Brain FFA uptake is elevated in morbid obesity, and is irreversible six months after bariatric surgery: a positron emission tomography study

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Running title: Free fatty acids and brain metabolism

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Abstract

**Background and aims:** Preclinical studies have shown that brain FFA handling can be involved in the control of whole-body homeostasis, but data in humans are scanty. We investigated whether there are differences in brain fatty acid uptake (BFAU) between morbidly obese and lean subjects, and the effect of weight loss following bariatric surgery (BS).

**Materials and methods:** We measured BFAU with 14(R, S)-[18F]fluoro-6-thia-heptadecanoic acid (FTHA) and positron emission tomography (PET) in 24 morbidly obese and 14 lean women. Obese subjects were re-studied 6 months after BS. We also assessed whether there is hypothalamic neuroinflammation in the obese using fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI).

**Results:** Obese subjects had higher BFAU than lean subjects (1.12 [0.61] vs 0.72 [0.50] µmol 100g⁻¹ min⁻¹, p=0.0002), driven by higher FFA availability. BFAU correlated positively with BMI (p=0.006, r=0.48), whole body fatty acid oxidation (p=0.006, r=0.47), and leptin levels (p=0.001, r=0.54). When BFAU, leptin, and BMI were included in the same model, the association between BFAU and leptin was the strongest. BFAU did not correlate with FLAIR-derived estimates of hypothalamic inflammation. Six months after BS, obese subjects achieved significant weight loss (-10 units of BMI). BFAU was not significantly changed (1.12 [0.61] vs 1.09 [0.39] µmol 100g⁻¹ min⁻¹, ns), likely due to the ongoing catabolic state. Finally, baseline BFAU predicted worse plasma glucose levels at 2 years of follow up.

**Conclusions:** BFAU is increased in morbidly obese as compared to lean subjects, and is unchanged six months after bariatric surgery. Baseline BFAU predicts worse plasma glucose levels...
at follow up, supporting the notion that the brain participates in the control of whole-body homeostasis.

Keywords: PET, obesity, bariatric surgery, free fatty acids, brain metabolism

Abbreviations: AV: arteriovenous; BFAU: brain fatty acid uptake; BMI: body mass index; BS: bariatric surgery; CT: computerized tomography; FDR: false discovery rate; FFA: free-fatty acids; FLAIR: fluid-attenuated inversion recovery; FTHA: 14(R, S)-[18F]fluoro-6-thia-heptadecanoic acid; FUR: fractional uptake rate; HFD: high fat diet; ICC: intraclass correlation coefficients; IQR: interquartile range; MBH: mediobasal hypothalamus; MRI: Magnetic resonance imaging; OGIS: oral glucose insulin sensitivity; PET: positron emission tomography; ROI: region of interest; SI: signal intensity; SPM: statistical parametric mapping; T2D: type 2 diabetes; WHP: waist-to-hip ratio
Introduction

Obesity is a major public health burden since it associates with an enhanced risk of cardiovascular disease, type 2 diabetes (T2D), stroke, neurodegenerative diseases, and many types of cancer\textsuperscript{1,2}. Obesity is linked with systemic low-grade inflammation\textsuperscript{3}. Several lines of research in animals further demonstrate that a high-fat diet (HFD) leads to neuroinflammation and, particularly, astrocyte proliferation and activation (\textit{i.e.}, astrogliosis). This process can occur even after only one day of HFD, and precede the establishment of systemic inflammation\textsuperscript{4}.

For a long time, the brain was thought not to use free fatty acids (FFA). This notion has been challenged by preclinical and clinical studies. In the brain, astrocytes oxidize FFA\textsuperscript{5}, and oligodendrocytes have also been shown to take up FFA\textsuperscript{6}. Astrocytes are able to deposit fatty acids into lipids, which are later released to the extracellular space and taken up by neurons\textsuperscript{7}. Studies of brain FFA handling in humans are scarce. Almost 10 years ago, using positron emission tomography (PET) and two fatty acid analogues we showed that brain fatty acid uptake (BFAU) was increased in subjects with metabolic syndrome as compared to lean controls, and that BFAU decrease after weight loss\textsuperscript{8}. Despite interest in the central effects of fatty acids, these findings have not been replicated.

In the current work, we sought to determine whether our previous findings on BFAU hold true in morbidly obese women as compared to lean women. In addition, we examined the effects of bariatric surgery on BFAU. In particular, we hypothesized that morbidly obese women have higher BFAU than lean women, and that after major surgery-induced weight loss BFAU is decreased.

Since obese subjects often consume a high-fat diet, we further hypothesized that neuroinflammation might also be present. Therefore, we measured the ratio of hypothalamic-to-amygdala signal intensity from fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI), which has been previously shown to reflect hypothalamic inflammation\textsuperscript{4,9,10}. Finally, we analyzed
follow-up data to test whether baseline BFAU predicts any metabolic outcome after bariatric surgery, such as plasma glucose levels and HbA1c.

Materials and Methods

**Participants and study design** We studied 24 morbidly obese women and 14 age- and sex-matched healthy nonobese controls. Obese patients were studied before and 6 months after bariatric surgery, controls were studied only once. Inclusion and exclusion criteria and the surgical techniques have been previously described in detail. Based on ADA criteria, nine obese patients had T2D and fifteen were nondiabetic, including four with impaired glucose tolerance and one with impaired fasting glucose before the operation. Nine of the obese subjects had a diagnosis of arterial hypertension. Seven obese subjects underwent laparoscopic Roux-en-Y gastric bypass and fourteen laparoscopic sleeve gastrectomy (3 subjects did not undergo surgery). The protocol was approved by the Ethics Committee of the Hospital District of Southwestern Finland, and all subjects gave written informed consent before participating in the study (NCT01373892).

**Study protocol** Clinical screening, anthropometric and biochemical measurements were performed as described. Subjects then underwent positron emission tomography/computerized tomography (PET/CT) measurements in the fasting state using a hybrid GE discovery STE and VCT scanners (General Electric Medical Systems, Milwaukee, WI, USA). In obese patients, the imaging studies were performed before the standard 4-week very-low calorie diet that preceded surgery. Following an overnight (10-12 hr) fast, 2 catheters were inserted in the antecubital veins, one for the administration of radiolabeled tracers and the other for arterialized blood sampling. Then, an intravenous bolus (185 ± 46 MBq) of the long-chain fatty acid analog 14(R,S)-[^18F]fluoro-6-thia-heptadecanoic acid ([^18F]-FTHA)) was given, following which the dynamic PET imaging of the brain started and continued for 40 min. Blood samples were drawn during the entire scanning period to measure FFA as well as radioactivity levels.
**Quantification of brain fatty acid uptake**  Brain fractional uptake rate (FUR) of $[^{18}F]$-FTHA was calculated by dividing brain radioactivity by the integral of the plasma unmetabolized radioactivity curve\(^{14}\). Brain FFA uptake (BFAU, in $\mu$mol $100g^{-1}$ min$^{-1}$) was then calculated at voxel level as the product of FUR and serum FFA concentration\(^8\).

**Indirect calorimetry**  Open-system indirect calorimetry (Deltatrac®) was used for the measurement of $O_2$ consumption ($VO_2$) and $CO_2$ production ($VCO_2$), from which whole-body energy expenditure, and substrate oxidation rates were calculated as previously described\(^{15}\).

**Calculations**  Insulin sensitivity was estimated by the OGIS (Oral Glucose Insulin Sensitivity) method, and expressed in mL min$^{-1}$ m$^{-2}$\(^{16}\).

**FLAIR-derived measurement and reliability**  To measure mediobasal hypothalamic (MBH) gliosis, the signal intensity (SI) was retrospectively measured from coronally oriented fluid-attenuated inversion recovery (FLAIR) images from magnetic resonance imaging (MRI), as previously described\(^4,9,10\). FLAIR images were gathered as part of the study protocol by Philips 1.5T device (Philips Ingenuity), with the following image parameters: TR 11,000 ms, TE 140 ms, TI 2,800 ms, slice thickness 5 mm. Briefly, circular regions of interest (ROIs, about 4 mm$^2$) were placed on one coronal slice onto MBH, which was identified as a bilateral most caudal brain region situated between the optic chiasm anteriorly and the mamillary bodies posteriorly. The amygdala, visible on the same slice in the medial temporal lobe, was chosen as the reference region (ROI about 20 mm$^2$) for the normalization of MBH SI, and the MBH-amygdala SI ratio served as the primary outcome measure of hypothalamic gliosis. All measurements were carried out by a fellowship-trained neuroradiologist (J.H.). To assess reliability of measurement, thirty cases were independently analyzed by another fellowship-trained neuroradiologist (M.N.), blind to the assessment by the other reader. We assessed inter-rater reliability by variability (absolute difference between measurements by two raters, divided by the average of those measurements), and intraclass correlation coefficients (ICC), where $<0.5$ is poor, 0.5-0.75 is moderate, 0.75-0.9 is good, and $>0.9$
is excellent reliability. We found low inter-rater variability (3.6%), suggesting low measurement error, and an ICC estimate of 0.63, suggesting moderate agreement.

Analytical methods  Plasma glucose was measured in the laboratory of the Turku PET Centre in duplicate using the glucose oxidase technique (Analox GM7 or GM9, Analox Instruments Ltd., London, UK). Glycosylated hemoglobin (HbA1c) was measured with ion-exchange high performance liquid chromatography (Variant II Haemoglobin A1c, Bio-Rad Laboratories, CA, USA). Serum insulin was determined by time-resolved immunofluorometric assay (AutoDELFIA, Perkin Elmer Life and Analytical Sciences). Serum FFA were measured with a photometric enzymatic assay (Wako Chemicals GmbH, Neuss, Germany) on Modular P800 automatic analyzer (Roche Diagnostics, Mannheim, Germany). Serum high-sensitivity C-reactive protein (hs-CRP) was analysed with the sandwich immunoassay method using an Innotrac Aio1 immunoanalyzer (Innotrac Diagnostics, Turku Finland). Serum adipokines were analysed in duplicate by using Milliplex Human Serum Adipokine (Panel A) kit [cat.no HADK1-61K-A] containing interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor alpha (TNFα), and leptin.

Statistical analysis  Data are presented as mean ± SD (or median [IQR] for non-normally distributed variables). Whole-brain statistical analysis was performed with statistical parametric mapping (SPM12) toolbox tuning on Matlab. Linear regressions were performed in SPM to evaluate correlations between BFAU and single regressors (BMI, OGIS, leptin, whole-body lipid oxidation, hypothalamus/amygda signal ratio) while controlling for confounding factors (in the SPM contrast, the controlling variables were set to a value of 0). The statistical threshold in SPM analysis was set at a cluster level and corrected with false discovery rate (FDR) with p<0.05. Fatty acid uptake values were extracted from nine regions of interest (ROI) (global, CER-A, anterior cerebellum; CER-P, posterior cerebellum; FRO, frontal lobe; LIMB, limbic lobe; MID, midbrain; OCC, occipital lobe; PAR, parietal lobe; TEMP, temporal lobe) with Marsbar plug-in for Matlab and correlated against the selected parameters using either Pearson ($r$), or Spearman ($\rho$) correlation analysis, as
appropriate. Further statistical analyses were done using JMP version 13.0 (SAS Institute, Cary, NC, USA). A \( p \) value <0.05 was considered statistically significant.

**Results**

*Clinical and metabolic characteristics of the study participants*

HbA\(_{1c}\) and fasting glucose were the only parameters where statistically significant differences were found between T2D and non-diabetic obese participants, and for this reason the data of T2D and non-diabetic subjects were pooled for further analysis. As expected, obese participants had higher measures of adiposity (WHR, fat mass, BMI), higher fasting FFA and glycerol levels, and worse insulin sensitivity, as compared to age-matched lean controls. Obese participants also had high circulating inflammatory markers (Table 1).

**Before surgery**

Brain FFA uptake was higher in the obese as compared to lean subjects (1.12 [0.61] vs 0.72 [0.50] \( \mu \)mol 100g\(^{-1}\)min\(^{-1}\), \( p=0.002 \)), globally (Figure 1) as well as in the various brain regions explored (Table 2). This increase was driven by higher FFA availability (0.80 ± 0.22 vs 0.55 ± 0.17 mmol/L, \( p=0.001 \)) as the fractional uptake rates were similar in the two groups. In contrast, the FLAIR hypothalamus/amygdala signal ratio did not differ between obese and control subjects (Table 2).

**After surgery**

Post-surgical data were available for 21 subjects (3 subjects did not undergo surgery). As expected, 6 months after the operation participants had lost significant amount of body weight (26 ± 8 kg). Serum FFA levels were not decreased (0.80 ± 0.22 vs 0.77 ± 0.17 mmol/L, ns), likely because of the ongoing catabolic state. As FUR was also unchanged, BFAU was not altered by surgery (Table 2 and Figure 2A). The FLAIR hypothalamus/amygdala signal ratio was also
unchanged (Table 2). Insulin sensitivity (as indexed by OGIS), HbA1c levels, and circulating inflammatory markers were improved (Table 1).

**Correlations**

In the whole dataset, baseline BFAU was positively associated with BMI ($r=0.47, p=0.003$) and other markers of adiposity such as fat mass ($r=0.45, p=0.006$) and leptin ($r=0.54, p=0.0008$), and negatively with OGIS ($r=-0.45, p=0.007$). BFAU was also directly related to whole-body lipid oxidation ($r=0.52, p=0.001$) (Figure 2B). In contrast, the FLAIR hypothalamus/amygdala signal ratio did not correlate with BMI, circulating inflammatory markers, or BFAU (Figure 3). When accounting for BMI, the correlations that remained significant were those between BFAU and leptin ($p=0.05$), and between BFAU and whole-body lipid oxidation ($p=0.03$).

In the 18 subjects for whom we had follow-up data, baseline BFAU predicted higher plasma glucose levels at 2 years of follow up (Figure 2C). The association remained significant after adjusting for baseline plasma glucose levels. Baseline serum FFA also predicted higher plasma glucose levels at 2 years of follow up.

**Discussion**

Our data show that, in the postabsorptive state, morbidly obese subjects have higher brain fatty acid uptake compared to lean individuals, and that, six months after surgery, BFAU is essentially unchanged because of the ongoing catabolic state and high substrate availability. Thus, we replicate and extend our original findings in metabolic syndrome.

In the postabsorptive state, increased BFAU in the obese results from high substrate (circulating FFA) availability because there is no difference in the intrinsic avidity of the brain for FFA (as reflected by the fractional uptake rate). Thus, although the increased BFAU of the obese seems to be an epiphenomenon of their metabolic status (excess lipolysis, due to insulin resistance), the chronic excess of fatty acid supply could ultimately lead to central deleterious effects and,
eventually, deterioration of their metabolic health. Indeed, in the current data baseline BFAU, predicted worse plasma glucose levels at 2 years of follow up. This is consistent with our previous finding in morbidly obese subjects undergoing bariatric surgery studied with FDG-PET under euglycemic hyperinsulinemic conditions, where higher brain glucose uptake also predicted worse glycemic levels at 2 and 3 years of follow-up. Taken together, these and other findings, indicate that in humans brain substrate handling may be involved in the control of whole-body homeostasis.

A well-described central effect of FFA is the induction of hypothalamic inflammation. Assessing hypothalamic inflammation in vivo in humans is demanding. One method that has been proposed to depict hypothalamic inflammation, is the T2 hypothalamic/amygdala hyperintensity signal. However as previously argued this signal does not constitute definitive proof of increased gliosis, since infection, edema, and tumors may have a similar appearance. We therefore analyzed FLAIR-MRI data (a similar to T2 MRI sequence optimal in analysing small periventricular areas) to test whether brain FFA uptake would correlate with the hypothalamic/amygdala hyperintensity signal. Contrary to expectation, we found no such correlations. In addition, despite separate analysis of the FLAIR-MRI data by two expert neuroradiologists, we also failed to replicate previous findings, i.e., correlations of this ratio with BMI or inflammatory markers. This discrepancy may in part be explained by technical reasons. The hypothalamus is a very small brain region, and our FLAIR-MRI sequence was protocolled to cover the whole brain with 5-mm thick slices. These relatively thick slices may have caused ROI malpositioning, and preclude sensitivity for subtle changes in hypothalamic FLAIR signal. We found small measurement error (inter-rater variability), yet only moderate intraclass correlations (0.63). Previous studies have not assessed reliability of hypothalamic FLAIR measurements. This pattern of results suggests insufficient between-subject variability (i.e., identifiability of individuals) to make this ratio an optimal biomarker in metabolic studies. Therefore, further studies with advanced technique are necessary to
assess whether central inflammation occurs in human obesity and whether it is improved following bariatric surgery.

The brain has been long considered not to use FFA. However, this notion has been challenged \cite{5,8}. Using the palmitate analog FTHA, we find that brain FFA uptake ranges 0.3-1.3 µmol 100mL$^{-1}$ min$^{-1}$, which translates to a whole-brain uptake of 4-14 µmol min$^{-1}$. By comparison with previous PET studies using FTHA \cite{20-23}, the brain has a relatively high tissue-specific FFA uptake (fourth after liver, kidney and heart but higher than adipose tissue or resting skeletal muscle). Thus, brain FFA uptake makes a small contribution to whole-body FFA disposal but avidly extracts FFA from the plasma. In brain slice cultures, polyunsaturated fatty acid species (PUFA) are taken up preferentially \cite{6}. FTHA has no double bonds, and therefore behaves as a saturated fatty acid. Consequently, we cannot rule out that brain fatty acid uptake would have been higher had it been measured with a PUFA-analogue. Unfortunately thus far such a tracer is not available.

With regard to the intracellular fate of fatty acids in the brain, preclinical studies have shown that the only brain cell type capable of oxidizing fatty acids are the astrocytes \cite{5}. Astrocytes also contain lipid droplets which can be released as exosomes and subsequently taken up by neurons \cite{7}, for their structural needs. In the postabsorptive state, one could hypothesize that substrate uptake would be at least in part directed to oxidation and energy production. In their elegant review \cite{24} Schönfeld and Reiser conclude that activated fatty acids can be either esterified to membrane lipids or undergo β-oxidation in the mitochondria, and argue that excessive oxidation of fatty acids could be detrimental for the ‘economy’ of the brain cells. Accordingly, our previous study using both [$^{18}$F]-FTHA-PET and [$^{11}$C]-palmitate-PET (the first tracking total FFA uptake, the latter non-oxidative metabolism) concluded that not all of the fatty acids are oxidized by the brain \cite{8}.

The current results show group differences (obese vs lean participants), correlations (between BFAU and brain-derived or metabolic parameters), and the effect of bariatric surgery. The mechanistic background of these findings has not been specifically addressed but some clues help
formulating a mechanistic hypothesis, e.g., the strong association of BFAU with leptin. Leptin is an adipokine that crosses the blood-brain barrier and acts as a neuropeptide in the brain. Astrocytes, which have been identified as key metabolic players, express the leptin receptor in obesity\textsuperscript{24}. Chronic exposure to leptin by intracerebroventricular application has been shown to increase the expression of glial fibrillary acidic protein and vimentin, and to increase the expression of astrocyte structural proteins\textsuperscript{25}. The chronic hyperleptinemia of the obese leading to astrocyte activation might be the origin of the observed increase in BFAU. Future preclinical studies could compare BFAU in wild type and leptin deficient rats.

Our study has limitations. First, we only included women so that extrapolation of our findings to men should be done with caution. Second, because of the physics of the PET, small anatomical regions such as the hypothalamus could not be analyzed with sufficient resolution\textsuperscript{26}. Third, we used MRI to probe central nervous system inflammation. Though the analysis was performed by two independent researchers, with good agreement values between them, MRI may not be the optimal technique to detect brain inflammation.

In summary, morbidly obese subjects have higher brain fatty acid uptake as compared to lean individuals. BFAU is essentially unchanged after surgically-induced major weight loss, very likely because of the ongoing catabolic state and high substrate availability. Baseline BFAU predicts worse plasma glucose levels at follow up, supporting the notion that the brain participates in the control of whole-body glucose homeostasis.
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Authors’ contribution

E. R., J.H, M.B., L.P., M.N., J.C.H., L.N., analyzed data and literature and drafted the manuscript. J.C.H conducted the clinical PET studies. J.H. and M.N. performed the FLAIR analysis. P.S. operated the patients. P.I., L.N., P.N. conceived the study designs. P.I., L.N., E.F., P.N. reviewed the manuscript. All authors approved the final version of the manuscript.

P.N. 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.

Prior Presentation

Parts of this study were presented as abstract at the 55th EASD annual meeting at Berlin (September 2019).
References

Figure Legends

A.

B.

C.

Figure 1 – Brain clusters (as defined by FDR-corrected SPM one-sample t-test) for the comparison of brain fatty acid uptake (BFAU) between the two groups, and the corresponding bar graphs (A). Brain clusters for the association between BFAU and BMI (B), and circulating leptin levels (C). For the corresponding bar graphs and scatterplots, the global ROI was extracted and used.
Figure 2 – No difference in BFAU was found six months after bariatric surgery (A). Brain clusters for the association between BFAU and whole-body lipid oxidation (B). Brain clusters for the association between BFAU and the change in plasma glucose at 2 years of follow-up and the corresponding scatterplot (C).
**Figure 3** – Representative coronal FLAIR image through the hypothalamus. The zoomed image shows the placement of right and left ROIs in the hypothalamus, amygdala, and putamen. Lack of correlation between the FLAIR hypothalamus/amygdala signal ratio and BFAU (B), BMI (C), and circulating IL-8 (D).