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Complete List of Authors:	Vreeken, Debby; Rijnstate Hospital, Vitalys Clinic, Surgery; Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Anatomy Wiesmann, M; Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Anatomy Deden, Laura; Rijnstate Hospital, Vitalys Clinic, Surgery Arnoldussen, Ilse; Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Anatomy Aarts, Esther; Donders Institute for Brain, Cognition and Behaviour, Radboud university Kessels, Roy; Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Medical Psychology; Vincent van Gogh Institute for Psychiatry Kleemann, Robert; Netherlands Organization for Applied Scientific Research (TNO), Metabolic Health Research Hazebroek, Eric; Rijnstate Hospital, Vitalys Clinic, Surgery Aarts, Edo; Rijnstate Hospital, Vitalys Clinic, Surgery Kiliaan, Amanda; Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Anatomy
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Vreeken, D.[1,2,3], Wiesmann, M.[3], Deden, L.N.[1,2], Arnoldussen, I.A.C.[3], Aarts, E.[4], Kessels, R.P.C.[4,5,6], Kleemann, R.[7], Hazebroek, E.J.[1,2], Aarts, E.O.[1,2*], Kiliaan, A.J.[3*†]

[1] *Department of Surgery, Rijnstate Hospital, Arnhem, the Netherlands.*

[2] *Vitalys Clinic, Velp, the Netherlands.*

[3] *Department of Anatomy, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, the Netherlands.*

[4] *Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, the Netherlands.*

[5] *Department of Medical Psychology, Radboud University Medical Center, Nijmegen, the Netherlands.*

[6] *Vincent van Gogh Institute for Psychiatry, Venray, the Netherlands.*

[7] *Department of Metabolic Health Research, Netherlands Organization for Applied Scientific Research (TNO), Leiden, the Netherlands.*

[*] These authors contributed equally.

[†] **Corresponding author:**

Amanda J. Kiliaan, PhD

Donders Institute for Brain, Cognition, and Behaviour

Radboud university medical center

Department of Anatomy (109)

Geert Grooteplein 21N

6525 EZ Nijmegen, the Netherlands

Phone: +31 24 361 4378

Email: amanda.kiliaan@radboudumc.nl

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ABSTRACT

Introduction

Weight loss after bariatric surgery (BS) is associated with improved cognition and structural brain recovery. However, this improved cognition after BS is not equally exhibited across patients and even decline of cognitive function has been reported. Due to relatively short follow-up and small samples of BS patients in earlier performed studies, it is complicated to elaborate on long-term consequences of weight loss, obesity and related diseases.

The aim of the BARICO study (**BA**riatric surgery **R**ijnstate and Radboudumc neuro**I**maging and **C**ognition in **O**besity) is to determine the longitudinal effect of weight loss after BS on outcomes of brain function and structure, using sensitive neuropsychological tests and (functional) MRI parameters. Secondary endpoints are metabolic and inflammation status of adipose tissue, liver and gut, in relation to brain structure and function. Also, the relation between weight loss and gut microbiota composition change and its correlation with neuropsychological outcomes will be investigated.

Methods and analysis

Data on 150 Dutch patients (between 35 and 55 years old, men and women) will be collected at different time points ranging from two months before, up to ten years after surgery. Neuropsychological tests, questionnaires, blood, faeces and several tissues will be collected before, during and after surgery to measure cognition, microbiota, metabolic and inflammation status over time by blood analyses. A subgroup of 75 participants will undergo (functional) MRI scanning in relation to executive functioning using the Stroop task, grey and white matter volumes and cerebral blood flow. Regression analyses will be used to explore associations between weight loss and the outcome measures.

Ethics and dissemination

This study is approved by the medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17). Findings of this research will be published in peer-reviewed journals and conference presentations.

Trial registration

The Netherlands National Trial Register (trialregister.nl) 7288. Date registered: 29-June-2018.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- For the first time in bariatric research, neuroimaging, neuropsychological tests and metabolic and histopathological parameters will be combined to investigate the effect of weight loss after bariatric surgery on brain function and structure.
- Collecting and investigating also multiple metabolic parameters (obtained from blood, various tissue and microbiota) may help to reveal the relation and underlying mechanisms between obesity and brain function and structure.
- With a follow-up of 10 years after bariatric surgery, we will be able to gain knowledge about the long-term effects of weight loss on cognitive function.
- Only bariatric surgery patients are included in this study, so whether the results are generalizable to obese patients in general will require further investigation.

INTRODUCTION

For over two decades, obesity-induced diseases, such as cardiovascular disease, and type 2 diabetes are one of the major health-care challenges of today's society.(1) Besides these well-known metabolic complications, it has become clear that obesity may lead to structural brain changes, cognitive impairment and neurodegenerative diseases.(2-5) A direct relationship exists between increased body mass and cognitive impairment.(6-9) To improve and possibly reduce the amount of obesity-induced diseases and inhibit cognitive impairment and neurodegenerative diseases, sustainable long-term weight loss in obese patients is required. Non-surgical treatments for obesity, such as dietary restriction and physical activity, often show disappointing long-term effects, especially in patients with morbid obesity (BMI above 40 kg/m^2).(10, 11) Bariatric surgery (BS), decreases body mass rapidly, and especially the commonly performed Roux-en-Y gastric bypass (RYGB) leads to this rapid weight loss which is often accompanied by remission of type 2 diabetes mellitus, hypertension and hyperlipidaemia.(12, 13) RYGB is a restrictive surgical procedure, excluding the main part of the stomach, the duodenum and the first part of the jejunum from the passage of food, leading to, among others, hormonal and gut microbiota changes.(14, 15)

Besides weight loss and remission of comorbidities, RYGB surgery is also associated with improved cognitive functions.(16, 17) This may be related to multiple metabolic parameters, such as systolic blood pressure or triglyceride concentrations.(18) Metabolic complications may arise in obesity due to a disturbed interaction between metabolic organs such as the adipose tissue, liver and the gut. Especially in midlife (between the age of 35 and 55), it has been reported that obesity, may impair cognitive functioning and increase the risk for dementia. However, mechanisms involved in this organ-organ crosstalk are poorly understood.(4, 19-22) One proposed mechanism is the altered signalling of visceral and abdominal adipose tissue. Adipose tissue acts as an independent endocrine organ releasing several hormones, proteins and cytokines, referred to as adipokines. Obesity is associated with dysfunctional white adipose tissue and therefore an imbalance in adipokines, such as increased levels of leptin and angiotensinogen, and low levels of adiponectin and omentin.(23, 24)

Especially, visceral adipose tissue seems to produce unfavourable adipokines and is associated with more metabolic complications when compared to subcutaneous adipose tissue.(25-28) Importantly, distribution of fat tissue depots differs between sexes. Overall, men accumulate more abdominal and visceral fat than women.(28) Moreover, women have a higher level of adipokines such as leptin and adiponection.(29, 30) The disbalance in adipokines may induce inflammation in several organs such as the liver, gut and vascular endothelium. The last one causing atherosclerosis, which ultimately may lead to changes in cerebral blood flow (CBF).(23)

Secondly, signalling between and within other organs, such as the liver, might also be disturbed in obese patients. The liver secretes hepatokines, such as insulin-like growth factor 1, selenoprotein P, leukocyte cell-derived chemotaxin, fetuin B and hepassocin, which may indirectly affect brain function and structure.(31, 32)

Thirdly, gut microbiota composition in obese people differ from that of non-obese individuals affecting metabolic processes, weight and obesity-related comorbidities.(33, 34) Microbiota is involved in adiposity and homeostasis, but also influences energy balance via hunger and satiety signalling to the brain. Gut microbiota may also affect the brain by producing (precursors of) neurotransmitters and short chain fatty acids, or through cytokines via the immune system.(35, 36) Bariatric surgery leads to a fast change in gut microbiota composition through changes in food intake, intestinal modifications due to the surgery itself and metabolic improvements, which might eventually lead to changes in gut-brain communication.(15, 37, 38) Hence, the metabolic organs, such as the liver, gut and adipose tissue and the gut microbiota may constitute new therapeutic targets. Although long-term results are not yet clear, the gut microbiota has become a target for anti-obesity treatments.(35)

Obesity is associated with impaired cerebral blood flow (CBF), which may lead to inadequate oxygen and energy supply in the brain and eventually loss of white and grey matter integrity.(39, 40) Lower levels of CBF in the prefrontal cortex are associated with reduced performance on tests of executive function and episodic memory.(40, 41) Even in the prodromal stages of Alzheimer's disease, changes

in CBF can be detected with perfusion MRI (arterial spin labelling; ASL), which may be used as a very early biomarker for neurodegenerative disorders.(42) However, the technique needs further optimization and several consortia are working on implementation of ASL perfusion MRI for clinical applications to provide images of sufficient and diagnostic utility.(43)

Furthermore, obesity is associated with changes in grey and white matter, which can be visualized using diffusion tensor imaging (DTI) and voxel-based morphometry analyses based on T1 weighted scans.(44, 45) These structural changes are especially prominent in brain regions governing reward seeking, inhibitory control and appetite.(46, 47) There are indications that rapid recovery of structural abnormalities takes place after BS.(48, 49) However, long-term data are lacking here.

Additionally, impairment in attention span, executive function and memory are commonly reported in obese patients.(16, 17) Cognitive impairment revealed in obesity might be reversible and varies between cognitive domains, but long-term follow-up studies are scarce. The Longitudinal Assessment of Bariatric Surgery (LABS) parent project is the most extensive longitudinal study to date focusing on cognitive changes in patients after BS. Investigators showed lasting improvements in the cognitive domains of attention, executive function and memory.(17)

Rationale

Cognitive benefits after BS are not equally exhibited across patients and cognitive domains. However, the precise causes are still poorly understood and underlying molecular mechanisms remain elusive. From the relatively short follow-up duration and small samples of BS patients in the studies reviewed, it is difficult to elaborate on the long term consequences of obesity and its related diseases. In this study, the mechanisms underlying obesity-related cognitive disorders will be investigated by longitudinal studies correlating cognition to brain changes, blood serum and plasma values and gut microbiota composition. Lastly, metabolic and histopathological parameters (at the time-point of the surgery) will be obtained to study whether associations or correlations exist between obesity-associated metabolic dysfunction of particular organs and brain function and

structure. To our knowledge this is the first study in humans that investigates brain structure and function changes after BS-induced weight loss and possible linkage between adipose tissue, liver function and the gut microbiome. Additionally, this is the first study in bariatric research combining neuroimaging, cognition and extensive profiling of biological markers.

The primary aim of the BARICO study (**BA**riatric surgery **R**ijnstate and Radboudumc neuro**I**maging and **C**ognition in **O**besity) is to determine the long-term effect of weight loss after bariatric surgery on measures of brain function and structure. The secondary aim is to provide mechanism-based rationales responsible for functional and structural decline in obese individuals. Furthermore, the extensive molecular profiling of tissues (i.e. organ biopsies, blood plasma/serum, and microbiota) will provide information that can be used to characterize the pathological state of organs, and eventually monitor this state via molecular signatures in the circulation. It will also provide information to stratify obese patients based on specific molecular signatures and pathways into risk groups regarding a particular organ dysfunction (mechanism-based subgroups). This study will therefore contribute to the development of better health campaigns, health care and preventatives to attenuate the impact of obesity. This paper describes the design and protocol of the BARICO study.

METHODS AND ANALYSIS

Study population

Patients who have already been screened and found eligible for BS based on the Fried guidelines will be asked to participate.(50) Totally, 150 patients will be included in the study. Study specific inclusion criteria are: (a) patients willing to perform neuropsychological tests and complete self-report questionnaires and sign an informed consent document; (b) age between 35 and 55 years; (c) patients must undergo Roux-en-Y gastric bypass (RYGB). Exclusion criteria for this study are: (a) previous or current neurological or severe psychiatric illness; (b) pregnancy; (c) treatment with any antibiotics, probiotics, or prebiotics three months before or during the study (excluding preoperative

prophylaxis). A subgroup of 75 patients will be included in the MRI sub-study, extra inclusion criteria for this group are: (d) patients willing to undergo MRI scanning and perform tasks in the MRI scanner; (e) right handed (more homogeneous sample and less variance). The standard exclusion criteria for the MRI subgroup include: (d) claustrophobia; (e) epilepsy; (f) pacemakers and defibrillators; (g) nerve stimulators; (h) intracranial clips; (i) infraorbital or intraocular metallic fragments; (j) cochlear implants; (k) ferromagnetic implants; (l) lying circumference above the MRI space capacity; (m) colour blindness. The study has been approved by the medical research ethics committee CMO Region Arnhem-Nijmegen (NL63493.091.17) and is registered at the Netherlands Trial Register (trialregister.nl) 7288.

Study design

At different time points (4-8 weeks preoperative, 6, 24 months and 5, 10 years postoperative, figure 1) several cognitive tests and questionnaires will be assessed. Furthermore, fasting blood and faecal matter will be collected in all patients (N=150) (blood at all time points, faeces 4-8 weeks preoperative, 6 and 24 months postoperative, figure 1). During RYGB surgery, several tissue biopsies will be collected and processed. A schematic overview of the study is shown in figure 1. Furthermore, length and weight will be assessed at each time point. A subgroup of patients (N=75) will additionally receive a (f)MRI scan 4-8 weeks preoperative and 2 years postoperative. During the whole study period (ten years) patients will be contacted by letter and via telephone at least once a year to assure the best follow up rate.

Recruitment procedures and consent

Patients are informed about the study by mail and telephone at least one week prior to their standard information visit (four to eight weeks before RYGB surgery). During this visit, patients will individually receive more information about this study and its objectives. Afterwards, the researchers will further clarify the study and the patients can ask for additional information. If they decide to

participate and fulfil the inclusion criteria, informed consents will be signed. Although the obese population consists of more females than males, the aim is an equal sex distribution during the recruitment period (i.e., a study population consisting of >30% men and >30% women).(1) Recruitment will take place between August 2018 and August 2020.

Outcome measures

The primary outcome measures are the neuropsychological tests scores, CBF values, hippocampal volume, mean diffusivity and fractional anisotropy (representing respectively grey and white matter integrity) and BOLD responses during the Stroop task. Combining neuroimaging and neuropsychological tests will give us more information how and whether the structural brain changes are related to functional brain changes. Secondary measures comprise the (histopathological and biochemical determined) health status of the collected organs, gut microbiota composition changes (in jejunal mucosa and faeces) and profiling of circulating mediators in blood (plasma and serum), as well as lifestyle and dietary habits in relation to cognitive function and brain structure. Combining information on the pathological state of liver, gut and adipose tissue and circulating mediators from corresponding plasma/serum samples obtained prior to and at surgery will provide insight into organ cross-talk and allow identification of biomarker signatures for metabolic health. Differences in metabolic health of the subjects may be associated with specific signalling molecule-profiles, which may be related to cognitive function.

(f)MRI

Subjects will be scanned in a 3T MAGNETOM Skyra MR scanner (Siemens AG, Healthcare Sector, Erlangen, Germany) using a 32-channel head coil. The MRI protocol included a T1-weighted 3D magnetization-prepared rapid gradient-echo (MPRAGE) sequence for anatomical reference and analysis (TR/TI/TE 2300/1100/3.03 ms; 8° flip angle; voxel size: 1.0 × 1.0 × 1.0 mm), a fluid-attenuated inversion recovery (FLAIR) sequence for white matter lesion visualization (TR/TI

5000/1800 ms; voxel size: 1.0 × 1.0 × 1.0 mm), diffusion-weighted MRI scans using simultaneous Multi-slice echo planar imaging for probing microstructural properties (TR/TE 3275/91.4 ms; voxel size: 1.9 × 1.9 × 1.9 mm; 6x b=0 s/mm^2, 42x b=900 s/mm^2, 83x b=1800 s/mm^2). To allow for offline distortion correction of the images, 7 more b=0 s/mm^2 volumes will be acquired using the exact same sequence parameters except for the inverted k-space read-out trajectory. An arterial-spin labelling sequence will used for quantification of cerebral blood flow (TR/TE 2500/12 ms; voxel size: 4.0 × 4.0 × 4.0 mm) and a multi-band, multi-echo planar imaging sequence will be used to measure blood oxygen level dependent (BOLD) contrast during the Stroop task (TR/TE 1500/12.4, 34.3, 56.2 ms; 75° flip angle; voxel size: 2.5 × 2.5 × 2.5 mm; field of view 210 mm; 51 transversal slices in interleaved order). The complete scanning protocol takes 45 minutes. For both time points, the same MR scanner, head coil and sequences will be used. Following the project MRI quality assurance is guaranteed by regular phantom measurements.

Cognitive assessment

Cognitive performance of all participants will be tested using an extensive neuropsychological test battery as detailed below. To assess general cognitive performance the Montreal Cognitive Assessment (MoCA) will be used.(51) To test attentional functions, the Flexibility subtest from the Tests of Attentional Performance (TAP 2.3) will be used.(52) This flexibility task focuses on shifting attention between objects, since paying attention is not a static process. Working memory will be assessed via the Digit Span subtest from the Wechsler Adult Intelligence Scale Fourth Edition (WAIS-IV-NL).(53) Participants have to repeat a series of digits in forward or backward order, or sort them numerically. The Controlled Oral Word Association Test (COWAT) will be used to determine verbal fluency.(54) Participants have to come up with as many words beginning with three designated letters within 60 seconds (for each letter). Episodic memory will be assessed via the immediate and delayed Story Recall subtest from the Rivermead Behavioural Memory Test (RBMT).(55) To control and correct for differences in premorbid intelligence between participants, verbal IQ will be

estimated using the Dutch version of the National Adult Reading Test (NART) at baseline.(56) The MoCA, episodic memory test and COWAT have parallel versions, to avoid material-specific learning effects during the repeated testing. Additionally, the tests are standardized, have been validated for use across a wide age range and have good re-test reliability. Together these tests will provide a good overview on the overall cognitive performance of the patients, including aspects as working and episodic memory, attention, verbal fluency and executive function. Also, education level will be recorded in accordance with the Dutch education system using 7 categories (1 being the lowest level of education and 7 being the highest).(57)

Assessment of biological measurements

On several time points (see figure 1) fasting (at least 5 hrs.) blood samples of the participants will be collected. As standard procedure, classical parameters will be measured, such as several vitamins and lipids (triglycerides and cholesterol). Special interest is on circulating mediators of organ cross-talk, such as cytokines, oxylipids, adipokines, hormones and inflammation markers as well as metabolites (derived from organs or microbiota) assessed by metabolomics such as bile acids and bioactive (short chain) fatty acids, and other lipid species (untargeted lipidomics).

Besides blood samples, faeces will be collected at different time points (see figure 1) using “faeces collection kits for at home” in order to monitor gut-microbiota changes and relate them to cognition and brain structure and function readouts. To gain insight into the microbiota in the intestinal mucosa, mucosal swabs will be collected within the jejunum (two places; 150 and 250cm from Treitz ligament) and stomach pouch (all during the surgery).

As metabolically active organs such as the liver and adipose tissue interact directly and indirectly with the brain, biopsies of these organs will be collected and analysed on histopathological and molecular, biochemical level. The different tissues collected will be subcutaneous, mesenteric and omental adipose tissue, liver and jejunum. Tissue biopsies from these organs will be taken to assess potential pathophysiological processes and to eventually define mechanism-based subgroups.

Questionnaires

At several time-points (see figure 1) standardized questionnaires on lifestyle, education, success rate of the surgery and eating habits will be assessed. Most of the questionnaires are routine practice for patients undergoing BS at the Rijnstate hospital. Physical activity will be assessed via the Baecke questionnaire and depressive symptoms will be assessed with the Beck Depression Inventory (BDI-II).(58, 59) To estimate the participants' food/nutrient intake patients will be asked to fill out an eating diary of two days (a weekday and a weekend day). Quality of Life will be evaluated with the Short Form 36 (SF-36).(60) Lastly, the results of BS will be evaluated via the Bariatric Analysis and Report Outcome System (BAROS).(61)

More specifically: the Barratt impulsivity scale (BIS-11)(62) and Behavioural inhibition/activation system (BIS/BAS)(63) questionnaires on impulsivity and reward sensitivity are included as reward sensitivity and impulsivity have been suggested to contribute to overeating.(64) Indeed, some facets of impulsivity and reward sensitivity have shown to be relevant in eating- and weight regulation.(65)

Physical measurements

At several time points during the study weight, length, waist circumference and blood pressure of the participants will be measured. Body mass index (BMI) will be calculated as weight divided by height in meters squared.

Data management

Data management will be handled using Research Manager (RM, Cloud 9 Health Solutions©), an established software package and data management tool that follows Good Clinical Practice (GCP) guidelines. Every change in the data is recorded in a log system and can be traced. Participants will be identified only by a study specific identification code. One researcher will keep a separate

participant identification code list that matches the study-specific identifying codes with the participant's names. Documents will be maintained by the investigator in strict confidence.

Sample size

The power calculation for the neuropsychological tasks is based on the results of the Digit Span subtest performed in a comparable study population.(17) With an expected standardized effect size of at least 0.3 and a correlation of 0.7, a selected sample of 150 patients will be sufficient to reach 90% power. The power calculation for the MRI parameters is based on the changes in the FA parameter studied by Zhang *et al.*(49) With an expected standardized effect size of at least 0.03 and a correlation of 0.5 including 75 patients in the MRI group will be sufficient to reach 90% power. A significance level based on the sequentially rejective multiple testing procedure discussed by Bretz *et al.* (for the neuropsychological tests 3% and for the MRI parameters 2%) has been taken into account in the power calculation.(66) The inclusion of 150 patients with a subgroup of 75 for the MRI scan has been considered adequate to answer the research question with sufficient power.

Analysis of primary outcome measures

As primary outcome measure, baseline levels of the imaging parameters (such as mean diffusivity (MD) and fractional anisotropy (FA)) will be compared with the results of the neuroimaging outcome 2 years after the surgery, including total weight loss (%) as a factor in the model. Next, the scores of the cognitive tests on five different time points will be analysed and compared to the total weight loss (%). Separate linear mixed models will be used and adjusted for different covariates such as sex, age, IQ score and depressive symptoms etc. where appropriate. To correct for multiple outcome measures, the sequentially rejective multiple testing procedure described in Bretz *et al.* will be used.(66)

Analysis of secondary outcome measures

As secondary outcome measures, the metabolic and histopathological parameters (obtained analyses from tissues collected during the surgery) will be analysed cross-sectionally to examine correlations between these metabolic and histopathological parameters among each other and in relation to brain function and structure. Furthermore, potential mechanisms underlying the crosstalk along the gut-brain axis will be investigated by longitudinal analyses focusing on establishing correlations between brain structure/function changes and changes in circulation mediators or faecal microbiota composition. Pearson correlation analysis will be used to investigate potential correlations between variables.

Data monitoring

Every year, data monitoring and auditing will be conducted by an independent specialized monitor of the Rijnstate Hospital. Yearly, a summary of the progress will be submitted to the ethical committee and the Netherlands Trial Register (trialregister.nl) 7288.

DISCUSSION

The BARICO study is a longitudinal, prospective study focusing on the effect of weight loss after BS on cognitive function and brain structure, measured with sensitive neuropsychological tests covering the most important domains, fMRI activation during the Stroop task, and several MRI techniques, such as DTI and ASL. To clarify the impact of metabolic dysfunction in obesity on brain function and structure, blood plasma and stool samples will be collected and analysed longitudinally as well as biopsies of key metabolic organs which will be collected during the RYGB and analysed cross-sectionally.

Limited studies demonstrated improvement in several cognitive domains such as memory, attention and executive function after BS.(16, 17) Furthermore, obese individuals seem to have lower grey and white matter volumes and altered white matter densities. Several studies show rapid recovery of these brain structural abnormalities after BS(48, 49): for instance, Tuulari *et al.* showed a causal link

between weight loss and brain tissue recovery.(48) Approximately 25-30% of the patients is expected not to reach sufficient weight loss (<50% excess weight loss) and thus it will be possible to study the effect of weight loss after BS on brain function and structure.

The strength of this study is the long follow-up duration of two years for the neuroimaging parameters and ten years for the neuropsychological tests after surgery. Furthermore, the strict inclusion criterion with respect to age range ensures a good representation of mid-life patients. Moreover, most studies in BS patients include mainly women, but it is important to account for sex-differences caused by variation in fat tissue distribution.(28)

Another strength of this study is the combination of neuroimaging and neuropsychological tests. In combination with the analysed metabolic and histopathological parameters (obtained in blood, organ biopsies and microbiota), the relation between multiple metabolic parameters can be investigated, such as adipokines, bioactive lipids (e.g., SCFA) and organ dysfunction or neuroimaging and cognition parameters in a comprehensive way. Especially, since RYGB influences gut-brain communication and may lead to beneficial alterations in adipose function, recovery of brain function and structure may be expected.(15, 67) Longitudinal analyses of the microbiota together with analysis of functional gut-derived metabolites in the circulation and cognitive outcomes may allow identification of mediators derived from the gut microflora that are relevant for cognition and prevention of cognitive decline.

The BARICO study has the potential to be the first to demonstrate interactions between periphery and central nervous system after weight loss in humans, particularly the involvement of the brain, adipose tissue liver and gut microbiota.

In conclusion, the BARICO study will reveal the relation and underlying mechanisms between obesity and brain function and structure. This information can be used to develop better health care and preventatives against obesity and associated disorders.

ETHICS AND DISSEMINATION

The study protocol was authorized by the medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17). All patients will sign informed consent on enrolment in the study. Study results of the study will be submitted for publication in peer-reviewed journals.

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Contributors

EOA and AJK conceived and designed the study. DV wrote the article and developed the protocol together with EOA, AJK, EJH, and RK. EJH, EOA and AJK are the principal investigators and DV is the main investigator. MW, LND, IAA, EA, RK and RPCK are co- investigators in the participating centres. All authors critically reviewed the content and approved the final manuscript.

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Competing interests

The authors declare that they have no conflicts of interests.

Patient consent

Obtained

Ethics approval

Medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17).

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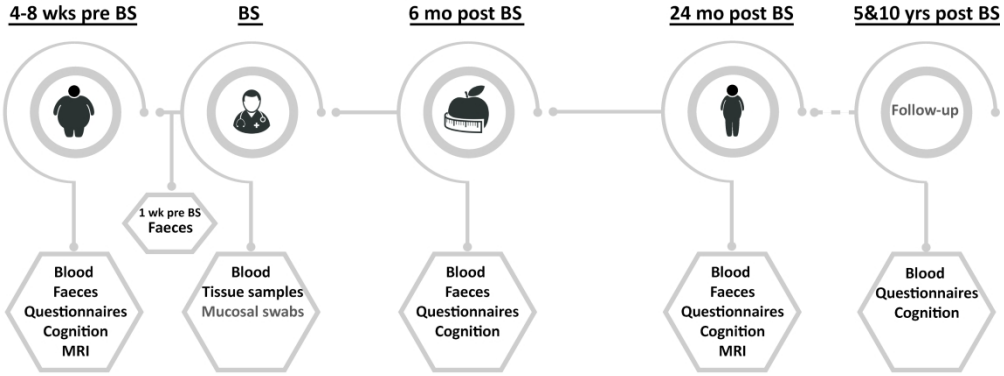
FIGURE LEGEND

Figure 1. Overview of the study design. Blood samples are taken during regular blood withdrawal at 6 time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota analyses will be performed at set time points on the faeces collected at home by the patients (4-8 wks. pre BS, 1 wk. pre BS, 6 mo. post BS, 24 mo. post BS) and mucosal swops will be collected during surgery. Furthermore, biopsies of liver, jejunum and adipose tissue depots (subcutaneous, mesenteric and omental) will be collected during surgery. Before (4-8 wks. pre BS) and several time points after surgery (6 mo. post BS, 24 mo. post BS and 5&10 yrs. post BS) patients will fill out questionnaires together with neuropsychological measurements to test cognitive function. A subgroup of patients (N=75) will be examined with MRI (4-8 wks. pre BS and 24 mo. post BS). MRI; magnetic resonance imaging. BS; Bariatric surgery. Wks; weeks. Mo; months. Yrs; years.



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Study rationale and protocol of the BARICO study: a longitudinal, prospective, observational study to evaluate effects of weight loss on brain function and structure after bariatric surgery.

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Vreeken, D.[1,2,3], Wiesmann, M.[3], Deden, L.N.[1,2], Arnoldussen, I.A.C.[3], Aarts, E.[4], Kessels, R.P.C.[4,5,6], Kleemann, R.[7], Hazebroek, E.J.[1,2], Aarts, E.O.[1,2*], Kiliaan, A.J.[3*†]

[1] *Department of Surgery, Rijnstate Hospital, Arnhem, the Netherlands.*

[2] *Vitalys Clinic, Velp, the Netherlands.*

[3] *Department of Anatomy, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, the Netherlands.*

[4] *Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, the Netherlands.*

[5] *Department of Medical Psychology, Radboud University Medical Center, Nijmegen, the Netherlands.*

[6] *Vincent van Gogh Institute for Psychiatry, Venray, the Netherlands.*

[7] *Department of Metabolic Health Research, Netherlands Organization for Applied Scientific Research (TNO), Leiden, the Netherlands.*

[*] These authors contributed equally.

[†] **Corresponding author:**

Amanda J. Kiliaan, PhD

Donders Institute for Brain, Cognition, and Behaviour

Radboud university medical center

Department of Anatomy (109)

Geert Grooteplein 21N

6525 EZ Nijmegen, the Netherlands

Phone: +31 24 361 4378

Email: amanda.kiliaan@radboudumc.nl

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ABSTRACT

Introduction

Weight loss after bariatric surgery (BS) is often associated with improved cognition and structural brain recovery. However, improved cognition after BS is not always exhibited by patients, in fact, in some cases there is even a decline in cognition. Long-term consequences of BS weight loss, in terms of obesity and related diseases, can be hard to determine due to studies having short follow-up periods and small sample sizes.

The aim of the BARICO study (**BA**riatric surgery **R**ijnstate and Radboudumc neuro**I**maging and **C**ognition in **O**besity) is to determine the long-term effect of weight loss after BS on brain function and structure, using sensitive neuropsychological tests and (functional) magnetic resonance imaging ((f)MRI). Secondary study endpoints are associated with changes in metabolic and inflammation status of adipose tissue, liver and gut, in relation to brain structure and function. Also, the possible correlation between weight loss, gut microbiota composition change and neuropsychological outcomes will be investigated.

Methods and analysis

Data from 150 Dutch BS patients (age between 35 and 55, men and women) will be collected at various time points between 2 months before and up to 10 years after surgery. Neuropsychological tests, questionnaires, blood, faeces and tissue samples will be collected before, during and after surgery to measure changes in cognition, microbiota, metabolic activity and inflammation over time. A subgroup of 75 participants will undergo (f)MRI in relation to executive functioning (determined by the Stroop task), grey and white matter volumes, and cerebral blood flow. Regression analyses will be used to explore associations between weight loss and outcome measures.

Ethics and dissemination

This study has been approved by the medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17). Research findings will be published in peer-reviewed journals and at conferences.

Trial registration

The Netherlands National Trial Register (trialregister.nl) 7288. Date registered: 29-June-2018.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- For the first time in bariatric research, neuroimaging, neuropsychological tests and metabolic and histopathological parameters will be combined to investigate the effect of weight loss on brain function and structure after bariatric surgery.
- Collecting and investigating multiple metabolic parameters (obtained from blood, various tissue and microbiota) may help to reveal the relationship, and underlying mechanisms, between obesity and brain function and structure.
- With a follow-up of 10 years after bariatric surgery, additional knowledge will be gathered on the long-term effects of weight loss on cognitive function.
- Only bariatric surgery patients are included in this study, so whether the results are generalizable to obese patients in general will require further investigation.

INTRODUCTION

For over two decades, obesity-induced diseases, such as cardiovascular disease, and type 2 diabetes mellitus (T2DM), have been one of the major health-care challenges of today's society.(1) Besides the well-known metabolic complications, obesity may lead to structural brain changes, cognitive impairment and neurodegenerative diseases.(2-5) Additionally, a direct relationship exists between increased body mass and cognitive impairment.(6-9) To improve and possibly reduce the amount of obesity-induced diseases, inhibit cognitive impairment and reduce neurodegenerative diseases, sustainable long-term weight loss in obese patients must be achieved. Non-surgical treatments for obesity, such as dietary restriction and physical activity, often show disappointing long-term effects, especially in patients with morbid obesity (body mass index (BMI) above 40 kg/m^2).(10, 11) Bariatric surgery (BS) is known to a rapid and sustainable decrease in body mass. In particular the commonly performed Roux-en-Y gastric bypass (RYGB) leads to rapid weight loss which is often accompanied by remission of T2DM, hypertension (HT) and dyslipidaemia (DL).(12, 13) RYGB is a restrictive and malabsorptive (for micronutrients) surgical procedure; it excludes the main part of the stomach, the duodenum and the first part of the jejunum from the passage of food, leading to, among others, hormonal and gut microbiota changes.(14, 15) Gut microbiota changes after RYGB comprise increases in gut microbiota diversity, increases in relative abundance of *Actinobacteria* and *Firmicutes* phyla and decreases in relative abundance of *Bacteroidetes* phyla. However, effects in reported studies are quite inconsistent and further research is needed. (16, 17)

Besides weight loss and remission of comorbidities, RYGB surgery is also associated with improved cognitive functions.(18, 19) This may be related to multiple metabolic parameters, such as systolic blood pressure or triglyceride concentrations.(20) Metabolic complications may also arise in obese patients due to a disturbed interaction between metabolic organs such as adipose tissue, liver and gut. This is especially a problem in midlife (between age 35 and 55) in which obesity has been reported to cause

cognitive decline and increase risk for developing dementia. However, mechanisms involved in this organ-organ crosstalk are poorly understood.(4, 21-24) Firstly, one proposed mechanism is the altered signalling of visceral and abdominal adipose tissue; adipose tissue acts as an independent endocrine organ releasing several hormones, proteins and cytokines, referred to as adipokines. Obesity is associated with dysfunctional white adipose tissue and therefore an imbalance in adipokines, such as increased levels of leptin and angiotensinogen, and low levels of adiponectin and omentin.(25, 26) Especially, visceral adipose tissue seems to produce unfavourable adipokines associated with more metabolic complications when compared to subcutaneous adipose tissue.(27-30) Importantly, the distribution of fat tissue depots differs between sexes. Overall, men accumulate more abdominal and visceral fat than women.(30) Moreover, women have a higher level of adipokines such as leptin and adiponectin.(31, 32) This disbalance in adipokines may induce inflammation in several organs such as the liver, gut and vascular endothelium. The latter causing atherosclerosis, ultimately leading to changes in cerebral blood flow (CBF).(25)

Secondly, signalling between, and within other organs, such as the liver, might be altered in obese patients. For example; the liver secretes hepatokines, such as insulin-like growth factor 1, selenoprotein P, leukocyte cell-derived chemotaxin, fetuin B and hepassocin, which may indirectly affect brain function and structure.(33, 34)

Thirdly, the gut microbiota composition in obese people differs from that of non-obese individuals, affecting metabolic processes, weight and obesity-related comorbidities.(35, 36) Microbiota is involved in adiposity and homeostasis but also influences energy balance via appetite and satiety signalling to the brain. Gut microbiota also affect the brain by producing (precursors of) neurotransmitters and short chain fatty acids, or through cytokines via the immune system.(37, 38) BS leads to a fast change in gut microbiota composition through changes in food intake, intestinal modifications due to the surgery itself, and metabolic improvements, eventually leading to changes in gut-brain communication.(15, 39, 40)

Hence, metabolic organs, such as liver, gut and adipose tissue and gut microbiota may constitute new therapeutic targets. Although long-term results are not yet clear, the gut microbiota has already become a target for anti-obesity treatments.(37)

Obesity is associated with impaired CBF, which may lead to inadequate oxygen and energy supply in the brain and eventually loss of white and grey matter integrity.(41, 42) Lower levels of CBF in the prefrontal cortex are associated with reduced performance on executive function and episodic memory tests.(42, 43) Even in the prodromal stages of Alzheimer's disease, changes in CBF can be detected with arterial spin labelling (ASL), which may be used as a very early biomarker for neurodegenerative disorders.(44) However, the technique requires further optimization and therefore several consortia are working on the implementation of ASL perfusion magnetic resonance imaging (MRI) for clinical applications to provide images of sufficient and diagnostic utility.(45)

Furthermore, obesity is associated with changes in grey and white matter, which can be visualized using diffusion tensor imaging (DTI) and voxel-based morphometry analyses based on T1 weighted scans.(46, 47) These structural changes are especially prominent in brain regions governing reward seeking, inhibitory control and appetite.(48, 49) There are indications that rapid recovery of structural abnormalities occur after BS, however long-term study data is lacking here. (50, 51)

Additionally, impairment in attention span, executive function and memory are commonly reported in obese patients.(18, 19) Cognitive impairment revealed in obesity might be reversible and varies between cognitive domains however long-term follow-up studies are scarce. The Longitudinal Assessment of Bariatric Surgery (LABS) parent project is the most extensive longitudinal study to date focusing on cognitive changes in patients after BS. Investigators showed lasting improvements three years after surgery in the cognitive domains of attention, executive function and memory.(19)

Rationale

Cognitive benefits after BS are not equally exhibited across patients and cognitive domains. However, precise causes are still poorly understood, and underlying molecular mechanisms remain elusive. From the relatively short follow-up duration and small samples of BS patients in the studies reviewed, it is difficult to elaborate on the long-term consequences of obesity and its related diseases. In this study, underlying mechanisms of obesity-related cognitive disorders will be investigated by longitudinal studies correlating cognition to brain changes, blood serum and plasma values, and gut microbiota composition. Lastly, metabolic and histopathological parameters (at the time-point of surgery) will be obtained to study whether associations or correlations exist between obesity-associated metabolic dysfunctions of particular organs and brain function and structure. To our knowledge this is the first study in humans investigating changes in brain structure and function, and changes in adipose tissue, liver function and the gut microbiome, after BS-induced weight loss. Additionally, this is the first study in bariatric research combining neuroimaging, cognition and extensive profiling of biological markers.

The primary aim of the BARICO study (**BA**riatric surgery **R**ijnstate and Radboudumc neuro**I**maging and **C**ognition in **O**besity) is to determine the long-term effect of weight loss on measures of brain function and structure after BS. The secondary aim is to provide mechanism-based rationales responsible for functional and structural decline in obese individuals. Therefore, the metabolic and inflammation status of organ biopsies will be determined together with molecular signatures via blood plasma/serum analyses. Furthermore, gut microbiota composition will be monitored over time to gain knowledge about the gut-brain axis.

This study will contribute to the development of better health campaigns, healthcare and preventatives to attenuate the impact of obesity. This paper describes the design and protocol of the BARICO study.

METHODS AND ANALYSIS

Study population

Patients who have been screened and found eligible for BS based on the Fried guidelines will be asked to participate.(52) In total, 150 patients will be included in the study. Study specific inclusion criteria are: (a) patients willing to perform neuropsychological tests, complete self-report questionnaires and sign an informed consent document; (b) age between 35 and 55 years; (c) patients must undergo RYGB. A laparoscopic antecolic antegastric RYGB procedure will be performed (biliopancreatic limb of 150 cm, alimentary limb of 100 cm). Exclusion criteria for this study are: (a) previous or current neurological or severe psychiatric illness; (b) pregnancy; (c) treatment with any antibiotics, probiotics, or prebiotics three months before or at any point during the study (excluding preoperative prophylaxis). A subgroup of 75 patients will be included in the MRI sub-study, extra inclusion criteria for this group are: (d) patients willing to undergo MRI scanning and perform tasks in the MRI scanner; (e) right handed (more homogeneous sample and less variance). The standard exclusion criteria for the MRI subgroup include: (d) claustrophobia; (e) epilepsy; (f) pacemakers and defibrillators; (g) nerve stimulators; (h) intracranial clips; (i) infraorbital or intraocular metallic fragments; (j) cochlear implants; (k) ferromagnetic implants; (l) circumference above the MRI space capacity; (m) colour blindness. The study has been approved by the medical research ethics committee CMO Region Arnhem-Nijmegen (NL63493.091.17) and is registered at the Netherlands Trial Register (trialregister.nl) 7288.

Study design

At several time points (4-8 weeks preoperative, 6, 24 months and 5, 10 years postoperative (figure 1)) a number of cognitive tests and questionnaires will be performed, and their results assessed. Furthermore, blood (after 8 hrs. period of fasting) and faecal matter will be collected from all patients (N=150) (blood at all time points, faeces 4-8 weeks preoperative, 6 and 24 months postoperative (figure 1)).

Intraoperatively, several tissue biopsies will be collected and processed. Medical evaluation, including anthropometric measurements and information on comorbidities, will be assessed 4-8 weeks preoperative and during all postoperative time points. A schematic overview of the study is shown in figure 1. A subgroup of patients (N=75) will additionally receive a (f)MRI scan 4-8 weeks preoperative and 24 months postoperative. During the whole study period (10 years) patients will be contacted by letter and via telephone at least once a year to ensure the best follow-up rate.

Recruitment procedures and consent

Patients are informed about the study by letter and telephone at least two weeks prior to their standard visit (4-8 weeks before RYGB surgery). During this visit, patients will individually receive more information about this study and its objectives. Afterwards, the researchers will further clarify the study and the patients can ask for additional information. If they decide to participate and fulfil the inclusion criteria, informed consents will be obtained. Although the obese population consists of more females than males, the aim is for an equal sex distribution during the recruitment period (i.e., a study population consisting of >30% men and >30% women).(1) Recruitment will take place between August 2018 and August 2020.

Outcome measures

The primary outcome measures are the neuropsychological tests scores, CBF values, hippocampal volume, mean diffusivity (MD) and fractional anisotropy (FA) (representing respectively grey and white matter integrity), and blood oxygen level dependent (BOLD) responses during the Stroop task. Combining neuroimaging and neuropsychological tests will give us more information on how and whether structural brain changes are related to functional brain changes. Secondary measures comprise of (histopathological and biochemical determined) health status of the collected tissue, gut microbiota

composition changes (in jejunal mucosa and faeces) and the profiling of circulating mediators in blood (plasma and serum), as well as lifestyle and dietary habits in relation to cognitive function and brain structure. Combining information on the pathological state of liver, gut, adipose tissue and circulating mediators from corresponding plasma/serum samples, obtained prior to and at surgery, will provide insight into organ cross-talk and allow identification of biomarker signatures for metabolic health. Differences in metabolic health of the subjects may be associated with specific signalling molecule-profiles, which may be related to cognitive function.

(f)MRI

Subjects will be scanned in a 3T MAGNETOM Skyra MR scanner (Siemens AG, Healthcare Sector, Erlangen, Germany) using a 32-channel head coil. The MRI protocol included: a T1-weighted 3D magnetization-prepared rapid gradient-echo (MPRAGE) sequence for anatomical reference and analysis (TR/TI/TE 2300/1100/3.03 ms; 8° flip angle; voxel size: 1.0 × 1.0 × 1.0 mm), a fluid-attenuated inversion recovery (FLAIR) sequence for white matter lesion visualization (TR/TI 5000/1800 ms; voxel size: 1.0 × 1.0 × 1.0 mm), and diffusion-weighted MRI scans using simultaneous multi-slice echo planar imaging for probing microstructural properties (TR/TE 3275/91.4 ms; voxel size: 1.9 × 1.9 × 1.9 mm; 6x b=0 s/mm^2, 42x b=900 s/mm^2, 83x b=1800 s/mm^2). To allow for offline distortion correction of the images, 7 more b=0 s/mm^2 volumes will be acquired using the exact same sequence parameters - except for the inverted k-space read-out trajectory. An ASL sequence will used for quantification of CBF (TR/TE 2500/12 ms; voxel size: 4.0 × 4.0 × 4.0 mm) and a multi-band, multi-echo planar imaging sequence will be used to measure BOLD contrast during the Stroop task (TR/TE 1500/12.4, 34.3, 56.2 ms; 75° flip angle; voxel size: 2.5 × 2.5 × 2.5 mm; field of view 210 mm; 51 transversal slices in interleaved order). The complete scanning protocol takes 45 minutes and for both time-points, the same: MR scanner, head coil, and

sequences will be used. Following the project MRI quality assurance is guaranteed by regular phantom measurements.

Cognitive assessment

Cognitive performance of all participants will be tested using an extensive neuropsychological test battery as detailed below. To assess general cognitive performance the Montreal Cognitive Assessment (MoCA) will be used.(53) To test attentional functions, the Flexibility subtest from the Tests of Attentional Performance (TAP 2.3) will be used.(54) This flexibility task focuses on shifting attention between objects. Working memory will be assessed via the Digit Span subtest from the Wechsler Adult Intelligence Scale Fourth Edition (WAIS-IV-NL).(55) Participants will have to repeat a series of digits in forward or backward order, or sort them numerically. The Controlled Oral Word Association Test (COWAT) will be used to determine verbal fluency.(56) Participants have to come up with as many words beginning with three designated letters within 60 seconds (for each letter). Episodic memory will be assessed via the immediate and delayed Story Recall subtest from the Rivermead Behavioural Memory Test (RBMT).(57) To control and correct for differences in premorbid intelligence between participants, verbal IQ will be estimated using the Dutch version of the National Adult Reading Test (NART) at baseline.(58) The MoCA, episodic memory test and COWAT have parallel versions, to avoid material-specific learning effects during the repeated testing. Additionally, the tests are standardized, have been validated for use across a wide age range and have good re-test reliability. Together these tests will provide a good overview on the overall cognitive performance of the patients, including aspects of working and episodic memory, attention, verbal fluency and executive function. Also, education level will be recorded in accordance with the Dutch education system using seven categories (one being the lowest level of education and seven being the highest).(59)

Assessment of biological measurements

At several time points (figure 1) fasting (at least 8 hrs.) blood samples from the participants will be collected. As standard procedure classical parameters, such as several vitamins (vitamin B12, D and folic acid) and lipids (triglycerides and cholesterol) will be measured. Special interest is taken on circulating mediators of organ cross-talk, such as: cytokines, oxylipids, adipokines, hormones and inflammation markers (e.g., C-reactive protein, serum amyloid A, vascular cell adhesion molecule 1, transforming growth factor beta), as well as metabolites (derived from organs or microbiota) assessed by metabolomics, such as bile acids and bioactive (short chain) fatty acids, and other lipid species (untargeted lipidomics).

Besides blood samples, faeces will be collected (figure 1) using "faeces collection kits for at home" in order to monitor gut-microbiota changes and relate them to cognition and brain structure and function readouts. Additionally, to gain insight into the microbiota in the intestinal mucosa, mucosal swabs will be collected during surgery within the jejunum (two places; 150 and 250cm from Treitz ligament) and stomach pouch.

As metabolically active organs such as the liver and adipose tissue interact directly and indirectly with the brain, biopsies of these organs will be collected and analysed on histopathological, and biochemical level. Tissue biopsies from subcutaneous, mesenteric and omental adipose tissue, liver and jejunum. Tissue biopsies from these organs will be taken to assess potential pathophysiological processes and to eventually define mechanism-based subgroups.

Questionnaires

At several time-points (figure 1) standardized questionnaires on lifestyle, education, success rate of the surgery and eating habits will be assessed. Most of the questionnaires are routine practice for patients undergoing BS at the Rijnstate hospital. Physical activity will be assessed via the Baecke questionnaire

and depressive symptoms will be assessed with the Beck Depression Inventory (BDI-II).(60, 61) To estimate the participants' food/nutrient intake and eating behaviour patients will be asked to fill out an eating diary of two days (a weekday and a weekend day). Quality of Life will be evaluated with the Short Form 36 (SF-36).(62) Lastly, the results of BS will be evaluated via the Bariatric Analysis and Report Outcome System (BAROS).(63)

More specifically: the Barratt impulsivity scale (BIS-11)(64) and Behavioural inhibition/activation system (BIS/BAS)(65) questionnaires on impulsivity and reward sensitivity are included as reward sensitivity and impulsivity have both previously been suggested to contribute to overeating.(66) Indeed, some facets of impulsivity and reward sensitivity have shown to be relevant in eating- and weight regulation.(67)

Medical evaluation

At several time points during the study (figure 1) a medical evaluation will take place where anthropometric measurements such as: body weight, length, waist circumference and blood pressure will be quantified. BMI will be calculated as weight divided by height in meters squared. Percentage excess weight loss (%EWL) (defined as weight loss divided by preoperative excess weight, with excess weight defined as the weight above a normal BMI of 25 kg/m^2) will be calculated during the time points after surgery, similar to percentage total body weight loss (%TBWL) (defined as weight loss divided by preoperative weight). The success of BS in terms of weight loss will be defined as a sustained weight loss larger than 50 %EWL.

Furthermore, data on comorbidities like T2DM, HT and DL and associated medication will be collected before the surgery and at all time-points after surgery. Comorbidities will be defined using following criteria: for T2DM a fasting plasma glucose of ≥ 7.0 mmol/L and HbA1c ≥ 48 mmol/mol (HbA1c ≥ 6.5%) or the use of oral antidiabetic or insulin medication; for HT the use of antihypertensive drug treatment; for DL the use of statins.

Data management

Data management will be handled using Research Manager (RM, Cloud 9 Health Solutions©), an established software package and data management tool that follows Good Clinical Practice (GCP) guidelines.(68) Every change in the data is recorded in a log system and can be traced. Participants will be identified only by a study specific identification code. One researcher will keep a separate participant identification code list that matches the study-specific identifying codes with the participant's names. Documents will be maintained by the investigator in strict confidence.

Sample size

The power calculation for the neuropsychological tasks was based on the results of the Digit Span subtest performed in a comparable study population.(19) With an expected standardized effect size of at least 0.3 and a correlation of 0.7, a selected sample of 150 patients will be sufficient to reach 90% power. The power calculation for the MRI parameters is based on changes in the FA parameter studied by Zhang *et al.*(51) With an expected standardized effect size of at least 0.03 and a correlation of 0.5 including 75 patients in the MRI group will be sufficient to reach 90% power. A significance level based on the sequentially rejective multiple testing procedure discussed by Bretz *et al.* (for the neuropsychological tests 3% and for the MRI parameters 2%) has been taken into account in the power calculation.(69) The inclusion of 150 patients with a subgroup of 75 for the MRI scan has been considered adequate to answer the research questions with sufficient power.

Analysis of primary outcome measures

As a primary outcome measure, baseline levels of the imaging parameters (such as MD and FA) will be compared with the results of the neuroimaging outcome 24 months after surgery (including %TBWL as a

factor in the model). Next, the scores of the cognitive tests from five different time points will be analysed and compared to %TBWL. Every dependent variable will be modelled in a separate linear mixed model. %TBWL will be used as a factor. Different variables, such as: depression score, age, and gender, will be (if appropriate) included in the model. For each model, we will decide which variables to include as a factor to reduce the amount of unexplained variation. To correct for multiple outcome measures, the sequentially rejective multiple testing procedure described in Bretz *et al.* will be used.(69) Data will be analysed using SPSS (version 25 for Windows) and R (version 3.5.1 for Windows). For the cognitive tests a p value of <0.03 and for the imaging parameters a p value of <0.02 will be considered as statistically significant.

Analysis of secondary outcome measures

As secondary outcome measures, the metabolic and histopathological parameters (obtained analyses from tissues collected during surgery) will be analysed cross-sectionally to examine correlations between and among each other, and in relation to brain function and structure. Furthermore, potential mechanisms underlying the crosstalk along the gut-brain axis will be investigated by longitudinal analyses focusing on establishing correlations between brain structure/function changes and changes in circulation mediators or faecal microbiota composition. Pearson correlation analysis will be used to investigate potential correlations between variables.

Data monitoring

Every year, data monitoring and auditing will be conducted by an independent specialised monitor from the Rijnstate Hospital. Yearly, a summary of the progress will be submitted to the ethical committee and the Netherlands Trial Register (trialregister.nl) 7288.

Patient and Public involvement

Patients and the public were not involved in the design of this study. Nevertheless, the results will be disseminated to the study participants via email, newsletters and social media platforms after the study results are published.

DISCUSSION

The BARICO study is a prospective study focusing on the effect of weight loss on cognitive function and brain structure after BS. This will be measured using sensitive neuropsychological tests covering the most important domains, fMRI activation during the Stroop task, and several MRI techniques, such as DTI and ASL. To clarify the impact of metabolic dysfunction in obesity on brain function and structure, blood plasma and stool samples will be collected and analysed longitudinally, and biopsies of key metabolic organs will be collected during the RYGB and analysed cross-sectionally.

After BS, there have only been a limited number of long-term studies demonstrating improvement in several cognitive domains, including memory, attention and executive function.(18, 19) Furthermore, it has been shown that obese individuals have lower grey and white matter volumes, and altered white matter densities, in comparison to healthy individuals with several studies showing a rapid recovery of these brain structural abnormalities after BS.(50, 51) For instance, Tuulari *et al.* showed a causal link between weight loss and brain tissue recovery.(50) Approximately 25-30% of the patients are not expected to reach sufficient weight loss (≤50 %EWL), and thus it will be possible to study the effect of weight loss after BS on brain function and structure.

Perhaps the strength of this study is in the long follow-up duration after surgery: 24 months for the neuroimaging parameters, and 10 years for the neuropsychological tests. Furthermore, the strict inclusion criterion with respect to age range ensures a good representation of mid-life patients.

Moreover, the majority of studies into BS patients are mostly composed of women but it is equally important to account for the variation in fat tissue distribution which is caused by differences in sex.(30) Another strength of this study is the combination of neuroimaging and neuropsychological tests. Alongside the analysis of metabolic and histopathological parameters (obtained in blood, organ biopsies and microbiota), meaning that the relation between multiple metabolic, neuroimaging and/or cognitive parameters can be investigated (e.g., adipokines, bioactive lipids (short-chain fatty acids) and organ dysfunction) in a comprehensive way. Since RYGB influences gut-brain communication, there may be beneficial alterations in adipose tissue functions, and/or recovery of brain function and structure following BS.(15, 70) Longitudinal analyses of the microbiota, together with analysis of functional gut-derived metabolites in the circulation and cognitive outcomes, may allow for the identification of mediators derived from gut microflora that are relevant to cognition and the prevention of cognitive decline.

The BARICO study has the potential to be the first to demonstrate interactions between the periphery and central nervous system after weight loss in humans, in particular it will question the roles and involvement of the brain, and adipose tissue, liver and gut microbiota, after weight loss caused by BS.

In conclusion, the BARICO study will reveal the relation and underlying mechanisms between obesity and brain function and structure. This information can be used to develop better health care as well as possible preventatives against obesity and associated disorders.

ETHICS AND DISSEMINATION

The study protocol was authorized by the medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17). All patients will sign informed consent forms upon enrolment in the study. Study results will be submitted for publication in peer-reviewed journals.

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Contributors

EOA and AJK conceived and designed the study. DV wrote the article and developed the protocol together with EOA, AJK, EJH, and RK. EJH, EOA and AJK are the principal investigators and DV is the main investigator. MW, LND, IAA, EA, RK and RPCK are co- investigators in the participating centres. All authors critically reviewed the content and approved the final manuscript.

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Competing interests

The authors declare that they have no conflicts of interests.

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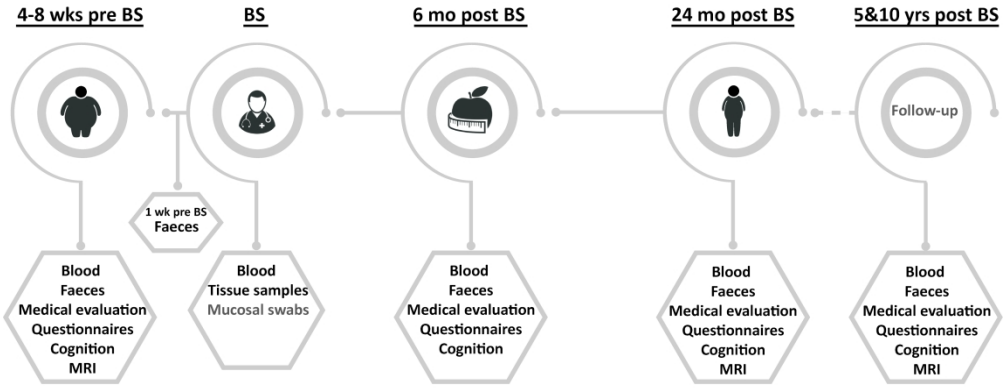
FIGURE LEGEND

Figure 1. Overview of the study design. Blood samples are taken during a regular blood withdrawal at six time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota analyses will be performed at set time points on the faeces (collected at home by the patients) (4-8 wks. pre BS, 1 wk. pre BS, 6 mo. post BS, 24 mo. post BS) and mucosal swabs (collected during surgery). Furthermore, biopsies of liver, jejunum and adipose tissue depots (subcutaneous, mesenteric and omental) will be collected during surgery. Before surgery, (4-8 wks. pre BS) and at several time points after, (6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS) a medical evaluation will take place and all patients will complete questionnaires and neuropsychological measurements to test cognitive function. A subgroup of patients (N=75) will be examined with MRI (4-8 wks. pre BS and 24 mo. post BS). MRI; magnetic resonance imaging. BS; Bariatric surgery. Wks; weeks. Mo; months. Yrs; years.



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Study rationale and protocol of the BARICO study: a longitudinal, prospective, observational study to evaluate effects of weight loss on brain function and structure after bariatric surgery.

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Study rationale and protocol of the BARICO study: a longitudinal, prospective, observational study to evaluate effects of weight loss on brain function and structure after bariatric surgery.

Vreeken, D.[1,2,3], Wiesmann, M.[3], Deden, L.N.[1,2], Arnoldussen, I.A.C.[3], Aarts, E.[4], Kessels, R.P.C.[4,5,6], Kleemann, R.[7], Hazebroek, E.J.[1,2], Aarts, E.O.[1,2*], Kiliaan, A.J.[3*†]

[1] *Department of Surgery, Rijnstate Hospital, Arnhem, the Netherlands.*

[2] *Vitalys Clinic, Velp, the Netherlands.*

[3] *Department of Anatomy, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, the Netherlands.*

[4] *Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, the Netherlands.*

[5] *Department of Medical Psychology, Radboud University Medical Center, Nijmegen, the Netherlands.*

[6] *Vincent van Gogh Institute for Psychiatry, Venray, the Netherlands.*

[7] *Department of Metabolic Health Research, Netherlands Organization for Applied Scientific Research (TNO), Leiden, the Netherlands.*

[*] These authors contributed equally.

[†] **Corresponding author:**

Amanda J. Kiliaan, PhD

Donders Institute for Brain, Cognition, and Behaviour

Radboud university medical center

Department of Anatomy (109)

Geert Grooteplein 21N

6525 EZ Nijmegen, the Netherlands

Phone: +31 24 361 4378

Email: amanda.kiliaan@radboudumc.nl

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ABSTRACT

Introduction

Weight loss after bariatric surgery (BS) is often associated with improved cognition and structural brain recovery. However, improved cognition after BS is not always exhibited by patients, in fact, in some cases there is even a decline in cognition. Long-term consequences of BS weight loss, in terms of obesity and related diseases, can be hard to determine due to studies having short follow-up periods and small sample sizes.

The aim of the BARICO study (**BA**riatric surgery **R**ijnstate and Radboudumc neuro**I**maging and **C**ognition in **O**besity) is to determine the long-term effect of weight loss after BS on brain function and structure, using sensitive neuropsychological tests and (functional) magnetic resonance imaging ((f)MRI). Secondary study endpoints are associated with changes in metabolic and inflammation status of adipose tissue, liver and gut, in relation to brain structure and function. Also, the possible correlation between weight loss, gut microbiota composition change and neuropsychological outcomes will be investigated.

Methods and analysis

Data from 150 Dutch BS patients (age between 35 and 55, men and women) will be collected at various time points between 2 months before and up to 10 years after surgery. Neuropsychological tests, questionnaires, blood, faeces and tissue samples will be collected before, during and after surgery to measure changes in cognition, microbiota, metabolic activity and inflammation over time. A subgroup of 75 participants will undergo (f)MRI in relation to executive functioning (determined by the Stroop task), grey and white matter volumes, and cerebral blood flow. Regression analyses will be used to explore associations between weight loss and outcome measures.

Ethics and dissemination

This study has been approved by the medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17). Research findings will be published in peer-reviewed journals and at conferences.

Trial registration

The Netherlands National Trial Register (trialregister.nl) 7288. Date registered: 29-June-2018.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- For the first time in bariatric research, neuroimaging, neuropsychological tests and metabolic and histopathological parameters will be combined to investigate the effect of weight loss on brain function and structure after bariatric surgery.
- Collecting and investigating multiple metabolic parameters (obtained from blood, various tissue and microbiota) may help to reveal the relationship, and underlying mechanisms, between obesity and brain function and structure.
- With a follow-up of 10 years after bariatric surgery, additional knowledge will be gathered on the long-term effects of weight loss on cognitive function.
- Only bariatric surgery patients are included in this study, so whether the results are generalizable to obese patients in general will require further investigation.

INTRODUCTION

For over two decades, obesity-induced diseases, such as cardiovascular disease, and type 2 diabetes mellitus (T2DM), have been one of the major health-care challenges of today's society.(1) Besides the well-known metabolic complications, obesity may lead to structural brain changes, cognitive impairment and neurodegenerative diseases.(2-5) Additionally, a direct relationship exists between increased body mass and cognitive impairment.(6-9) To improve and possibly reduce the amount of obesity-induced diseases, inhibit cognitive impairment and reduce neurodegenerative diseases, sustainable long-term weight loss in obese patients must be achieved. Non-surgical treatments for obesity, such as dietary restriction and physical activity, often show disappointing long-term effects, especially in patients with morbid obesity (body mass index (BMI) above 40 kg/m^2).(10, 11) Bariatric surgery (BS) is known to a rapid and sustainable decrease in body mass. In particular the commonly performed Roux-en-Y gastric bypass (RYGB) leads to rapid weight loss which is often accompanied by remission of T2DM, hypertension (HT) and dyslipidaemia (DL).(12, 13) RYGB is a restrictive and malabsorptive (for micronutrients) surgical procedure; it excludes the main part of the stomach, the duodenum and the first part of the jejunum from the passage of food, leading to, among others, hormonal and gut microbiota changes.(14, 15) Gut microbiota changes after RYGB comprise increases in gut microbiota diversity, increases in relative abundance of *Actinobacteria* and *Firmicutes* phyla and decreases in relative abundance of *Bacteroidetes* phyla. However, effects in reported studies are quite inconsistent and further research is needed. (16, 17)

Besides weight loss and remission of comorbidities, RYGB surgery is also associated with improved cognitive functions.(18, 19) This may be related to multiple metabolic parameters, such as systolic blood pressure or triglyceride concentrations.(20) Metabolic complications may also arise in obese patients due to a disturbed interaction between metabolic organs such as adipose tissue, liver and gut. This is especially a problem in midlife (between age 35 and 55) in which obesity has been reported to cause cognitive decline and increase risk for developing dementia. However, mechanisms involved in this organ-organ crosstalk are poorly understood.(4, 21-24) Firstly, one proposed

mechanism is the altered signalling of visceral and abdominal adipose tissue; adipose tissue acts as an independent endocrine organ releasing several hormones, proteins and cytokines, referred to as adipokines. Obesity is associated with dysfunctional white adipose tissue and therefore an imbalance in adipokines, such as increased levels of leptin and angiotensinogen, and low levels of adiponectin and omentin.(25, 26) Especially, visceral adipose tissue seems to produce unfavourable adipokines associated with more metabolic complications when compared to subcutaneous adipose tissue.(27-30) Importantly, the distribution of fat tissue depots differs between sexes. Overall, men accumulate more abdominal and visceral fat than women.(30) Moreover, women have a higher level of adipokines such as leptin and adiponectin.(31, 32) This disbalance in adipokines may induce inflammation in several organs such as the liver, gut and vascular endothelium. The latter causing atherosclerosis, ultimately leading to changes in cerebral blood flow (CBF).(25)

Secondly, signalling between, and within other organs, such as the liver, might be altered in obese patients. For example; the liver secretes hepatokines, such as insulin-like growth factor 1, selenoprotein P, leukocyte cell-derived chemotaxin, fetuin B and hepassocin, which may indirectly affect brain function and structure.(33, 34)

Thirdly, the gut microbiota composition in obese people differs from that of non-obese individuals, affecting metabolic processes, weight and obesity-related comorbidities.(35, 36) Microbiota is involved in adiposity and homeostasis but also influences energy balance via appetite and satiety signalling to the brain. Gut microbiota also affect the brain by producing (precursors of) neurotransmitters and short chain fatty acids, or through cytokines via the immune system.(37, 38) BS leads to a fast change in gut microbiota composition through changes in food intake, intestinal modifications due to the surgery itself, and metabolic improvements, eventually leading to changes in gut-brain communication.(15, 39, 40) Hence, metabolic organs, such as liver, gut and adipose tissue and gut microbiota may constitute new therapeutic targets. Although long-term results are not yet clear, the gut microbiota has already become a target for anti-obesity treatments.(37)

Obesity is associated with impaired CBF, which may lead to inadequate oxygen and energy supply in the brain and eventually loss of white and grey matter integrity.(41, 42) Lower levels of CBF in the prefrontal cortex are associated with reduced performance on executive function and episodic memory tests.(42, 43) Even in the prodromal stages of Alzheimer's disease, changes in CBF can be detected with arterial spin labelling (ASL), which may be used as a very early biomarker for neurodegenerative disorders.(44) However, the technique requires further optimization and therefore several consortia are working on the implementation of ASL perfusion magnetic resonance imaging (MRI) for clinical applications to provide images of sufficient and diagnostic utility.(45)

Furthermore, obesity is associated with changes in grey and white matter, which can be visualized using diffusion tensor imaging (DTI) and voxel-based morphometry analyses based on T1 weighted scans.(46, 47) These structural changes are especially prominent in brain regions governing reward seeking, inhibitory control and appetite.(48, 49) There are indications that rapid recovery of structural abnormalities occur after BS, however long-term study data is lacking here. (50, 51)

Additionally, impairment in attention span, executive function and memory are commonly reported in obese patients.(18, 19) Cognitive impairment revealed in obesity might be reversible and varies between cognitive domains however long-term follow-up studies are scarce. The Longitudinal Assessment of Bariatric Surgery (LABS) parent project is the most extensive longitudinal study to date focusing on cognitive changes in patients after BS. Investigators showed lasting improvements three years after surgery in the cognitive domains of attention, executive function and memory.(19)

Rationale

Cognitive benefits after BS are not equally exhibited across patients and cognitive domains. However, precise causes are still poorly understood, and underlying molecular mechanisms remain elusive. From the relatively short follow-up duration and small samples of BS patients in the studies reviewed, it is difficult to elaborate on the long-term consequences of obesity and its related diseases. In this study, underlying mechanisms of obesity-related cognitive disorders will be

investigated by longitudinal studies correlating cognition to brain changes, blood serum and plasma values, and gut microbiota composition. Lastly, metabolic and histopathological parameters (at the time-point of surgery) will be obtained to study whether associations or correlations exist between obesity-associated metabolic dysfunctions of particular organs and brain function and structure. To our knowledge this is the first study in humans investigating changes in brain structure and function, and changes in adipose tissue, liver function and the gut microbiome, after BS-induced weight loss. Additionally, this is the first study in bariatric research combining neuroimaging, cognition and extensive profiling of biological markers.

The primary aim of the BARICO study (**BA**riatric surgery **R**ijnstate and Radboudumc neuro**I**maging and **C**ognition in **O**besity) is to determine the long-term effect of weight loss on measures of brain function and structure after BS. The secondary aim is to provide mechanism-based rationales responsible for functional and structural decline in obese individuals. Therefore, the metabolic and inflammation status of organ biopsies will be determined together with molecular signatures via blood plasma/serum analyses. Furthermore, gut microbiota composition will be monitored over time to gain knowledge about the gut-brain axis.

This study will contribute to the development of better health campaigns, healthcare and preventatives to attenuate the impact of obesity. This paper describes the design and protocol of the BARICO study.

METHODS AND ANALYSIS

Study population

Patients who have been screened and found eligible for BS based on the Fried guidelines will be asked to participate.(52) In total, 150 patients will be included in the study. Study specific inclusion criteria are: (a) patients willing to perform neuropsychological tests, complete self-report

questionnaires and sign an informed consent document; (b) age between 35 and 55 years; (c) patients must undergo RYGB. A laparoscopic antecolic antegastric RYGB procedure will be performed (biliopancreatic limb of 150 cm, alimentary limb of 100 cm). Exclusion criteria for this study are: (a) previous or current neurological or severe psychiatric illness; (b) pregnancy; (c) treatment with any antibiotics, probiotics, or prebiotics three months before or at any point during the study (excluding preoperative prophylaxis). A subgroup of 75 patients will be included in the MRI sub-study, extra inclusion criteria for this group are: (d) patients willing to undergo MRI scanning and perform tasks in the MRI scanner; (e) right handed (more homogeneous sample and less variance). The standard exclusion criteria for the MRI subgroup include: (d) claustrophobia; (e) epilepsy; (f) pacemakers and defibrillators; (g) nerve stimulators; (h) intracranial clips; (i) infraorbital or intraocular metallic fragments; (j) cochlear implants; (k) ferromagnetic implants; (l) circumference above the MRI space capacity; (m) colour blindness. The study has been approved by the medical research ethics committee CMO Region Arnhem-Nijmegen (NL63493.091.17) and is registered at the Netherlands Trial Register (trialregister.nl) 7288.

Study design

At several time points (4-8 weeks preoperative, 6, 24 months and 5, 10 years postoperative (figure 1)) a number of cognitive tests and questionnaires will be performed, and their results assessed. Furthermore, blood (after 8 hrs. period of fasting) and faecal matter will be collected from all patients (N=150) (blood at all time points, faeces 4-8 weeks preoperative, 6 and 24 months postoperative (figure 1)). Intraoperatively, several tissue biopsies will be collected and processed. Medical evaluation, including anthropometric measurements and information on comorbidities, will be assessed 4-8 weeks preoperative and during all postoperative time points. A schematic overview of the study is shown in figure 1. A subgroup of patients (N=75) will additionally receive a (f)MRI scan 4-8 weeks preoperative and 24 months postoperative. During the whole study period (10 years)

patients will be contacted by letter and via telephone at least once a year to ensure the best follow-up rate.

Recruitment procedures and consent

Patients are informed about the study by letter and telephone at least two weeks prior to their standard visit (4-8 weeks before RYGB surgery). During this visit, patients will individually receive more information about this study and its objectives. Afterwards, the researchers will further clarify the study and the patients can ask for additional information. If they decide to participate and fulfil the inclusion criteria, informed consents will be obtained. Although the obese population consists of more females than males, the aim is for an equal sex distribution during the recruitment period (i.e., a study population consisting of >30% men and >30% women).(1) Recruitment will take place between August 2018 and August 2020.

Outcome measures

The primary outcome measures are the neuropsychological tests scores, CBF values, hippocampal volume, mean diffusivity (MD) and fractional anisotropy (FA) (representing respectively grey and white matter integrity), and blood oxygen level dependent (BOLD) responses during the Stroop task. Combining neuroimaging and neuropsychological tests will give us more information on how and whether structural brain changes are related to functional brain changes. Secondary measures comprise of (histopathological and biochemical determined) health status of the collected tissue, gut microbiota composition changes (in jejunal mucosa and faeces) and the profiling of circulating mediators in blood (plasma and serum), as well as lifestyle and dietary habits in relation to cognitive function and brain structure. Combining information on the pathological state of liver, gut, adipose tissue and circulating mediators from corresponding plasma/serum samples, obtained prior to and at surgery, will provide insight into organ cross-talk and allow identification of biomarker signatures for

metabolic health. Differences in metabolic health of the subjects may be associated with specific signalling molecule-profiles, which may be related to cognitive function.

(f)MRI

Subjects will be scanned in a 3T MAGNETOM Skyra MR scanner (Siemens AG, Healthcare Sector, Erlangen, Germany) using a 32-channel head coil. The MRI protocol included: a T1-weighted 3D magnetization-prepared rapid gradient-echo (MPRAGE) sequence for anatomical reference and analysis (TR/TI/TE 2300/1100/3.03 ms; 8° flip angle; voxel size: 1.0 × 1.0 × 1.0 mm), a fluid-attenuated inversion recovery (FLAIR) sequence for white matter lesion visualization (TR/TI 5000/1800 ms; voxel size: 1.0 × 1.0 × 1.0 mm), and diffusion-weighted MRI scans using simultaneous multi-slice echo planar imaging for probing microstructural properties (TR/TE 3275/91.4 ms; voxel size: 1.9 × 1.9 × 1.9 mm; 6x b=0 s/mm^2, 42x b=900 s/mm^2, 83x b=1800 s/mm^2). To allow for offline distortion correction of the images, 7 more b=0 s/mm^2 volumes will be acquired using the exact same sequence parameters - except for the inverted k-space read-out trajectory. An ASL sequence will used for quantification of CBF (TR/TE 2500/12 ms; voxel size: 4.0 × 4.0 × 4.0 mm) and a multi-band, multi-echo planar imaging sequence will be used to measure BOLD contrast during the Stroop task (TR/TE 1500/12.4, 34.3, 56.2 ms; 75° flip angle; voxel size: 2.5 × 2.5 × 2.5 mm; field of view 210 mm; 51 transversal slices in interleaved order). The complete scanning protocol takes 45 minutes and for both time-points, the same: MR scanner, head coil, and sequences will be used. Following the project MRI quality assurance is guaranteed by regular phantom measurements.

Cognitive assessment

Cognitive performance of all participants will be tested using an extensive neuropsychological test battery as detailed below. To assess general cognitive performance the Montreal Cognitive Assessment (MoCA) will be used.(53) To test attentional functions, the Flexibility subtest from the Tests of Attentional Performance (TAP 2.3) will be used.(54) This flexibility task focuses on shifting

attention between objects. Working memory will be assessed via the Digit Span subtest from the Wechsler Adult Intelligence Scale Fourth Edition (WAIS-IV-NL).(55) Participants will have to repeat a series of digits in forward or backward order, or sort them numerically. The Controlled Oral Word Association Test (COWAT) will be used to determine verbal fluency.(56) Participants have to come up with as many words beginning with three designated letters within 60 seconds (for each letter). Episodic memory will be assessed via the immediate and delayed Story Recall subtest from the Rivermead Behavioural Memory Test (RBMT).(57) To control and correct for differences in premorbid intelligence between participants, verbal IQ will be estimated using the Dutch version of the National Adult Reading Test (NART) at baseline.(58) The MoCA, episodic memory test and COWAT have parallel versions, to avoid material-specific learning effects during the repeated testing. Additionally, the tests are standardized, have been validated for use across a wide age range and have good re-test reliability. Together these tests will provide a good overview on the overall cognitive performance of the patients, including aspects of working and episodic memory, attention, verbal fluency and executive function. Also, education level will be recorded in accordance with the Dutch education system using seven categories (one being the lowest level of education and seven being the highest).(59)

Assessment of biological measurements

At several time points (figure 1) fasting (at least 8 hrs.) blood samples from the participants will be collected. As standard procedure classical parameters, such as several vitamins (vitamin B12, D and folic acid) and lipids (triglycerides and cholesterol) will be measured. Special interest is taken on circulating mediators of organ cross-talk, such as: cytokines, oxylipids, adipokines, hormones and inflammation markers (e.g., C-reactive protein, serum amyloid A, vascular cell adhesion molecule 1, transforming growth factor beta), as well as metabolites (derived from organs or microbiota) assessed by metabolomics, such as bile acids and bioactive (short chain) fatty acids, and other lipid species (untargeted lipidomics).

Besides blood samples, faeces will be collected (figure 1) using "faeces collection kits for at home" in order to monitor gut-microbiota changes and relate them to cognition and brain structure and function readouts. Additionally, to gain insight into the microbiota in the intestinal mucosa, mucosal swabs will be collected during surgery within the jejunum (two places; 150 and 250cm from Treitz ligament) and stomach pouch.

As metabolically active organs such as the liver and adipose tissue interact directly and indirectly with the brain, biopsies of these organs will be collected and analysed on histopathological, and biochemical level. Tissue biopsies from subcutaneous, mesenteric and omental adipose tissue, liver and jejunum. Tissue biopsies from these organs will be taken to assess potential pathophysiological processes and to eventually define mechanism-based subgroups.

Questionnaires

At several time-points (figure 1) standardized questionnaires on lifestyle, education, success rate of the surgery and eating habits will be assessed. Most of the questionnaires are routine practice for patients undergoing BS at the Rijnstate hospital. Physical activity will be assessed via the Baecke questionnaire and depressive symptoms will be assessed with the Beck Depression Inventory (BDI-II).(60, 61) To estimate the participants' food/nutrient intake and eating behaviour patients will be asked to fill out an eating diary of two days (a weekday and a weekend day). Quality of Life will be evaluated with the Short Form 36 (SF-36).(62) Lastly, the results of BS will be evaluated via the Bariatric Analysis and Report Outcome System (BAROS).(63)

More specifically: the Barratt impulsivity scale (BIS-11)(64) and Behavioural inhibition/activation system (BIS/BAS)(65) questionnaires on impulsivity and reward sensitivity are included as reward sensitivity and impulsivity have both previously been suggested to contribute to overeating.(66) Indeed, some facets of impulsivity and reward sensitivity have shown to be relevant in eating- and weight regulation.(67)

Medical evaluation

At several time points during the study (figure 1) a medical evaluation will take place where anthropometric measurements such as: body weight, length, waist circumference and blood pressure will be quantified. BMI will be calculated as weight divided by height in meters squared. Percentage excess weight loss (%EWL) (defined as weight loss divided by preoperative excess weight, with excess weight defined as the weight above a normal BMI of 25 kg/m^2) will be calculated during the time points after surgery, similar to percentage total body weight loss (%TBWL) (defined as weight loss divided by preoperative weight). The success of BS in terms of weight loss will be defined as a sustained weight loss larger than 50 %EWL.

Furthermore, data on comorbidities like T2DM, HT and DL and associated medication will be collected before the surgery and at all time-points after surgery. Comorbidities will be defined using following criteria: for T2DM a fasting plasma glucose of ≥ 7.0 mmol/L and HbA1c ≥ 48 mmol/mol (HbA1c ≥ 6.5%) or the use of oral antidiabetic or insulin medication; for HT the use of antihypertensive drug treatment; for DL the use of statins.

Data management

Data management will be handled using Research Manager (RM, Cloud 9 Health Solutions©), an established software package and data management tool that follows Good Clinical Practice (GCP) guidelines.(68) Every change in the data is recorded in a log system and can be traced. Participants will be identified only by a study specific identification code. One researcher will keep a separate participant identification code list that matches the study-specific identifying codes with the participant's names. Documents will be maintained by the investigator in strict confidence.

Sample size

The power calculation for the neuropsychological tasks was based on the results of the Digit Span subtest performed in a comparable study population.(19) With an expected standardized effect size

of at least 0.3 and a correlation of 0.7, a selected sample of 150 patients will be sufficient to reach 90% power. The power calculation for the MRI parameters is based on changes in the FA parameter studied by Zhang *et al.*(51) With an expected standardized effect size of at least 0.03 and a correlation of 0.5 including 75 patients in the MRI group will be sufficient to reach 90% power. A significance level based on the sequentially rejective multiple testing procedure discussed by Bretz *et al.* (for the neuropsychological tests 3% and for the MRI parameters 2%) has been taken into account in the power calculation.(69) The inclusion of 150 patients with a subgroup of 75 for the MRI scan has been considered adequate to answer the research questions with sufficient power.

Analysis of primary outcome measures

As a primary outcome measure, baseline levels of the imaging parameters (such as MD and FA) will be compared with the results of the neuroimaging outcome 24 months after surgery (including %TBWL as a factor in the model). Next, the scores of the cognitive tests from five different time points will be analysed and compared to %TBWL. Every dependent variable will be modelled in a separate linear mixed model. %TBWL will be used as a factor. Different variables, such as: depression score, age, and gender, will be (if appropriate) included in the model. For each model, we will decide which variables to include as a factor to reduce the amount of unexplained variation. To correct for multiple outcome measures, the sequentially rejective multiple testing procedure described in Bretz *et al.* will be used (more information in the supplementary material).(69) Data will be analysed using SPSS (version 25 for Windows) and R (version 3.5.1 for Windows). For the cognitive tests a *p* value of <0.03 and for the imaging parameters a *p* value of <0.02 will be considered as statistically significant.

Analysis of secondary outcome measures

As secondary outcome measures, the metabolic and histopathological parameters (obtained analyses from tissues collected during surgery) will be analysed cross-sectionally to examine correlations between and among each other, and in relation to brain function and structure. Furthermore,

potential mechanisms underlying the crosstalk along the gut-brain axis will be investigated by longitudinal analyses focusing on establishing correlations between brain structure/function changes and changes in circulation mediators or faecal microbiota composition. Pearson correlation analysis will be used to investigate potential correlations between variables.

Data monitoring

Every year, data monitoring and auditing will be conducted by an independent specialised monitor from the Rijnstate Hospital. Yearly, a summary of the progress will be submitted to the ethical committee and the Netherlands Trial Register (trialregister.nl) 7288.

Patient and Public involvement

Patients and the public were not involved in the design of this study. Nevertheless, the results will be disseminated to the study participants via email, newsletters and social media platforms after the study results are published.

DISCUSSION

The BARICO study is a prospective study focusing on the effect of weight loss on cognitive function and brain structure after BS. This will be measured using sensitive neuropsychological tests covering the most important domains, fMRI activation during the Stroop task, and several MRI techniques, such as DTI and ASL. To clarify the impact of metabolic dysfunction in obesity on brain function and structure, blood plasma and stool samples will be collected and analysed longitudinally, and biopsies of key metabolic organs will be collected during the RYGB and analysed cross-sectionally.

After BS, there have only been a limited number of long-term studies demonstrating improvement in several cognitive domains, including memory, attention and executive function.(18, 19) Furthermore, it has been shown that obese individuals have lower grey and white matter volumes, and altered white matter densities, in comparison to healthy individuals with several studies showing a rapid

recovery of these brain structural abnormalities after BS.(50, 51) For instance, Tuulari *et al.* showed a causal link between weight loss and brain tissue recovery.(50) Approximately 25-30% of the patients are not expected to reach sufficient weight loss (≤50 %EWL), and thus it will be possible to study the effect of weight loss after BS on brain function and structure.

Perhaps the strength of this study is in the long follow-up duration after surgery: 24 months for the neuroimaging parameters, and 10 years for the neuropsychological tests. Furthermore, the strict inclusion criterion with respect to age range ensures a good representation of mid-life patients. Moreover, the majority of studies into BS patients are mostly composed of women but it is equally important to account for the variation in fat tissue distribution which is caused by differences in sex.(30)

Another strength of this study is the combination of neuroimaging and neuropsychological tests. Alongside the analysis of metabolic and histopathological parameters (obtained in blood, organ biopsies and microbiota), meaning that the relation between multiple metabolic, neuroimaging and/or cognitive parameters can be investigated (e.g., adipokines, bioactive lipids (short-chain fatty acids) and organ dysfunction) in a comprehensive way. Since RYGB influences gut-brain communication, there may be beneficial alterations in adipose tissue functions, and/or recovery of brain function and structure following BS.(15, 70) Longitudinal analyses of the microbiota, together with analysis of functional gut-derived metabolites in the circulation and cognitive outcomes, may allow for the identification of mediators derived from gut microflora that are relevant to cognition and the prevention of cognitive decline.

The BARICO study has the potential to be the first to demonstrate interactions between the periphery and central nervous system after weight loss in humans, in particular it will question the roles and involvement of the brain, and adipose tissue, liver and gut microbiota, after weight loss caused by BS.

In conclusion, the BARICO study will reveal the relation and underlying mechanisms between obesity and brain function and structure. This information can be used to develop better health care as well as possible preventatives against obesity and associated disorders.

ETHICS AND DISSEMINATION

The study protocol was authorized by the medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17). All patients will sign informed consent forms upon enrolment in the study. Study results will be submitted for publication in peer-reviewed journals.

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Contributors

EOA and AJK conceived and designed the study. DV wrote the article and developed the protocol together with EOA, AJK, EJH, and RK. EJH, EOA and AJK are the principal investigators and DV is the main investigator. MW, LND, IAA, EA, RK and RPCK are co- investigators in the participating centres. All authors critically reviewed the content and approved the final manuscript.

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Competing interests

The authors declare that they have no conflicts of interests.

Ethics approval

Medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17).

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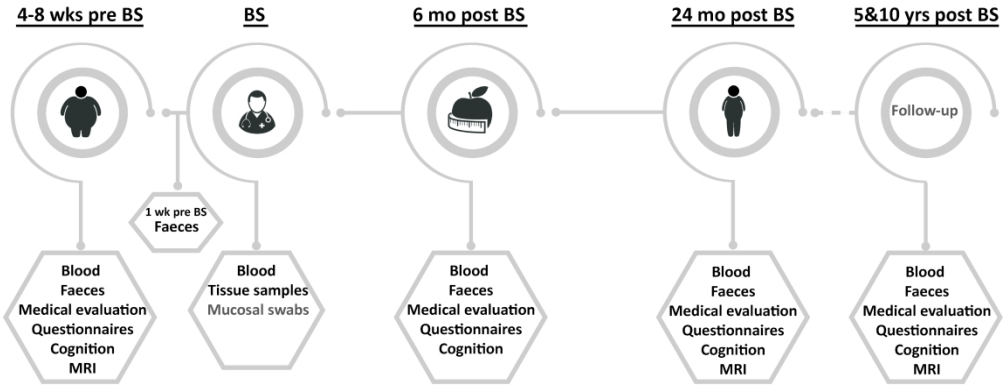
FIGURE LEGEND

Figure 1. Overview of the study design. Blood samples are taken during a regular blood withdrawal at six time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota analyses will be performed at set time points on the faeces (collected at home by the patients) (4-8 wks. pre BS, 1 wk. pre BS, 6 mo. post BS, 24 mo. post BS) and mucosal swabs (collected during surgery). Furthermore, biopsies of liver, jejunum and adipose tissue depots (subcutaneous, mesenteric and omental) will be collected during surgery. Before surgery, (4-8 wks. pre BS) and at several time points after, (6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS) a medical evaluation will take place and all patients will complete questionnaires and neuropsychological measurements to test cognitive function. A subgroup of patients (N=75) will be examined with MRI (4-8 wks. pre BS and 24 mo. post BS). MRI; magnetic resonance imaging. BS; Bariatric surgery. Wks; weeks. Mo; months. Yrs; years.



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SUPPLEMENTARY MATERIAL

Since multiple outcome measures will be studied, correction for this is applied using the sequentially rejective multiple testing procedure described in Bretz *et al.* (2008)(69). As we are highly interested in both the neuropsychological tests and the MRI parameters, the MRI parameters and the neuropsychological parameters are clustered. A significance level of 0.05 is used, and an alpha level of 0.03 is allocated to the neuropsychological tests and 0.02 to the MRI parameters. The neuropsychological tests and neuroimaging tests will be tested with a multiple testing procedure (supplementary figure 1). The neuropsychological tests will initially be tested at 3/5 of the overall type I error rate (i.e. 0.03 two-sided) and neuroimaging parameters at 2/5 of it (i.e. 0.02 two-sided). Alpha will be reallocated when shown that the corresponding hypothesis is rejected. Based on the literature a specific hypothesis sequence will be tested (the sequence for the neuropsychological tests is: digit span, TAP flexibility task, story immediate/delayed recall, verbal fluency and MoCA; for the MRI parameters: DTI parameters, ASL measures, BOLD response of the Stroop test and grey and white matter volumes). Within each test separately correction for multiple testing will be included, for example for multiple brain areas analysed within a MRI parameter.

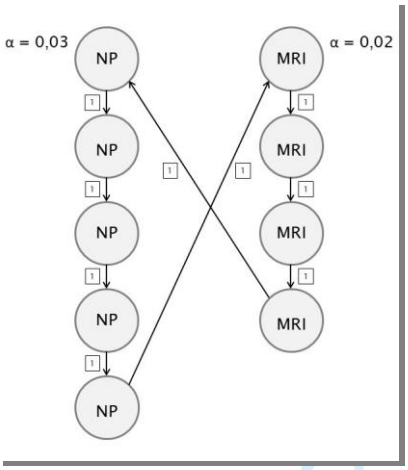

FIGURE LEGEND

Supplementary figure 1. Multiple testing sequence. NP: neuropsychological tests, MRI: MRI parameters.