabolic health (10)—this might include the brain.

REFERENCES

1. Schulingkamp RJ, Pagano TC, Hung D, Raffa RB. Insulin receptors and insulin action in the brain: review and clinical implications. Neurosci Biobehav Rev 2000;24:855–872
2. Banks WA, Jaspan JB, Kastin AJ. Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. Peptides 1997;18:1257–1262
3. Hasselbalch SG, Knudsen GM, Videbaek C, et al. No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism in humans. Diabetes 1999;48:1915–1921
4. Tomlinson DR, Gardiner NJ. Glucose neurotoxicity. Nat Rev Neurosci 2008;9:36–45
5. CITED?Cranston I, Marsden P, Matyka K, et al. Regional differences in cerebral blood flow and glucose utilization in diabetic man: the effect of insulin. J Cereb Blood Flow Metab 1998;18:130–140
6. Hirvonen J, Virtanen KA, Nummenmaa L, et al. Effects of insulin on brain glucose metabolism in impaired glucose tolerance. Diabetes 2011;60:443–447
7. Bingham EM, Hopkins D, Smith D, et al. The role of insulin in human brain glucose metabolism: an 18fluoro-deoxyglucose positron emission tomography study. Diabetes 2002;51:3384–3390
8. Colditz GA, Willett WC, Stampfer MJ, et al. Weight as a risk factor for clinical diabetes in women. Am J Epidemiol 1990;132:501–513
9. 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
10. Maggard MA, Shugarman LR, Suttorp M, et al. Meta-analysis: surgical treatment of obesity. Ann Intern Med 2005;142:549–559
11. Helmiö M, Victorzon M, Ovaska J, et al. SLEEVEPASS: a randomized prospective multicenter study comparing laparoscopic sleeve gastrectomy and gastric bypass in the treatment of morbid obesity: preliminary results. Surg Endosc 2012;26:2521–2526
12. Nuutila P, Raitakari M, Laine H, et al. Role of blood flow in regulating insulin-stimulated glucose uptake in humans. Studies using bradykinin, $[^{15}O]$water, and $[^{18}F]$fluoro-deoxy-glucose and positron emission tomography. J Clin Invest 1996;97:1741–1747
13. Alenius S, Ruotsalainen U. Bayesian image reconstruction for emission tomography based on median root prior. Eur J Nucl Med 1997;24:258–265
14. Kern W, Benedict C, Schultes B, et al. Low cerebrospinal fluid insulin levels in obese humans. Diabetologia 2006;49:2790–2792
15. Hallschmid M, Schultes B. Central nervous insulin resistance: a promising target in the treatment of metabolic and cognitive disorders? Diabetologia 2009;52:2264–2269
16. Pagotto U. Where does insulin resistance start? The brain. Diabetes Care 2009;32(Suppl. 2):S174–S177
17. Korf ES, van Straaten EC, de Leeuw FE, et al. Diabetes mellitus, hypertensionand medial temporal lobe atrophy: the LADIS study. Diabet Med 2007;2:166–171
18. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 2008;294:E15–E26