Hypothalamic inflammation improves through bariatric surgery and hypothalamic volume predicts short-term weight loss response in adults with or without type 2 diabetes.

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Twitter Summary:

Radiologic hypothalamic alterations that may suggest inflammation were present in obesity and improved after bariatric surgery. Larger hypothalamic volume negatively impacts weight loss trajectories.

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ABSTRACT:

OBJECTIVE: Preclinical research implicates hypothalamic inflammation (HI) in obesity and type 2 diabetes pathophysiology. However, their pathophysiological relevance and potential reversibility need to be better defined. We sought to evaluate the effect of bariatric surgery (BS) on radiologic biomarkers of HI and the association between the severity of such radiologic alterations and post-BS weight loss (WL)-trajectories. The utility of cerebrospinal fluid large extracellular vesicles (CSF-lEV) enriched for microglial and astrocyte markers in studying HI was also explored.

RESEARCH DESIGN AND METHODS: We included 72 individuals with obesity (20 with/52 without type 2 diabetes) and 24 controls. Participants underwent lumbar puncture and 3T magnetic resonance imaging at baseline and 1-year post-BS. We assessed mean hypothalamic diffusivity (MD) (higher values indicate lesser microstructural integrity) and the volume of the whole and main hypothalamic subregions. CSF-lEV enriched for glial and astrocyte markers were determined by flow cytometry.

RESULTS: Compared with controls, obesity and type 2 diabetes groups showed a larger volume and higher MD in the hypothalamic tubular inferior region, the area encompassing the Arcuate nucleus. These radiological alterations were positively associated with baseline anthropometric and metabolic measures and improved post-BS. A larger baseline tubular inferior hypothalamic volume was independently related to lesser WL one and two years after BS. CSF-lEV did not differ between groups and were unrelated to WL-trajectories.

CONCLUSIONS: These findings suggest HI improvement after BS and may support a role for HI in modulating the weight loss response to these interventions.

ARTICLE HIGHLIGHTS

- **Why did we undertake this study?**

  Neuroimaging studies have demonstrated hypothalamic radiologic alterations that might indicate inflammation in association with obesity and type 2 diabetes. Their pathophysiological consequences and reversibility need to be better defined.

- **What is the specific question(s) we wanted to answer?**

  We aimed to examine the effect of bariatric surgery on radiologic biomarkers of hypothalamic inflammation and to explore whether these biomarkers modulate post-surgery weight loss trajectories in people with and without type 2 diabetes.

- **What did we find?**

  Radiologic biomarkers of hypothalamic inflammation improved after surgery. A larger baseline hypothalamic volume was related to lesser post-surgery weight loss.

- **What are the implications of our findings?**

  These findings may reinforce the participation of hypothalamic inflammation in human obesity pathophysiology and its potential as a therapeutic target.

Preclinical studies suggest the participation of hypothalamic inflammation (HI) in obesity and type 2 diabetes pathophysiology (1). In mice, a high-fat diet (HFD) leads to an inflammatory response, primarily mediated by glial cells (astrocytes and microglia) and mainly located in the Arcuate nucleus (Arc) (1–3). This inflammation has been related to diminished responsiveness to peripheral anorexigenic signals and has been causally associated with obesity (1–3). While the direct participation of HI in type 2 diabetes is less well-established, a significant direct contribution has also been proposed (1,4,5).

In vivo, brain inflammatory processes might be detectable through different Magnetic Resonance Imaging (MRI) modalities. T1-weighted MRI images offer detailed macrostructural and morphological information, while T2-weighted MRI images and Diffusion Tensor Imaging (DTI) metrics from Diffusion-Weighted Imaging (DWI) like mean diffusivity (MD) reveal magnetic properties and microstructural characteristics of brain tissue. Previous cross-sectional neuroimaging studies have consistently identified micro and macrostructural hypothalamic radiologic abnormalities in individuals with obesity or type 2 diabetes (6–12). These abnormalities included increased signal on T2-weighted MRI, increased MD on DWI, and hypothalamic enlargement in T1-weighted MRI. Although all of them might indicate the presence of HI, none of these measures is specific for inflammation. Of note, both MD, which reflects water diffusivity in the brain tissue, and T2 signal, which reflects water content, increase in the presence of inflammation, but also in other conditions such as atrophy (6). Similarly, brain volume is influenced by various components like cell number or size, synaptic and vascular density, and extracellular water content. Thus, although increased grey matter volume is often viewed as a sign of neuronal integrity, it may also indicate the presence of inflammation and the existence of cytotoxic or vasogenic edema (7).

On the other hand, the physiopathological relevance of the above-mentioned radiologic alterations still needs to be fully defined. Very few prospective studies have assessed the

relationship between radiologic biomarkers of hypothalamic inflammation, future adiposity gain, and type 2 diabetes development and whether these structural biomarkers are related to weight loss (WL)-trajectories after lifestyle interventions or bariatric surgery (BS) has not been explored (13,15,16). Additionally, although preclinical models showed that HI and its metabolic consequences can be reversed by caloric restriction or BS, whether HI might also be reversible in humans remains unclear (17,18). Of note, the few studies investigating the impact of BS on hypothalamic microstructure showed mixed results (6,11,19). The limited sample in these previous studies (n=10 to 28) and the possibility that MRI might not be sensitive enough to detect gliosis reversion might explain negative findings (6,11,18,19).

In this scenario, longitudinal multimodal studies including a larger number of participants and combining both micro and macrostructural radiological evaluations with the analysis of novel biochemical biomarkers indicative of glial activation might improve our understanding of the involvement of HI in obesity and type 2 diabetes pathophysiology and might add insight into hypothalamic cell dynamics in response to BS. Among these biochemical biomarkers, extracellular vesicles (EV) might be especially interesting. EV are small blebs (30-500 nm) surrounded by a phospholipid bilayer and released by most cell types in response to different stimuli(20). Previous studies have demonstrated that microglia shed EV upon activation, and an increased concentration of EV enriched for markers of glial cell origin isolated from the cerebrospinal fluid (CSF) has been described in patients with diseases associated with glial activation (21–23).

Our main aims were to compare micro and macro hypothalamic structure between individuals with obesity (with and without type 2 diabetes) and healthy controls, to assess the reversibility of radiological hypothalamic alterations one year after BS, and to explore the association between these pre- and post-BS MRI-derived metrics and the extent of WL at short-term after

BS. As a secondary aim, we analyzed the utility of CSF-EV enriched for markers of glial cell origin in studying HI *in vivo*.

RESEARCH DESIGN AND METHODS

Study design and participants

We conducted a prospective longitudinal study across two centers between 2017 and 2021, involving 72 individuals with obesity eligible for BS at Hospital Clínic de Barcelona (52 without type 2 diabetes [obesity group] and 20 with type 2 diabetes [diabetes group]), frequency-matched by age and sex in a 3:1 ratio with controls (n=24) sourced from the Sant Pau Initiative on Neurodegeneration (SPIN) cohort (20).

Inclusion criteria were age between 18 and 70 years, BMI ≥40 Kg/m^2 or ≥35 Kg/m^2 in the presence of obesity-related comorbidities. Exclusion criteria were a personal history of cardiovascular or chronic inflammatory diseases, neurodegenerative or unstable psychiatric disorders, common MRI-scan and LP contraindications, and a body weight change ≥5.0% in the 3 months before the baseline assessment. Type 2 diabetes and normal glucose tolerance were defined according to ADA guidelines(25). Participants with type 2 diabetes using more than once daily basal insulin and presenting microvascular complications or HbA1c>8.5% were also excluded.

The study protocol was approved by the institutional ethical committee of both study centers (Reg. HCB/2018/0619; Reg. IIBSP-DOW-2014-30). Written informed consent was obtained from all the study participants.

Study procedures

The obesity and type 2 diabetes groups were evaluated before and 1 year after BS. Forty-seven of 71 (66.2%) study participants included in the obesity and type 2 diabetes groups underwent Roux-en-Y gastric bypass (RYGB) and 24 (33.8%) sleeve gastrectomy (SG). The proportion of

individuals with type 2 diabetes undergoing RYGB and SG was similar (RYGB: 55.0% *vs.* SG: 45.0%, p=0.212). Body weight after BS was recorded for 2 years. The control group was assessed on a single occasion. Study procedures included body composition analysis by dual-energy X-ray absorptiometry (DXA), fasting blood analysis, mixed meal tolerance test (MMTT), structural T1-weighted and DWI scans, and lumbar puncture (LP). The flow diagram for study participants is presented in Figure S1. The MRI acquisition site and protocol were modified in the second year of the study. Thus, DWI sequences were only available for a subgroup of participants (69.0%). Participants with or without DWI sequences were comparable in age, sex, and BMI (Table S1). However, in the DWI subgroup, participants with obesity were slightly younger than controls and individuals with type 2 diabetes (p=0.064).

Metabolic assessment

MMTT and DXA, including estimated visceral adipose tissue (eVAT), were conducted as previously described (26). HOMA-IR and Matsuda indexes were calculated (27,28).

Lumbar puncture

Neurologists with expertise in the procedure performed the LP. CSF was collected by free-flow/dripping following international consensus recommendations (29).

Isolation of large EV from cerebrospinal fluid

Large EV (lEV) were isolated from CSF as previously described, with slight modifications (26). A detailed description of these analyses can be consulted in the Supplemental Methods section of the Supplementary material.

CSF analyses were conducted in control group participants with available CSF and those from the obesity and diabetes groups with available CSF samples at the baseline and post-BS assessments (Figure S1). Individuals included in the CSF analyses did not differ in age or BMI from those not included. Sex proportion was also maintained except for a larger representation of female participants in the control group (Table S1).

Magnetic resonance imaging acquisition and processing

MRI data were acquired at two different sites in Barcelona, Spain: Hospital Clínic (Hosp-C) – 3T Siemens MAGNETOM Prisma scanner– and Hospital del Mar (Hosp-M) – 3T Philips Achieva scanner–. Baseline and longitudinal MRI scans were obtained at the identical site for every individual participant. DWI was only available at Hosp-C. High-resolution 3D T1-weighted structural images were acquired in the sagittal plane (Hosp-C: Repetition Time [TR]=2,300ms, Echo Time [TE]=2.98ms, voxel size=1.0x1.0x1.0mm; Hosp-M: TR=6,672ms, TE=60ms, voxel size=1.64x1.64x1.64mm) for anatomical segmentation. A single-shot 2D spin-echo echo-planar imaging (EPI) sequence was employed (TR=7,700ms, TE=89ms, voxel size=2.05x2.05x2.0mm), applying diffusion gradients along 30 directions with a b-value of 1000 s/mm² and a baseline image without diffusion weighting. All the MRI were inspected by an expert neuroradiologist in each center before the analyses to check for incidental lesions and quality control.

From the structural T1-weighted images, putamen and intracranial volumes (ICV) were estimated using the automated *FreeSurfer* v7.0 (https://surfer.nmr.mgh.harvard.edu/). The automated protocol introduced by Billot et al. was used to segment the hypothalamus and its subunits: anterior-superior (a-sHyp); anterior-inferior (a-iHyp); superior tuberal (supTub); inferior tuberal (infTub); and the posterior hypothalamus (posHyp) (30). The hypothalamic segmentations were visually inspected by trained neuroimaging processing technicians.

The regional volumes were normalized by ICV to assess intergroup differences. Raw hypothalamic volumes were used for paired data before and after BS. Further details concerning the correction for ICV are available at https://surfer.nmr.mgh.harvard.edu/fswiki/eTIV.

The hypothalamic location and composition are presented in Figure 1. Hypothalamic subunit images were generated with ITK-SNAP (open-source software for segmenting anatomical structures; http://www.itksnap.org/).

Processing of DWI included eddy current-induced distortion correction, denoising with MRtrix3 (an open-source, cross-platform software for medical image processing; https://www.mrtrix.org/), and EPI distortion correction. DTI data were estimated using diffusion imaging in Python (Dipy) (31) to subsequently derive MD maps. Finally, the mean MD for the entire hypothalamus and its main subunits was calculated. To overcome challenges in accurately quantifying MD in small hypothalamic subunits, larger regions comprising both right and left sides (supTub, infTub, posHyp) were defined. Notably, the analysis excluded regions a-sHyp and a-iHyp due to the limited number of voxels corresponding to these areas.

Statistical analysis

Data are presented as median (25^{th}-75^{th} percentile), mean±standard deviation (SD), or number (percentage).

Among primary study variables, MD and raw hypothalamic volumes displayed a normal distribution, and head-standardized hypothalamic volumes and CSF-lEV concentration exhibited a non-normal distribution even after log transformation.

Inter-group differences assessment and correlation analyses were conducted using parametric (ANOVA and ANCOVA with Bonferroni-corrected post-tests and Pairwise Pearson) or non-parametric tests (Kruskal-Wallis's rank test, followed by Wilcoxon-Mann-Whitney test and Spearman's rank test).

Pre- and post-BS changes in raw hypothalamic and putamen volumes and MD were assessed in the whole cohort with repeated measures ANCOVA test. An interaction term was introduced in these models to test the effect of type 2 diabetes status and the type of BS on the study

outcomes. Stratified analyses were conducted when a significant interaction was present. Pre- and post-BS CSF- lEV profiles were compared using the Wilcoxon signed-rank test.

The hypothalamic volumetric changes were calculated as the percentage of initial volume minus the final volume (1-year post-BS) divided by the initial volume. The association between baseline and post-BS MRI-derived metrics and the extent of WL achieved 1 and 2 years after BS was analyzed with linear regression analyses. Backward stepwise linear regression models were applied to evaluate the contribution of those variables associated with the percentage of total weight loss (TWL). A p-value threshold of 0.1 was used to limit the total number of variables at each step. The association between baseline CSF-lEV concentrations and weight loss was explored by applying Spearman's rank test.

The statistical analysis was performed with STATA/IC 15.0 (StataCorp.; College Station, TX, USA) for Windows. All p-values are two-sided, and the significance level was defined as a p-value <0.05.

RESULTS

1. Obesity and type 2 diabetes were linked to hypothalamic microstructural alterations and enlargement of the hypothalamic subregion encompassing the Arcuate nucleus.

Demographical, clinical, anthropometrical, biochemical, and neuroimaging data of the study groups at baseline is displayed in Table 1.

a) Hypothalamic mean diffusivity.

In univariate ANOVA analysis, hypothalamic MD tended to be higher in obesity and diabetes groups than in controls. When examining various hypothalamic subregions, obese and diabetes groups showed higher MD in the infTub subunit than controls, with no differences in posterior and supTub regions or putamen (selected as reference region) (Table 1).

In correlation analyses, higher hypothalamic MD was associated with higher hs-CRP, larger BMI, higher fasting plasma glucose (FPG), and lower peripheral insulin sensitivity measured by the Matsuda index (Figure S2). However, in a multivariable linear regression analysis incorporating age, sex, BMI, and these metabolic variables, only age (β: 0.350, p=0.027), female sex (β: -0.397, p=0.012), and BMI (β: 0.550, p=0.048) remained significantly associated with hypothalamic MD.

In ANCOVA analyses, after adjusting for age and sex, differences in whole hypothalamic MD between the three study groups were reinforced (p_{group}=0.005). In post-hoc comparisons, both obesity and diabetes groups (p=0.002 and p=0.035, respectively) showed higher age and sex-adjusted whole hypothalamic MD compared to controls without differences between them (p=0.298). These differences were present across all the explored hypothalamic subunits, including posterior (p_{group}=0.014), infTub (p_{group}=0.006), and supTub (p_{group}=0.006). In contrast, the lack of association between study groups and putamen MD was unmodified with the inclusion of age and sex as covariates (p_{group}=0.311).

b) Hypothalamic volumes.

As shown in Table 1, obesity and diabetes groups showed larger head-standardized right infTub subunit (the region comprising the Arc) than controls, without differences between them. A similar tendency was found for the left infTub subunit, albeit statistical significance was not achieved (p=0.074). No significant volumetric differences were observed between the three study groups for the whole left or right hypothalamus, other hypothalamic subunits, or the putamen.

In correlation analyses, infTub volumes were positively and bilaterally associated with hs-CRP, eVAT and HbA1c (Figure S3). No significant associations between left or right infTub volumes and age, BMI, or indices of insulin sensitivity were observed.

c) Cerebrospinal fluid - lEV profile.

The concentration of CSF-lEV enriched for markers of microglial or astrocyte origins was comparable between the study groups (Table 1). There were no associations between microglia/astrocyte-derived CSF-lEV and age, anthropometric, metabolic, inflammatory, or neuroimaging variables (Table S2).

2. Microstructural hypothalamic alterations improved, and hypothalamic volume decreased after bariatric surgery.

Post-BS changes in anthropometrical, metabolic, and biochemical variables in the diabetes and obesity groups are detailed in Table S3.

a) Hypothalamic mean diffusivity.

MD hypothalamic values decreased 1 year after BS ($15.5\pm0.2*10^{-4}$ *vs*. $15.2 \pm0.2*10^{-4}$, p=0.035). Hypothalamic MD changes were comparable in individuals with or without type 2 diabetes ($p_{time*type\ 2\ diabetes}$=0.820) and between those who underwent RYGB or SG ($p_{time*type\ of\ BS}$ =0.750). The post-BS reduction in MD was mainly mediated by MD decreases in the infTub and supTub subunits (Figure S4). There were no changes in putaminal MD values ($24.4\pm0.1*10^{-3}$ *vs*. $24.5\pm0.2*10^{-3}$, p=0.264).

Baseline between-groups differences in age- and sex-adjusted hypothalamic MD were no longer observed after surgery (p_{group}=0.083).

b) Hypothalamic volumes.

At the 1-year evaluation, there was a reduction in the left but not in the right whole hypothalamic volume (p<0.001 and p=0.681, respectively). When analyzing the different hypothalamic regions, significant volume size reductions were observed in the left a-iHyp (p=0.037), bilateral a-sHyp (left: p=0.013; right: p=0.010), left posHyp (p=0.004), left infTub (p=0.015), and left supTub (p=0.023) subunits (Figure 2[A], Table S3). No variation in the putamen volume was observed bilaterally (left: p=0.587; right: p=0.184).

The extent of volumetric changes in the whole left hypothalamus did not vary according to the type of BS ($p_{time*type\ of\ BS}$=0.277) but differed by pre-BS type 2 diabetes status ($p_{time*type\ 2\ diabetes}$=0.014). In stratified analyses, volumetric changes in the diabetes group were non-significant and numerically minor compared to the obesity group (Figure 2[B-C], Table S3).

At the post-BS assessment, individuals with type 2 diabetes antedating surgery, but not those without, still presented larger head-standardized right infTub volume than controls (p=0.004 and p=0.236, in post-hoc analyses, respectively).

c) Cerebrospinal fluid lEV profile.

At the 1-year follow-up, the concentration of CSF-lEV was not significantly modified: 10,145.4 (6,491.8-12,420.6) pre-BS *vs.* 10,454.7 (7,529.1-13,269.2) post-BS, p=0.079. The concentration of CSF-lEV enriched for markers of microglial and astrocyte origins significantly increased post-BS (Figure S5).

Changes in CSF-lEV profile did not vary according to the pre-BS type 2 diabetes status or by type of BS (p >0.05 for all;).

The post-BS CSF-lEV profile in the diabetes and obesity groups did not significantly differ from that observed in controls (Figure S5).

3. Prospective association between biomarkers of hypothalamic inflammation and short-term weight loss trajectories.

The mean TWL at the 1- and 2-year post-BS evaluation was 31.5±8.5% and 31.8±9.1%, respectively. TWL was comparable between individuals undergoing RYGB and SG (1-year: p=0.145; 2-year: p=0.242) and obesity and diabetes groups (1-year: p=0.464; 2-year: p=0.375).

a) MRI-derived metrics.

Among the different baseline MRI-derived metrics, only infTub subunit size was associated with TWL in univariate linear regression analyses (Table 2). Larger infTub subunit volumes at baseline were bilaterally associated with lesser TWL 1 and 2 years after BS (Model 1). This

association was independent of age, sex, baseline BMI, type 2 diabetes, and type of BS (Model 2 and Model 3) In backward stepwise linear regression analyses including infTub volume, age, sex, type of BS, baseline BMI, and pre-BS type 2 diabetes status, only the infTub subunit size at baseline (β: -0.098, p=0.014) remained as a significant predictor of 1-year TWL, explaining 8.2% of the TWL variability at this time point. At the 2-year follow-up, only larger infTub volume at baseline (β: -0.124, p=0.007), undergoing SG instead of RYGB (β: 4.331, p=0.088), and older age (β: -0.240, p=0.065) remained significantly associated to a lesser TWL. These variables explained 18.6% of weight variability at this time point.

No association was observed between the 1-year MRI-derived hypothalamic metrics and the TWL at the 2-year follow-up (data not shown).

b) Cerebrospinal fluid - lEV profile.

No associations were observed between baseline or 1-year concentrations of CSF-lEV enriched for markers of microglia or astrocyte origin and the TWL at 1- or 2 years post-BS (data not shown).

CONCLUSIONS

Our study reinforces the existence of radiologic hypothalamic alterations that might indicate HI in individuals with obesity (with or without type 2 diabetes) and expands previous literature by adding insight into their improvement through BS. Furthermore, it supports the physiological importance of such neuroimaging findings by demonstrating an independent association with worse weight outcomes after BS.

First, in our study, as in previous ones, obesity was found to be associated with the presence of altered hypothalamic microstructure that might indicate inflammation and hypothalamic enlargement within the subregion where the Arc is located (10,13,14,28–32). Furthermore, our multimodal neuroimaging assessment strengthens the indication that inflammation is the potential underlying mechanism for the observed volumetric differences. Of note, we found a

co-localized increase in hypothalamic volume and MD and a joint decrease in these measures at the post-BS reassessment. As previously mentioned, increased brain volume may arise from heightened cell number or size, increased synaptic or vascular density, or vasogenic edema. However, only this latter condition is linked to MD increases, as heightened cellularity or vascularization restricts water diffusion, causing decreased MD (33,34). Our neuroimaging findings were also consistent with earlier pre-clinical studies conducted in mice, showing increased vascular permeability and augmentation in hypothalamic volume attributable to vasogenic edema in response to prolonged exposure to a HFD (35–37).

On the other hand, we did not observe any differences in hypothalamic volumetrics or MD between individuals with or without type 2 diabetes. This is in contrast to a recent prospective study showing a progressive rise in gliosis (measured as T2 relaxation time) in the medio-basal hypothalamus (MBH) across normal glucose tolerance, pre-diabetes, and type 2 diabetes states independently of adiposity (13). The limited number of participants with diabetes and MD data in our study and the possibility that microstructural indices might be more sensitive than volume to detect HI might explain this discrepancy. Nonetheless, we observed a positive association between HbA1c and infTub volume that might support a association between HI and altered glucose homeostasis.

Second, one year after BS, we detected a significant decrease in hypothalamic MD and a size reduction of the left hypothalamus, suggesting a partial reversal of HI. Volumetric reduction affecting other than the infTub subunit might indicate that obesity-associated HI is not restricted to Arc. In this regard, in the study by Brown et al., with a larger sample size than ours (n=1,111 adults from the Human Connectome Project), the positive association between BMI and hypothalamic volumes was more widespread (14). Thus, it might be possible that our study was underpowered to fully detect baseline volumetric differences but powered enough to capture its changes after BS. Similarly, although the significance of lateralized volumetric

changes in our study is unclear, it deserves further investigation. Pre-clinical studies suggest a lateralized hypothalamic control for food intake and energy metabolism with dominance for the right side on the anorexigenic response (37).

Our data might also suggest impaired hypothalamic recovery in people with type 2 diabetes. Although the extent of MD changes was comparable, hypothalamic volumetric changes were smaller in participants with diabetes than in those without. In addition, at the post-BS assessment, individuals with type 2 diabetes still showed increased hypothalamic volume in the Arc area compared to controls.

Only three previous studies have evaluated changes in hypothalamic microstructure after BS. In contrast to our findings, Kreutzer et al.(6) and Rebelos et al.(19) reported no significant changes, at 6 and 10 months, respectively. Discrepancies with our study may arise from limited sample sizes (n=10 in Kreutzer et al. and n=24 in Rebelos et al.), varying neuroimaging protocols, and differences in the time elapsed between BS and the radiologic reassessment. Conversely, Van de Sande-Lee et al. (13) found a significant reduction in hypothalamic relaxation time 9 months post-RYGB in a cohort of 11 participants with and 17 without diabetes. Contrary to our findings, they observed larger radiologic improvement in individuals with diabetes. However, in this research, hypothalamic microstructure in the non-type 2 diabetes group was similar to that observed in controls. Additionally, at the post-BS assessment, T2 relaxation time was still longer in participants with type 2 diabetes, which would also suggest more permanent hypothalamic damage in this group. Further studies, with larger sample sizes and longer follow-ups, would be necessary to define better whether type 2 diabetes modulates the effects of BS on HI and to establish the clinical relevance of post-BS hypothalamic changes on sustaining BS benefits on body adiposity and glucose homeostasis (39).

Third, as a main addition to the literature, we demonstrated an association between structural biomarkers of HI, specifically at the Arc location, and post-BS WL-trajectories. In our study, a larger infTub subunit at baseline was independently and bilaterally associated with lesser WL in the short term after BS. This finding might reinforce the pathological significance of the baseline volumetric and microstructural alterations observed in our study. It also complements data from previous studies showing an association between a greater degree of microstructural MBH alterations and increased susceptibility to future adiposity gain and the development of metabolic complications (13,15,16).

Finally, we did not observe between-group differences in the glial cell-derived CSF-lEV nor an association between its concentrations and anthropometric, metabolic, or neuroimaging variables. Furthermore, contrary to our hypothesis, we detected a significant increase in the CSF-lEV concentration post-BS. As we only evaluated lEV concentration but not cargo, our analyses cannot distinguish between glial phenotypes or localize the regions primarily involved in glial and astrocyte-derived lEV production. Thus, the significance of post-BS increases in the analyzed CSF-lEV remains to be elucidated. In any case, our data did not support its utility in studying HI.

Our study has limitations. The lower-than-anticipated number of individuals with DWI acquisitions might have limited our statistical power to detect differences between groups in radiologic measures at the baseline and post-BS evaluations. Women were overrepresented, and thus, our results might not be generalizable to men. The applied hypothalamic segmentation protocol cannot delineate the Arc in an isolated manner but locates it within the infTub subunit, which also comprises other nuclei. Also, both MD and volume lack specificity for inflammation, preventing the establishment of histopathological changes underlying the neuroimaging findings. Our study cannot disentangle if hypothalamic radiologic improvement was primarily mediated by WL, BS, or dietary changes. In this regard, HFD has been identified

as the primary mechanism involved in the development of HI in mice. CSF-lEV cargo was not examined, so whether BS impacts lEV functional properties deserves further research. Finally, our study was observational, and causal relationships cannot be established.

In conclusion, people with obesity present micro and macrostructural hypothalamic alterations that might indicate inflammation. These radiologic alterations are partially reversible through BS and independently associated with a poorer WL response. These data suggest HI as a potential target for treating obesity and its co-morbidities.

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analysis and interpretation. A.P, A.J, G.C-B, E.O, and J.V facilitated the literature research and review. A.J and J.F designed the study. All authors helped interpret the data, read the final version of the manuscript, and approve its submission. A.P and A.J are the guarantors of this study and, as such, had full access to all the data in the study and take responsibility for the integrity and accuracy.

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Table 1. Baseline clinical, anthropometric, biochemical, neuroimaging data and cerebrospinal fluid-lEV concentrations.

	Control group (n=24)	Obesity group (n=52)	Type 2 diabetes group (n=20)	$p_{\text{groups baseline}}$
Clinical and metabolic variables				
Age (years)	51.9 ± 9.9	47.5 ± 9.4	51.1 ± 5.3	0.085
Females (n, %)	17 (70.8)	46 (88.5)	15 (75.0)	0.136
BMI (Kg/m^2)	23.7 ± 2.6	44.0 ± 4.6*	42.9 ±4.0*	<0.001
Body weight (Kg)	65.4 ± 10.4	117.1 ± 17.9*	114.2 ± 15.0*	<0.001
eVAT (cm^3)	348.5 (230.0 - 604.5)	2,194 (1,720 - 2,701)*	3,011 (2,564.0 - 3,821.0)*†	<0.001
SBP (mmHg)	116.7 ± 13.7	132.6 ± 17.4*	136.0 ± 20.3*	<0.001
DBP (mmHg)	73.2 ± 7.3	82.8 ± 11.3*	84.7 ± 11.5*	<0.001
FPG (mg/dl)	87.5 (83.5 - 92.0)	90.0 (86.0 - 96.0)*	121.0 (108.5 - 142.0)*†	<0.001
HbA1c (%)	5.5 (5.1 - 5.7)	5.5 (5.2 - 5.9)	6.4 (5.9 - 7.2)*†	<0.001
HbA1c (mmol/mol)	37.0 (32.0 - 39.0)	37.0 (33.0 - 41.0)	46.0 (41.0 - 55.0)*†	<0.001
Total cholesterol (mg/dl)	200.0 ± 38.5	187.5 ± 37.2	192.9 ± 36.2	0.439
LDL (mg/dl)	129.9 ± 35.9	117.7 ± 27.0	109.0 ± 25.7	0.128
HDL (mg/dl)	61.2 ± 12.8	48.8 ± 10.8*	46.5 ± 9.6*	<0.001
TG (mg/dl)	74.7 (59.5 - 84.4)	104.0 (80.5 - 149.0)*	158.5 (102.0 - 214.0)*†	<0.001
hs-CRP (mg/dl)	0.08 (0.04 - 0.10)	0.63 (0.44 - 0.96)*	0.67 (0.33 - 1.31)*	<0.001
HOMA-IR	1.2 (0.9 - 1.5)	3.9 (2.8 - 6.9)*	9.1 (7.3 - 11.0)*†	<0.001
Matsuda index	6.9 (5.9 - 8.0)	2.6 (1.7 - 3.5)*	1.4 (0.9 - 1.9)*†	0.001
Mean diffusivity (mm^2/s)$^{\Delta}$				
Whole hypothalamus	14.6 ± 0.2 $*10^{-4}$	15.9 ± 0.2 $*10^{-4}$	15.6 ± 0.1 $*10^{-4}$	0.056
Posterior hypothalamus	15.9 ± 0.2 $*10^{-4}$	16.8 ± 0.2 $*10^{-4}$	16.9 ± 0.1 $*10^{-4}$	0.254
Tubular-inferior hypothalamus	15.6 ± 0.2 $*10^{-4}$	17.1 ± 0.2 $*10^{-4*}$	16.7 ± 0.1 $*10^{-4}$	0.028
Tubular-superior hypothalamus	12.8 ± 0.2 $*10^{-4}$	13.8 ± 0.2 $*10^{-4}$	13.5 ± 0.1 $*10^{-4}$	0.106
Putamen	24.5 ± 10.6$*10^{-3}$	24.4 ± 6.7$*10^{-3}$	24.9 ± 12.6$*10^{-3}$	0.389
Hypothalamic subunits and putamen volumes normalized to ICV$^{\Delta}$				

Anterior-inferior	Left	1.3 (1.1 - 1.5)*10^{-5}	1.4 (1.2 - 1.6)*10^{-5}	1.6 (1.3 - 1.8)*10^{-5}	0.075
	Right	1.3 (1.0 - 1.4)*10^{-5}	1.3 (1.1 - 1.6)*10^{-5}	1.5 (1.2 - 1.7)*10^{-5}	0.184
Anterior-superior	Left	1.6 (1.5 - 1.9)*10^{-5}	1.8 (1.5 - 2.0)*10^{-5}	1.7 (1.6 - 2.0)*10^{-5}	0.473
	Right	1.6 (1.4 - 1.8)*10^{-5}	1.7 (1.5 - 2.1)*10^{-5}	1.8 (1.4 - 2.1)*10^{-5}	0.179
Posterior	Left	8.0 (7.5 - 9.0)*10^{-5}	8.7 (7.6 - 10.3)*10^{-5}	8.5 (8.2 - 9.3)*10^{-5}	0.306
	Right	8.6 (7.8 - 9.3)*10^{-5}	8.9 (8.2 - 10.4)*10^{-5}	9.2 (8.0 - 9.9)*10^{-5}	0.282
Tubular-inferior	Left	9.4 (8.9 - 9.8)*10^{-5}	10.0 (9.0 - 11.3)*10^{-5}	10.0 (9.4 - 11.1)*10^{-5}	0.074
	Right	8.6 (8.5 - 9.0)*10^{-5}	9.3 (8.4 - 9.8)*10^{-5*}	9.2 (8.6 - 10.1)*10^{-5*}	0.030
Tubular-superior	Left	7.6 (7.0 - 8.4)*10^{-5}	8.2 (7.3 - 9.5)*10^{-5}	7.8 (7.0 - 8.8)*10^{-5}	0.128
	Right	7.5 (7.0 - 8.5)*10^{-5}	8.0 (7.5 - 9.4)*10^{-5}	7.9 (7.4 - 9.1)*10^{-5}	0.211
Whole	Left	28.4 (26.3 - 30.5)*10^{-5}	29.2 (27.6 - 34.6)*10^{-5}	29.5 (27.9 - 31.3) *10^{-5}	0.120
	Right	27.6 (25.8 - 29.5)*10^{-5}	29.1 (27.1 - 33.7)*10^{-5}	29.4 (27.7 - 31.9)*10^{-5}	0.093
Putamen	Left	3.15 (2.90 - 3.41)*10^{-3}	3.38 (3.00 - 3.67)*10^{-3}	3.26 (2.93 - 3.57)*10^{-3}	0.158
	Right	3.21 (3.01 - 3.54)*10^{-3}	3.37 (3.07 - 3.73)*10^{-3}	3.35 (3.00 - 3.58)*10^{-3}	0.384
Cerebrospinal fluid – lEV (lEV/µl CSF)$^{\Delta}$					
$CSFE^+$		12,959.5 (10,739.2 - 13,933.9)	9,282.6 (6,182.2 - 12,183.4)	10,893.9 (6,797.3 - 16,784.5)	0.074
$CSFE^+/IB4^+$		5,975.0 (2,845.3 - 10,840.9)	6,331.8 (4,649.9 - 8,063.4)	4,149.4 (3,082.0 - 12,557.6)	0.762
$CSFE^+/CD14^+$		11,484.8 (5,490.6 - 16,453.74)	9,891.4 (5,529.7 - 14,729.5)	4,933.2 (3,611.9 - 14,337.3)	0.500
$CSFE^+/ACSA^+$		5,090.8 (2,711.6 - 9,342.6)	3,806.8 (870.8 - 6,491.0)	2,827.7 (1,115.9 - 8,484.4)	0.745

Data are presented as median (25th-75th percentile), mean±standard deviation, or number (percentage).

Inter-group differences were assessed using both parametric (ANOVA with Bonferroni-corrected post-tests) and non-parametric tests (Kruskal-Wallis's rank test, followed by Wilcoxon-Mann-Whitney test) as deemed appropriate for the data.

$^{*}p<0.05$ compared with the control group; $^{\dagger}p<0.05$ compared with the obesity group.

$^{\Delta}$ The available sample for volumetric data corresponds to N=22 for controls, N=44 for the obesity and N=18 for the type 2 diabetes groups; for mean diffusivity, it corresponds to N=16 for controls, N=29 for the obesity and N=13 for the type 2 diabetes groups; for CSF-lEV, it corresponds to N=16 for controls, N=11 for the type 2 diabetes and N=26 for the obesity groups.

$CSFE^+$: lEV positive for carboxyfluorescein succinimidyl ester (indicative of membrane integrity); $CSFE^+/ACSA^+$: lEV positive for CSFE and astrocyte cell surface antigen (a marker of astrocyte origin); $CSF^+/CD14^+$: lEV positive for CFSE and CD14 (a marker of microglial origin); $CSFE^+/IB4^+$: lEV positive for CFSE and isolectin B4 (a marker of microglial origin); DBP: diastolic blood pressure; eVAT: estimated visceral adipose tissue; FPG: fasting plasma glucose; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; hs-CRP: high sensitivity C reactive protein; lEV: large extracellular vesicles; TG: triglycerides; SBP: systolic blood pressure.

Table 2. Regression analysis between tubular inferior volume at baseline and total weight loss percentage at one and two years after bariatric surgery.

		Tubular inferior volume			Left tubular inferior volume			Right tubular inferior volume		
		β	SD	p-value	β	SD	p-value	β	SD	p-value
1-year TWL	Model 1	-0.098	0.039	0.014	-0.160	0.075	0.036	-0.188	0.073	0.007
	Model 2	-0.119	0.045	0.011	-0.187	0.090	0.042	-0.224	0.081	0.008
	Model 3	-0.116	0.048	0.019	-0.174	0.096	0.074	-0.219	0.084	0.012
2-year TWL	Model 1	-0.128	0.046	0.008	-0.242	0.086	0.007	-0.208	0.089	0.023
	Model 2	-0.133	0.055	0.020	-0.220	0.101	0.032	-0.228	0.104	0.033
	Model 3	-0.133	0.058	0.026	-0.216	0.107	0.050	-0.229	0.106	0.032

Model 1: unadjusted; **Model 2:** adjusted by age, sex, and type of bariatric surgery (Roux-en-Y gastric bypass or sleeve gastrectomy); **Model 3:** adjusted by age, sex, type of bariatric surgery (Roux-en-Y gastric bypass or sleeve gastrectomy), pre-bariatric surgery type 2 diabetes status, and baseline body mass index.

TWL: percentage of total body weight loss. SD: standard deviation

Figure Legends

Figure 1. Hypothalamus location in the brain and specific subunits.

A: anterior; P: posterior; L: left; R: right; S: superior, I: inferior.

a-iHyp: the anterior-inferior hypothalamus; a-sHyp: the anterior-superior hypothalamus; infTub: the inferior tuberal hypothalamus; supTub: the superior tuberal hypothalamus; posHyp: the posterior hypothalamus.

Figure 2. Percentual volumetric hypothalamic changes (**A**) and their statistical significance (**B**) across hypothalamic subunits following bariatric surgery in the whole cohort and in the obese and type 2 diabetes groups.

Volumetric changes were calculated as the percentage of initial volume minus the final volume (1-year post-BS) divided by the initial volume. Calid colors represent a volumetric decrease after surgery, and cold colors represent a volumetric increase.

A: anterior; P: posterior; L: left; R: right. a-iHyp: the anterior-inferior hypothalamus; a-sHyp: the anterior-superior hypothalamus; infTub: the inferior tuberal hypothalamus; supTub: the superior tuberal hypothalamus; posHyp: the posterior hypothalamus.