

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [info.bmjopen@bmj.com](mailto:info.bmjopen@bmj.com)

# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025464
Article Type:	Protocol
Date Submitted by the Author:	16-Jul-2018
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
Keywords:	Obesity, Weight loss, Bariatric surgery, Neuroimaging, Cognition

SCHOLARONE™  
Manuscripts

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33

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

3  
4 Vreeken, D.<sup>1,2,3</sup>, Wiesmann, M.<sup>3</sup>, Deden, L.N.<sup>1,2</sup>, Arnoldussen, I.A.C.<sup>3</sup>, Aarts, E.<sup>4</sup>, Kessels, R.P.C.<sup>4,5,6</sup>,  
5 Kleemann, R.<sup>7</sup>, Hazebroek, E.J.<sup>1,2</sup>, Aarts, E.O.<sup>1,2\*</sup>, Kiliaan, A.J.<sup>3\*\*</sup>

6  
7 <sup>1</sup> *Department of Surgery, Rijnstate Hospital, Arnhem, the Netherlands.*

8 <sup>2</sup> *Vitalys Clinic, Velp, the Netherlands.*

9 <sup>3</sup> *Department of Anatomy, Donders Institute for Brain, Cognition and Behaviour, Radboud university*  
10 *medical center, Nijmegen, the Netherlands.*

11 <sup>4</sup> *Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, the*  
12 *Netherlands.*

13 <sup>5</sup> *Department of Medical Psychology, Radboud University Medical Center, Nijmegen, the Netherlands.*

14 <sup>6</sup> *Vincent van Gogh Institute for Psychiatry, Venray, the Netherlands.*

15 <sup>7</sup> *Department of Metabolic Health Research, Netherlands Organization for Applied Scientific Research*  
16 *(TNO), Leiden, the Netherlands.*

17  
18 \*These authors contributed equally.

19 <sup>†</sup> **Corresponding author:**

20 Amanda J. Kiliaan, PhD

21 Donders Institute for Brain, Cognition, and Behaviour

22 Radboud university medical center

23 Department of Anatomy (109)

24 Geert Grooteplein 21N

25 6525 EZ Nijmegen, the Netherlands

26 Phone: +31 24 361 4378

27 Email: [amanda.kiliaan@radboudumc.nl](mailto:amanda.kiliaan@radboudumc.nl)

28  
29 **Short title: The BARICO study, effect of weight loss on brain function**

30 Words count (excluding title page, abstract, references and figure): 3782

31 Words count abstract: 300

32  
33 **Keywords: obesity, weight loss, bariatric surgery, neuroimaging, cognition**

## 34 **ABSTRACT**

### 35 **Introduction**

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

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

### 49 **Methods and analysis**

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

### 59 **Ethics and dissemination**

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

63

64 **Trial registration**

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

For peer review only

## 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.

## 78 INTRODUCTION

79 For over two decades, obesity-induced diseases, such as cardiovascular disease, and type 2 diabetes  
80 are one of the major health-care challenges of today's society.(1) Besides these well-known  
81 metabolic complications, it has become clear that obesity may lead to structural brain changes,  
82 cognitive impairment and neurodegenerative diseases.(2-5) A direct relationship exists between  
83 increased body mass and cognitive impairment.(6-9) To improve and possibly reduce the amount of  
84 obesity-induced diseases and inhibit cognitive impairment and neurodegenerative diseases,  
85 sustainable long-term weight loss in obese patients is required. Non-surgical treatments for obesity,  
86 such as dietary restriction and physical activity, often show disappointing long-term effects,  
87 especially in patients with morbid obesity (BMI above 40 kg/m<sup>2</sup>). (10, 11) Bariatric surgery (BS),  
88 decreases body mass rapidly, and especially the commonly performed Roux-en-Y gastric bypass  
89 (RYGB) leads to this rapid weight loss which is often accompanied by remission of type 2 diabetes  
90 mellitus, hypertension and hyperlipidaemia.(12, 13) RYGB is a restrictive surgical procedure,  
91 excluding the main part of the stomach, the duodenum and the first part of the jejunum from the  
92 passage of food, leading to, among others, hormonal and gut microbiota changes.(14, 15)  
93 Besides weight loss and remission of comorbidities, RYGB surgery is also associated with improved  
94 cognitive functions.(16, 17) This may be related to multiple metabolic parameters, such as systolic  
95 blood pressure or triglyceride concentrations.(18) Metabolic complications may arise in obesity due  
96 to a disturbed interaction between metabolic organs such as the adipose tissue, liver and the gut.  
97 Especially in midlife (between the age of 35 and 55), it has been reported that obesity, may impair  
98 cognitive functioning and increase the risk for dementia. However, mechanisms involved in this  
99 organ-organ crosstalk are poorly understood.(4, 19-22) One proposed mechanism is the altered  
100 signalling of visceral and abdominal adipose tissue. Adipose tissue acts as an independent endocrine  
101 organ releasing several hormones, proteins and cytokines, referred to as adipokines. Obesity is  
102 associated with dysfunctional white adipose tissue and therefore an imbalance in adipokines, such as  
103 increased levels of leptin and angiotensinogen, and low levels of adiponectin and omentin.(23, 24)

1  
2  
3 104 Especially, visceral adipose tissue seems to produce unfavourable adipokines and is associated with  
4  
5 105 more metabolic complications when compared to subcutaneous adipose tissue.(25-28) Importantly,  
6  
7 106 distribution of fat tissue depots differs between sexes. Overall, men accumulate more abdominal and  
8  
9 107 visceral fat than women.(28) Moreover, women have a higher level of adipokines such as leptin and  
10  
11 108 adiponectin.(29, 30) The disbalance in adipokines may induce inflammation in several organs such  
12  
13 109 as the liver, gut and vascular endothelium. The last one causing atherosclerosis, which ultimately  
14  
15 110 may lead to changes in cerebral blood flow (CBF).(23)

16  
17 111 Secondly, signalling between and within other organs, such as the liver, might also be disturbed in  
18  
19 112 obese patients. The liver secretes hepatokines, such as insulin-like growth factor 1, selenoprotein P,  
20  
21 113 leukocyte cell-derived chemotaxin, fetuin B and hepassocin, which may indirectly affect brain  
22  
23 114 function and structure.(31, 32)

24  
25 115 Thirdly, gut microbiota composition in obese people differ from that of non-obese individuals  
26  
27 116 affecting metabolic processes, weight and obesity-related comorbidities.(33, 34) Microbiota is  
28  
29 117 involved in adiposity and homeostasis, but also influences energy balance via hunger and satiety  
30  
31 118 signalling to the brain. Gut microbiota may also affect the brain by producing (precursors of)  
32  
33 119 neurotransmitters and short chain fatty acids, or through cytokines via the immune system.(35, 36)  
34  
35 120 Bariatric surgery leads to a fast change in gut microbiota composition through changes in food  
36  
37 121 intake, intestinal modifications due to the surgery itself and metabolic improvements, which might  
38  
39 122 eventually lead to changes in gut-brain communication.(15, 37, 38) Hence, the metabolic organs,  
40  
41 123 such as the liver, gut and adipose tissue and the gut microbiota may constitute new therapeutic  
42  
43 124 targets. Although long-term results are not yet clear, the gut microbiota has become a target for anti-  
44  
45 125 obesity treatments.(35)

46  
47 126 Obesity is associated with impaired cerebral blood flow (CBF), which may lead to inadequate oxygen  
48  
49 127 and energy supply in the brain and eventually loss of white and grey matter integrity.(39, 40) Lower  
50  
51 128 levels of CBF in the prefrontal cortex are associated with reduced performance on tests of executive  
52  
53 129 function and episodic memory.(40, 41) Even in the prodromal stages of Alzheimer's disease, changes  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 130 in CBF can be detected with perfusion MRI (arterial spin labelling; ASL), which may be used as a very  
4  
5 131 early biomarker for neurodegenerative disorders.(42) However, the technique needs further  
6  
7 132 optimization and several consortia are working on implementation of ASL perfusion MRI for clinical  
8  
9 133 applications to provide images of sufficient and diagnostic utility.(43)

10  
11 134 Furthermore, obesity is associated with changes in grey and white matter, which can be visualized  
12  
13 135 using diffusion tensor imaging (DTI) and voxel-based morphometry analyses based on T1 weighted  
14  
15 136 scans.(44, 45) These structural changes are especially prominent in brain regions governing reward  
16  
17 137 seeking, inhibitory control and appetite.(46, 47) There are indications that rapid recovery of  
18  
19 138 structural abnormalities takes place after BS.(48, 49) However, long-term data are lacking here.

20  
21 139 Additionally, impairment in attention span, executive function and memory are commonly reported  
22  
23 140 in obese patients.(16, 17) Cognitive impairment revealed in obesity might be reversible and varies  
24  
25 141 between cognitive domains, but long-term follow-up studies are scarce. The Longitudinal Assessment  
26  
27 142 of Bariatric Surgery (LABS) parent project is the most extensive longitudinal study to date focusing on  
28  
29 143 cognitive changes in patients after BS. Investigators showed lasting improvements in the cognitive  
30  
31 144 domains of attention, executive function and memory.(17)

32  
33  
34 145

#### 35 36 146 **Rationale**

37  
38 147 Cognitive benefits after BS are not equally exhibited across patients and cognitive domains. However,  
39  
40 148 the precise causes are still poorly understood and underlying molecular mechanisms remain elusive.

41  
42 149 From the relatively short follow-up duration and small samples of BS patients in the studies  
43  
44 150 reviewed, it is difficult to elaborate on the long term consequences of obesity and its related  
45  
46 151 diseases. In this study, the mechanisms underlying obesity-related cognitive disorders will be  
47  
48 152 investigated by longitudinal studies correlating cognition to brain changes, blood serum and plasma  
49  
50 153 values and gut microbiota composition. Lastly, metabolic and histopathological parameters (at the  
51  
52 154 time-point of the surgery) will be obtained to study whether associations or correlations exist  
53  
54 155 between obesity-associated metabolic dysfunction of particular organs and brain function and  
55  
56  
57  
58  
59  
60

1  
2  
3 156 structure. To our knowledge this is the first study in humans that investigates brain structure and  
4  
5 157 function changes after BS-induced weight loss and possible linkage between adipose tissue, liver  
6  
7 158 function and the gut microbiome. Additionally, this is the first study in bariatric research combining  
8  
9 159 neuroimaging, cognition and extensive profiling of biological markers.  
10

11  
12 160

13 161 The primary aim of the BARICO study (**BA**riatric surgery **Rijn**state and Radboudumc neuro**Im**aging  
14  
15 162 and **C**ognition in **O**besity) is to determine the long-term effect of weight loss after bariatric surgery  
16  
17 163 on measures of brain function and structure. The secondary aim is to provide mechanism-based  
18  
19 164 rationales responsible for functional and structural decline in obese individuals. Furthermore, the  
20  
21 165 extensive molecular profiling of tissues (i.e. organ biopsies, blood plasma/serum, and microbiota) will  
22  
23 166 provide information that can be used to characterize the pathological state of organs, and eventually  
24  
25 167 monitor this state via molecular signatures in the circulation. It will also provide information to  
26  
27 168 stratify obese patients based on specific molecular signatures and pathways into risk groups  
28  
29 169 regarding a particular organ dysfunction (mechanism-based subgroups). This study will therefore  
30  
31 170 contribute to the development of better health campaigns, health care and preventatives to  
32  
33 171 attenuate the impact of obesity. This paper describes the design and protocol of the BARICO study.  
34  
35

36  
37 172

## 38 173 **METHODS AND ANALYSIS**

### 39 174 **Study population**

40  
41  
42 175 Patients who have already been screened and found eligible for BS based on the Fried guidelines will  
43  
44 176 be asked to participate.(50) Totally, 150 patients will be included in the study. Study specific inclusion  
45  
46 177 criteria are: (a) patients willing to perform neuropsychological tests and complete self-report  
47  
48 178 questionnaires and sign an informed consent document; (b) age between 35 and 55 years; (c)  
49  
50 179 patients must undergo Roux-en-Y gastric bypass (RYGB). Exclusion criteria for this study are: (a)  
51  
52 180 previous or current neurological or severe psychiatric illness; (b) pregnancy; (c) treatment with any  
53  
54 181 antibiotics, probiotics, or prebiotics three months before or during the study (excluding preoperative  
55  
56  
57  
58  
59  
60

1  
2  
3 182 prophylaxis). A subgroup of 75 patients will be included in the MRI sub-study, extra inclusion criteria  
4  
5 183 for this group are: (d) patients willing to undergo MRI scanning and perform tasks in the MRI scanner;  
6  
7 184 (e) right handed (more homogeneous sample and less variance). The standard exclusion criteria for  
8  
9 185 the MRI subgroup include: (d) claustrophobia; (e) epilepsy; (f) pacemakers and defibrillators; (g)  
10  
11 186 nerve stimulators; (h) intracranial clips; (i) infraorbital or intraocular metallic fragments; (j) cochlear  
12  
13 187 implants; (k) ferromagnetic implants; (l) lying circumference above the MRI space capacity; (m)  
14  
15 188 colour blindness. The study has been approved by the medical research ethics committee CMO  
16  
17 189 Region Arnhem-Nijmegen (NL63493.091.17) and is registered at the Netherlands Trial Register  
18  
19 190 (trialregister.nl) 7288.  
20  
21  
22

191

### 192 **Study design**

23  
24  
25  
26 193 At different time points (4-8 weeks preoperative, 6, 24 months and 5, 10 years postoperative, figure  
27  
28 194 1) several cognitive tests and questionnaires will be assessed. Furthermore, fasting blood and faecal  
29  
30 195 matter will be collected in all patients (N=150) (blood at all time points, faeces 4-8 weeks  
31  
32 196 preoperative, 6 and 24 months postoperative, figure 1). During RYGB surgery, several tissue biopsies  
33  
34 197 will be collected and processed. A schematic overview of the study is shown in figure 1. Furthermore,  
35  
36 198 length and weight will be assessed at each time point. A subgroup of patients (N=75) will additionally  
37  
38 199 receive a (f)MRI scan 4-8 weeks preoperative and 2 years postoperative. During the whole study  
39  
40 200 period (ten years) patients will be contacted by letter and via telephone at least once a year to assure  
41  
42 201 the best follow up rate.  
43  
44

202

### 203 **Recruitment procedures and consent**

44  
45  
46  
47  
48  
49 204 Patients are informed about the study by mail and telephone at least one week prior to their  
50  
51 205 standard information visit (four to eight weeks before RYGB surgery). During this visit, patients will  
52  
53 206 individually receive more information about this study and its objectives. Afterwards, the researchers  
54  
55 207 will further clarify the study and the patients can ask for additional information. If they decide to  
56  
57  
58  
59

1  
2  
3 208 participate and fulfil the inclusion criteria, informed consents will be signed. Although the obese  
4  
5 209 population consists of more females than males, the aim is an equal sex distribution during the  
6  
7 210 recruitment period (i.e., a study population consisting of >30% men and >30% women).(1)  
8  
9 211 Recruitment will take place between August 2018 and August 2020.

10  
11 212

### 13 213 **Outcome measures**

15 214 The primary outcome measures are the neuropsychological tests scores, CBF values, hippocampal  
16  
17 215 volume, mean diffusivity and fractional anisotropy (representing respectively grey and white matter  
18  
19 216 integrity) and BOLD responses during the Stroop task. Combining neuroimaging and  
20  
21 217 neuropsychological tests will give us more information how and whether the structural brain changes  
22  
23 218 are related to functional brain changes. Secondary measures comprise the (histopathological and  
24  
25 219 biochemical determined) health status of the collected organs, gut microbiota composition changes  
26  
27 220 (in jejunal mucosa and faeces) and profiling of circulating mediators in blood (plasma and serum), as  
28  
29 221 well as lifestyle and dietary habits in relation to cognitive function and brain structure. Combining  
30  
31 222 information on the pathological state of liver, gut and adipose tissue and circulating mediators from  
32  
33 223 corresponding plasma/serum samples obtained prior to and at surgery will provide insight into organ  
34  
35 224 cross-talk and allow identification of biomarker signatures for metabolic health. Differences in  
36  
37 225 metabolic health of the subjects may be associated with specific signalling molecule-profiles, which  
38  
39 226 may be related to cognitive function.

40  
41 227

### 43 228 **(f)MRI**

44  
45 229 Subjects will be scanned in a 3T MAGNETOM Skyra MR scanner (Siemens AG, Healthcare Sector,  
46  
47 230 Erlangen, Germany) using a 32-channel head coil. The MRI protocol included a T1-weighted 3D  
48  
49 231 magnetization-prepared rapid gradient-echo (MPRAGE) sequence for anatomical reference and  
50  
51 232 analysis (TR/TI/TE 2300/1100/3.03 ms; 8° flip angle; voxel size: 1.0 × 1.0 × 1.0 mm), a fluid-  
52  
53 233 attenuated inversion recovery (FLAIR) sequence for white matter lesion visualization (TR/TI

1  
2  
3 234 5000/1800 ms; voxel size: 1.0 × 1.0 × 1.0 mm), diffusion-weighted MRI scans using simultaneous  
4  
5 235 Multi-slice echo planar imaging for probing microstructural properties (TR/TE 3275/91.4 ms; voxel  
6  
7 236 size: 1.9 × 1.9 × 1.9 mm; 6x b=0 s/mm<sup>2</sup>, 42x b=900 s/mm<sup>2</sup>, 83x b=1800 s/mm<sup>2</sup>). To allow for offline  
8  
9 237 distortion correction of the images, 7 more b=0 s/mm<sup>2</sup> volumes will be acquired using the exact same  
10  
11 238 sequence parameters except for the inverted k-space read-out trajectory. An arterial-spin labelling  
12  
13 239 sequence will used for quantification of cerebral blood flow (TR/TE 2500/12 ms; voxel size: 4.0 × 4.0  
14  
15 240 × 4.0 mm) and a multi-band, multi-echo planar imaging sequence will be used to measure blood  
16  
17 241 oxygen level dependent (BOLD) contrast during the Stroop task (TR/TE 1500/12.4, 34.3, 56.2 ms; 75°  
18  
19 242 flip angle; voxel size: 2.5 × 2.5 × 2.5 mm; field of view 210 mm; 51 transversal slices in interleaved  
20  
21 243 order). The complete scanning protocol takes 45 minutes. For both time points, the same MR  
22  
23 244 scanner, head coil and sequences will be used. Following the project MRI quality assurance is  
24  
25 245 guaranteed by regular phantom measurements.  
26  
27  
28 246  
29

### 30 247 **Cognitive assessment**

31  
32 248 Cognitive performance of all participants will be tested using an extensive neuropsychological test  
33  
34 249 battery as detailed below. To assess general cognitive performance the Montreal Cognitive  
35  
36 250 Assessment (MoCA) will be used.(51) To test attentional functions, the Flexibility subtest from the  
37  
38 251 Tests of Attentional Performance (TAP 2.3) will be used.(52) This flexibility task focuses on shifting  
39  
40 252 attention between objects, since paying attention is not a static process. Working memory will be  
41  
42 253 assessed via the Digit Span subtest from the Wechsler Adult Intelligence Scale Fourth Edition (WAIS-  
43  
44 254 IV-NL).(53) Participants have to repeat a series of digits in forward or backward order, or sort them  
45  
46 255 numerically. The Controlled Oral Word Association Test (COWAT) will be used to determine verbal  
47  
48 256 fluency.(54) Participants have to come up with as many words beginning with three designated  
49  
50 257 letters within 60 seconds (for each letter). Episodic memory will be assessed via the immediate and  
51  
52 258 delayed Story Recall subtest from the Rivermead Behavioural Memory Test (RBMT).(55) To control  
53  
54 259 and correct for differences in premorbid intelligence between participants, verbal IQ will be  
55  
56  
57  
58  
59

1  
2  
3 260 estimated using the Dutch version of the National Adult Reading Test (NART) at baseline.(56) The  
4  
5 261 MoCA, episodic memory test and COWAT have parallel versions, to avoid material-specific learning  
6  
7 262 effects during the repeated testing. Additionally, the tests are standardized, have been validated for  
8  
9 263 use across a wide age range and have good re-test reliability. Together these tests will provide a  
10  
11 264 good overview on the overall cognitive performance of the patients, including aspects as working and  
12  
13 265 episodic memory, attention, verbal fluency and executive function. Also, education level will be  
14  
15 266 recorded in accordance with the Dutch education system using 7 categories (1 being the lowest level  
16  
17 267 of education and 7 being the highest).(57)  
18

19  
20  
21 268

### 22 269 **Assessment of biological measurements**

23  
24 270 On several time points (see figure 1) fasting (at least 5 hrs.) blood samples of the participants will be  
25  
26 271 collected. As standard procedure, classical parameters will be measured, such as several vitamins  
27  
28 272 and lipids (triglycerides and cholesterol). Special interest is on circulating mediators of organ cross-  
29  
30 273 talk, such as cytokines, oxylipids, adipokines, hormones and inflammation markers as well as  
31  
32 274 metabolites (derived from organs or microbiota) assessed by metabolomics such as bile acids and  
33  
34 275 bioactive (short chain) fatty acids, and other lipid species (untargeted lipidomics).  
35

36 276 Besides blood samples, faeces will be collected at different time points (see figure 1) using “faeces  
37  
38 277 collection kits for at home” in order to monitor gut-microbiota changes and relate them to cognition  
39  
40 278 and brain structure and function readouts. To gain insight into the microbiota in the intestinal  
41  
42 279 mucosa, mucosal swabs will be collected within the jejunum (two places; 150 and 250cm from Treitz  
43  
44 280 ligament) and stomach pouch (all during the surgery).  
45

46  
47 281 As metabolically active organs such as the liver and adipose tissue interact directly and indirectly with  
48  
49 282 the brain, biopsies of these organs will be collected and analysed on histopathological and molecular,  
50  
51 283 biochemical level. The different tissues collected will be subcutaneous, mesenteric and omental  
52  
53 284 adipose tissue, liver and jejunum. Tissue biopsies from these organs will be taken to assess potential  
54  
55 285 pathophysiological processes and to eventually define mechanism-based subgroups.  
56

286

**287 Questionnaires**

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

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

300

**301 Physical measurements**

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

305

**306 Data management**

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

1  
2  
3 311 participant identification code list that matches the study-specific identifying codes with the  
4  
5 312 participant's names. Documents will be maintained by the investigator in strict confidence.

6  
7 313

#### 8 9 314 **Sample size**

10  
11 315 The power calculation for the neuropsychological tasks is based on the results of the Digit Span  
12  
13 316 subtest performed in a comparable study population.(17) With an expected standardized effect size  
14  
15 317 of at least 0.3 and a correlation of 0.7, a selected sample of 150 patients will be sufficient to reach  
16  
17 318 90% power. The power calculation for the MRI parameters is based on the changes in the FA  
18  
19 319 parameter studied by Zhang *et al.*(49) With an expected standardized effect size of at least 0.03 and  
20  
21 320 a correlation of 0.5 including 75 patients in the MRI group will be sufficient to reach 90% power. A  
22  
23 321 significance level based on the sequentially rejective multiple testing procedure discussed by Bretz *et*  
24  
25 322 *al.* (for the neuropsychological tests 3% and for the MRI parameters 2%) has been taken into account  
26  
27 323 in the power calculation.(66) The inclusion of 150 patients with a subgroup of 75 for the MRI scan has  
28  
29 324 been considered adequate to answer the research question with sufficient power.

30  
31  
32 325

#### 33 34 326 **Analysis of primary outcome measures**

35  
36 327 As primary outcome measure, baseline levels of the imaging parameters (such as mean diffusivity  
37  
38 328 (MD) and fractional anisotropy (FA)) will be compared with the results of the neuroimaging outcome  
39  
40 329 2 years after the surgery, including total weight loss (%) as a factor in the model. Next, the scores of  
41  
42 330 the cognitive tests on five different time points will be analysed and compared to the total weight  
43  
44 331 loss (%). Separate linear mixed models will be used and adjusted for different covariates such as sex,  
45  
46 332 age, IQ score and depressive symptoms etc. where appropriate. To correct for multiple outcome  
47  
48 333 measures, the sequentially rejective multiple testing procedure described in Bretz *et al.* will be  
49  
50 334 used.(66)

51  
52  
53 335

#### 54 55 336 **Analysis of secondary outcome measures**



1  
2  
3 337 As secondary outcome measures, the metabolic and histopathological parameters (obtained analyses  
4  
5 338 from tissues collected during the surgery) will be analysed cross-sectionally to examine correlations  
6  
7 339 between these metabolic and histopathological parameters among each other and in relation to  
8  
9 340 brain function and structure. Furthermore, potential mechanisms underlying the crosstalk along the  
10  
11 341 gut-brain axis will be investigated by longitudinal analyses focusing on establishing correlations  
12  
13 342 between brain structure/function changes and changes in circulation mediators or faecal microbiota  
14  
15 343 composition. Pearson correlation analysis will be used to investigate potential correlations between  
16  
17 344 variables.  
18

19  
20 345

#### 21 346 **Data monitoring**

22  
23 347 Every year, data monitoring and auditing will be conducted by an independent specialized monitor of  
24  
25 348 the Rijnstate Hospital. Yearly, a summary of the progress will be submitted to the ethical committee  
26  
27 349 and the Netherlands Trial Register (trialregister.nl) 7288.  
28

29  
30 350

#### 31 351 **DISCUSSION**

32  
33 352 The BARICO study is a longitudinal, prospective study focusing on the effect of weight loss after BS on  
34  
35 353 cognitive function and brain structure, measured with sensitive neuropsychological tests covering  
36  
37 354 the most important domains, fMRI activation during the Stroop task, and several MRI techniques,  
38  
39 355 such as DTI and ASL. To clarify the impact of metabolic dysfunction in obesity on brain function and  
40  
41 356 structure, blood plasma and stool samples will be collected and analysed longitudinally as well as  
42  
43 357 biopsies of key metabolic organs which will be collected during the RYGB and analysed cross-  
44  
45 358 sectionally.  
46

47  
48 359 Limited studies demonstrated improvement in several cognitive domains such as memory, attention  
49  
50 360 and executive function after BS.(16, 17) Furthermore, obese individuals seem to have lower grey and  
51  
52 361 white matter volumes and altered white matter densities. Several studies show rapid recovery of  
53  
54 362 these brain structural abnormalities after BS(48, 49): for instance, Tuulari *et al.* showed a causal link  
55  
56  
57  
58  
59

1  
2  
3 363 between weight loss and brain tissue recovery.(48) Approximately 25-30% of the patients is expected  
4  
5 364 not to reach sufficient weight loss (<50% excess weight loss) and thus it will be possible to study the  
6  
7 365 effect of weight loss after BS on brain function and structure.

8  
9 366 The strength of this study is the long follow-up duration of two years for the neuroimaging  
10  
11 367 parameters and ten years for the neuropsychological tests after surgery. Furthermore, the strict  
12  
13 368 inclusion criterion with respect to age range ensures a good representation of mid-life patients.  
14  
15 369 Moreover, most studies in BS patients include mainly women, but it is important to account for sex-  
16  
17 370 differences caused by variation in fat tissue distribution.(28)

18  
19 371 Another strength of this study is the combination of neuroimaging and neuropsychological tests. In  
20  
21 372 combination with the analysed metabolic and histopathological parameters (obtained in blood,  
22  
23 373 organ biopsies and microbiota), the relation between multiple metabolic parameters can be  
24  
25 374 investigated, such as adipokines, bioactive lipids (e.g., SCFA) and organ dysfunction or neuroimaging  
26  
27 375 and cognition parameters in a comprehensive way. Especially, since RYGB influences gut-brain  
28  
29 376 communication and may lead to beneficial alterations in adipose function, recovery of brain function  
30  
31 377 and structure may be expected.(15, 67) Longitudinal analyses of the microbiota together with  
32  
33 378 analysis of functional gut-derived metabolites in the circulation and cognitive outcomes may allow  
34  
35 379 identification of mediators derived from the gut microflora that are relevant for cognition and  
36  
37 380 prevention of cognitive decline.

38  
39  
40 381 The BARICO study has the potential to be the first to demonstrate interactions between periphery  
41  
42 382 and central nervous system after weight loss in humans, particularly the involvement of the brain,  
43  
44 383 adipose tissue liver and gut microbiota.

45  
46  
47 384 In conclusion, the BARICO study will reveal the relation and underlying mechanisms between obesity  
48  
49 385 and brain function and structure. This information can be used to develop better health care and  
50  
51 386 preventatives against obesity and associated disorders.

52  
53 387

54  
55 388 **ETHICS AND DISSEMINATION**

1  
2  
3 389 The study protocol was authorized by the medical review ethics committee CMO Region Arnhem and  
4  
5 390 Nijmegen (NL63493.091.17). All patients will sign informed consent on enrolment in the study. Study  
6  
7 391 results of the study will be submitted for publication in peer-reviewed journals.  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

1  
2  
3 392 **Acknowledgements**

4  
5 393 Not applicable.

6  
7 394

8  
9 395 **Contributors**

10  
11 396 EOA and AJK conceived and designed the study. DV wrote the article and developed the protocol

12  
13 397 together with EOA, AJK, EJH, and RK. EJH, EOA and AJK are the principal investigators and DV is the

14  
15 398 main investigator. MW, LND, IAA, EA, RK and RPCK are co- investigators in the participating centres.

16  
17 399 All authors critically reviewed the content and approved the final manuscript.

18  
19 400

20  
21 401 **Funding**

22  
23 402 This work is supported by a grant of the Rijnstate-Radboudumc promotion fund. The

24  
25 403 histopathological and biochemical analyses will be performed in collaboration with the Netherlands

26  
27 404 Organisation for Applied Scientific Research (TNO) Metabolic Health Research (Leiden, the

28  
29 405 Netherlands) with support from TNO's Research program Biomedical Health and the Shared Research

30  
31 406 Program GLoBAL, an initiative of Radboudumc, Rijnstate and TNO.

32  
33 407

34  
35 408 **Competing interests**

36  
37 409 The authors declare that they have no conflicts of interests.

38  
39 410

40  
41 411 **Patient consent**

42  
43 412 Obtained

44  
45 413

46  
47 414 **Ethics approval**

48  
49 415 Medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17).

50  
51 52  
53  
54  
55  
56  
57  
58  
59  
60

416 **REFERENCES**

- 417 1. WHO. Obesity and overweight; Fact sheet 2018 [cited 2018 23-02].
- 418 2. Espeland MA, Erickson K, Neiberg RH, Jakicic JM, Wadden TA, Wing RR, et al. Brain and white matter  
419 hyperintensity volumes after 10 years of random assignment to lifestyle intervention. *Diabetes care*. 2016;39(5):764-71.
- 420 3. Anstey K, Cherbuin N, Budge M, Young J. Body mass index in midlife and late-life as a risk factor for  
421 dementia: a meta-analysis of prospective studies. *Obes Rev*. 2011;12(5):426-37.
- 422 4. Emmerzaal TL, Kiliaan AJ, Gustafson DR. 2003-2013: a decade of body mass index, Alzheimer's disease, and  
423 dementia. *J Alzheimer's Dis*. 2015;43(3):739-55.
- 424 5. Maayan L, Hoogendoorn C, Sweat V, Convit A. Disinhibited eating in obese adolescents is associated with  
425 orbitofrontal volume reductions and executive dysfunction. *Obesity (Silver Spring)*. 2011;19(7):1382-7.
- 426 6. Cournot M, Marquie J, Ansiau D, Martinaud C, Fonds H, Ferrieres J, et al. Relation between body mass index and  
427 cognitive function in healthy middle-aged men and women. *Neurology*. 2006;67(7):1208-14.
- 428 7. Gunstad J, Lhotsky A, Wendell CR, Ferrucci L, Zonderman AB. Longitudinal examination of obesity and cognitive  
429 function: results from the Baltimore longitudinal study of aging. *Neuroepidemiology*. 2010;34(4):222-9.
- 430 8. Prickett C, Brennan L, Stolwyk R. Examining the relationship between obesity and cognitive function: a systematic  
431 literature review. *Obes Res Clin Pract*. 2015;9(2):93-113.
- 432 9. Bastard J-P, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between  
433 obesity, inflammation, and insulin resistance. *Eur Cytokine Netw*. 2006;17(1):4-12.
- 434 10. Gloy VL, Briel M, Bhatt DL, Kashyap SR, Schauer PR, Mingrone G, et al. Bariatric surgery versus non-surgical  
435 treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. *BMJ*. 2013;347:f5934.
- 436 11. Europe W. Body mass index - BMI 2018 [08-03-2018]. Available from: [http://www.euro.who.int/en/health-](http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi)  
437 [topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi](http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi).
- 438 12. Lee WJ, Chong K, Ser KH, Lee YC, Chen SC, Chen JC, et al. Gastric bypass vs sleeve gastrectomy for type 2 diabetes  
439 mellitus: a randomized controlled trial. *Arch Surg*. 2011;146(2):143-8.
- 440 13. Gisella Carranza-Leon B, Puzifferri N, Adams-Huet B, Jabbour I, Lingvay I. Metabolic response 4years after gastric  
441 bypass in a complete cohort with type 2 diabetes mellitus. *Diabetes Res Clin Pract*. 2018;137:224-30.
- 442 14. Kim KS, Sandoval DA. Endocrine Function after Bariatric Surgery. *Compr Physiol*. 2017;7(3):783-98.
- 443 15. Ballsmider LA, Vaughn AC, David M, Hajnal A, Di Lorenzo PM, Czaja K. Sleeve gastrectomy and Roux-en-Y gastric  
444 bypass alter the gut-brain communication. *Neural Plast*. 2015;2015:601985.
- 445 16. Handley JD, Williams DM, Caplin S, Stephens JW, Barry J. Changes in cognitive function following bariatric surgery:  
446 a systematic review. *Obes Surg*. 2016;26(10):2530-7.
- 447

- 1  
2  
3 448 17. Alosco ML, Galioto R, Spitznagel MB, Strain G, Devlin M, Cohen R, et al. Cognitive function after bariatric surgery:  
4 449 evidence for improvement 3 years after surgery. *Am J Surg*. 2014;207(6):870-6.  
5  
6 450 18. Tuulari JJ. Effects of Obesity and Weight Loss Following Bariatric Surgery on Brain Function, Structural Integrity  
7 451 and Metabolism. 2015.  
8  
9 452 19. Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kåreholt I, Winblad B, et al. Obesity and vascular risk factors at  
10 453 midlife and the risk of dementia and Alzheimer disease. *Arch Neurol*. 2005;62(10):1556-60.  
11  
12 454 20. Whitmer R, Gustafson D, Barrett-Connor E, Haan M, Gunderson E, Yaffe K. Central obesity and increased risk of  
13 455 dementia more than three decades later. *Neurology*. 2008;71(14):1057-64.  
14  
15 456 21. Whitmer RA, Gunderson EP, Barrett-Connor E, Quesenberry CP, Yaffe K. Obesity in middle age and future risk of  
16 457 dementia: a 27 year longitudinal population based study. *BMJ*. 2005;330(7504):1360.  
17  
18 458 22. Whitmer RA, Gunderson EP, Quesenberry CP, Zhou J, Yaffe K. Body mass index in midlife and risk of Alzheimer  
19 459 disease and vascular dementia. *Curr Alzheimer Res*. 2007;4(2):103-9.  
20  
21 460 23. Arnoldussen IA, Kiliaan AJ, Gustafson DR. Obesity and dementia: adipokines interact with the brain. *Eur*  
22 461 *Neuropsychopharmacol*. 2014;24(12):1982-99.  
23  
24 462 24. Jaganathan R, Ravindran R, Dhanasekaran S. Emerging Role of Adipocytokines in Type 2 Diabetes as Mediators of  
25 463 Insulin Resistance and Cardiovascular Disease. *Can J Diabetes*. 2017.  
26  
27 464 25. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. *Trends Endocrinol Metab*. 2000;11(8):327-32.  
28  
29 465 26. Arner P. Not all fat is alike. *The Lancet*. 1998;351(9112):1301-2.  
30  
31 466 27. Foster MT, Pagliassotti MJ. Metabolic alterations following visceral fat removal and expansion: Beyond anatomic  
32 467 location. *Adipocyte*. 2012;1(4):192-9.  
33  
34 468 28. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for  
35 469 obesity complications. *Mol Aspects Med*. 2013;34(1):1-11.  
36  
37 470 29. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin  
38 471 concentrations in normal-weight and obese humans. *N Engl J Med*. 1996;334(5):292-5.  
39  
40 472 30. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, et al. Relationship of adiponectin to body fat  
41 473 distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia*.  
42 474 2003;46(4):459-69.  
43  
44 475 31. Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. *Nat Rev*  
45 476 *Endocrinol*. 2017;13(9):509-20.  
46  
47 477 32. Stefan N, Haring H-U. The role of hepatokines in metabolism. *Nat Rev Endocrinol*. 2013;9(3):144-52.  
48  
49 478 33. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest*. 2011;121(6):2126-32.  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 479 34. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*.  
4 480 2012;489(7415):242-9.  
5  
6 481 35. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. The microbiota-gut-brain axis in obesity. *Lancet*  
7 482 *Gastroenterol Hepatol*. 2017;2(10):747-56.  
8  
9 483 36. Wang HX, Wang YP. Gut Microbiota-brain Axis. *Chin Med J (Engl)*. 2016;129(19):2373-80.  
10  
11 484 37. Aron-Wisnewsky J, Dore J, Clement K. The importance of the gut microbiota after bariatric surgery. *Nat Rev*  
12 485 *Gastroenterol Hepatol*. 2012;9(10):590-8.  
13  
14 486 38. Peat CM, Kleiman SC, Bulik CM, Carroll IM. The Intestinal Microbiome in Bariatric Surgery Patients. *Eur Eat Disord*  
15 487 *Rev*. 2015;23(6):496-503.  
16  
17 488 39. Cipolla MJ. Chapter 5: Control of Cerebral Blood Flow. *The Cerebral Circulation. Integrated Systems Physiology:*  
18 489 *From Molecule to Function*. San Rafael (CA)2009. p. 29-36.  
19  
20 490 40. Willeumier KC, Taylor DV, Amen DG. Elevated BMI is associated with decreased blood flow in the prefrontal cortex  
21 491 using SPECT imaging in healthy adults. *Obesity (Silver Spring)*. 2011;19(5):1095-7.  
22  
23 492 41. Alosco ML, Spitznagel MB, Raz N, Cohen R, Sweet LH, Colbert LH, et al. Obesity interacts with cerebral  
24 493 hypoperfusion to exacerbate cognitive impairment in older adults with heart failure. *Cerebrovasc Dis Extra*. 2012;2(1):88-  
25 494 98.  
26  
27 495 42. Wierenga CE, Hays CC, Zlata ZZ. Cerebral blood flow measured by arterial spin labeling MRI as a preclinical  
28 496 marker of Alzheimer's disease. *J Alzheimer's Dis*. 2014;42 (Suppl 4):S411-9.  
29  
30 497 43. Alsop DC, Detre JA, Golay X, Gunther M, Hendrikse J, Hernandez-Garcia L, et al. Recommended implementation of  
31 498 arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the  
32 499 European consortium for ASL in dementia. *Magn Reson Med*. 2015;73(1):102-16.  
33  
34 500 44. Kullmann S, Callaghan MF, Heni M, Weiskopf N, Scheffler K, Haring HU, et al. Specific white matter tissue  
35 501 microstructure changes associated with obesity. *Neuroimage*. 2016;125:36-44.  
36  
37 502 45. Debette S, Wolf C, Lambert JC, Crivello F, Soumare A, Zhu YC, et al. Abdominal obesity and lower gray matter  
38 503 volume: a Mendelian randomization study. *Neurobiol Aging*. 2014;35(2):378-86.  
39  
40 504 46. Karlsson HK, Tuulari JJ, Hirvonen J, Lepomaki V, Parkkola R, Hiltunen J, et al. Obesity is associated with white  
41 505 matter atrophy: a combined diffusion tensor imaging and voxel-based morphometric study. *Obesity (Silver Spring)*.  
42 506 2013;21(12):2530-7.  
43  
44 507 47. Arnoldussen IAC, Wiesmann M, Pelgrim CE, Wielemaker EM, van Duyvenvoorde W, Amaral-Santos PL, et al.  
45 508 Butyrate restores HFD-induced adaptations in brain function and metabolism in mid-adult obese mice. *Int J Obes (Lond)*.  
46 509 2017;41(6):935-44.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 510 48. Tuulari JJ, Karlsson HK, Antikainen O, Hirvonen J, Pham T, Salminen P, et al. Bariatric Surgery Induces White and  
4 511 Grey Matter Density Recovery in the Morbidly Obese: A Voxel-Based Morphometric Study. *Hum Brain Mapp.*  
5 512 2016;37(11):3745-56.  
6  
7 513 49. Zhang Y, Ji G, Xu M, Cai W, Zhu Q, Qian L, et al. Recovery of brain structural abnormalities in morbidly obese  
8 514 patients after bariatric surgery. *Int J Obes (Lond).* 2016;40(10):1558-65.  
9  
10 515 50. Fried M, Hainer V, Basdevant A, Buchwald H, Deitel M, Finer N, et al. Interdisciplinary European Guidelines on  
11 516 Surgery of Severe Obesity. *Obes Facts.* 2008;1(1):52-9.  
12  
13 517 51. Nasreddine Z, Philips NA, Bédirian V, S. C, V. W, Collin I, et al. The Montreal Cognitive Assessment, MoCA: A Brief  
14 518 Screening Tool For Mild Cognitive Impairment. *J Am Geriatr Soc.* 2005;53(4):695-9.  
15  
16 519 52. Zimmerman P, Fimm B. Test for Attentional Performance (TAP), Manual. Würselen, Germany: Psytest. 1994.  
17  
18 520 53. Wechsler D. Wechsler adult intelligence scale-Fourth Edition (WAIS-IV). San Antonio, TX: NCS Pearson.  
19 521 2008;22:498.  
20  
21 522 54. Schmand B, Groenink, S.C., van den Dungen, M. Letterfluency: psychometrische eigenschappen en Nederlandse  
22 523 normen. *Tijdschr Gerontol Geriatr.* 2008;39(2):64-76.  
23  
24 524 55. Wilson B, Cockburn J, Baddeley A. Rivermead Behavioural Memory Test. London: Thames Valley Test Company;  
25 525 1985.  
26  
27 526 56. Schmand B, Bakker D, Saan R, Louman J. The Dutch Reading Test for Adults: a measure of premorbid intelligence  
28 527 level. *Tijdschr Gerontol Geriatr.* 1991;22(1):15-9.  
29  
30 528 57. Verhage F. Intelligentie en leeftijd: Onderzoek bij Nederlanders van twaalf tot zevenenzeventig jaar. Assen: Van  
31 529 Gorcum; 1964.  
32  
33 530 58. Baecke JA, Burema, J., Frijters, J.E. A short questionnaire for the measurement of habitual physical activity in  
34 531 epidemiological studies. *Am J Clin Nutr.* 1980;36(5):936-42.  
35  
36 532 59. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry.*  
37 533 1961;4:561-71.  
38  
39 534 60. Ware JE, Sherbourne, C.D. The MOS 36-Item Short-Form Health Survey (SF-36): I. Conceptual Framework and Item  
40 535 Selection. *Medical Care.* 1992;30(6):473-83.  
41  
42 536 61. Oria HE, Moorehead M.K. . Bariatric analysis and reporting outcome system (BAROS). *Obes Surg.* 1998;8(5):487-  
43 537 99.  
44  
45 538 62. Patton JH, Stanford MS, Barratt ES. Factor structure of the Barratt impulsiveness scale. *J Clin Psychol.*  
46 539 1995;51(6):768-74.  
47  
48 540 63. Carver CS, White T.L. . Behavioral Inhibition, Behavioral Activation, and Affective Responses to Impending Reward  
49 541 and Punishment: The BIS/BAS Scales. *J Pers Soc Psychol.* 1994;67(2):319-33.  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



- 1  
2  
3 542 64. Michaud A, Vainik U, Garcia-Garcia I, Dagher A. Overlapping Neural Endophenotypes in Addiction and Obesity.  
4 543 Frontiers in endocrinology. 2017;8:127.  
5  
6 544 65. Meule A, Hofmann J, Weghuber D, Blechert J. Impulsivity, perceived self-regulatory success in dieting, and body  
7 545 mass in children and adolescents: A moderated mediation model. Appetite. 2016;107:15-20.  
8  
9 546 66. Bretz F, Maurer W, Brannath W, Posch M. A graphical approach to sequentially rejective multiple test procedures.  
10 547 Stat Med. 2009;28(4):586-604.  
11  
12 548 67. Hoffstedt J, Andersson DP, Eriksson Hogling D, Theorell J, Naslund E, Thorell A, et al. Long-term Protective  
13 549 Changes in Adipose Tissue After Gastric Bypass. Diabetes Care. 2017;40(1):77-84.  
14  
15  
16 550  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 551 **FIGURE LEGEND**  
4

5 552

6  
7 553 **Figure 1.** Overview of the study design. Blood samples are taken during regular blood withdrawal at 6  
8  
9 554 time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota  
10  
11 555 analyses will be performed at set time points on the faeces collected at home by the patients (4-8  
12  
13 556 wks. pre BS, 1 wk. pre BS, 6 mo. post BS, 24 mo. post BS) and mucosal swops will be collected during  
14  
15 557 surgery. Furthermore, biopsies of liver, jejunum and adipose tissue depots (subcutaneous,  
16  
17 558 mesenteric and omental) will be collected during surgery. Before (4-8 wks. pre BS) and several time  
18  
19 559 points after surgery (6 mo. post BS, 24 mo. post BS and 5&10 yrs. post BS) patients will fill out  
20  
21 560 questionnaires together with neuropsychological measurements to test cognitive function. A  
22  
23 561 subgroup of patients (N=75) will be examined with MRI (4-8 wks. pre BS and 24 mo. post BS). MRI;  
24  
25 562 magnetic resonance imaging. BS; Bariatric surgery. Wks; weeks. Mo; months. Yrs; years.  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

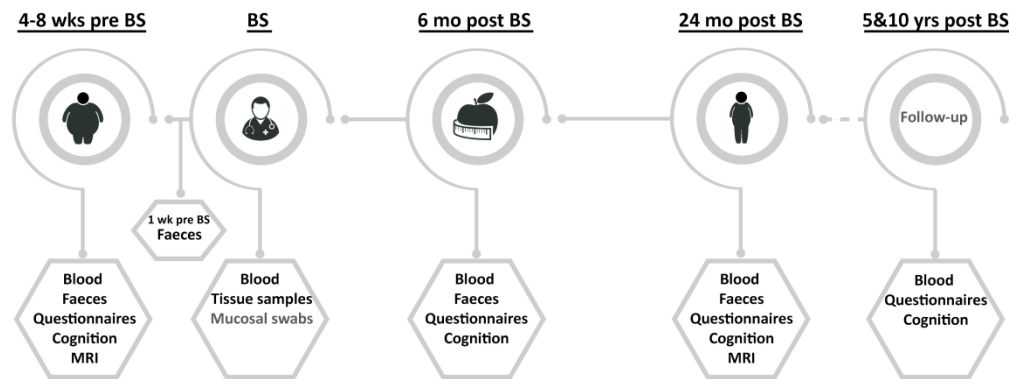


Figure 1. Overview of the study design. Blood samples are taken during regular blood withdrawal at 6 time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota analyses will be performed at set time points on the faeces collected at home by the patients (4-8 wks. pre BS, 1 wk. pre BS, 6 mo. post BS, 24 mo. post BS) and mucosal swabs 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.

301x122mm (300 x 300 DPI)

# BMJ Open

## 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.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025464.R1
Article Type:	Protocol
Date Submitted by the Author:	22-Oct-2018
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
<b>Primary Subject Heading</b>:	Neurology
Secondary Subject Heading:	Research methods, Nutrition and metabolism, Radiology and imaging, Surgery
Keywords:	Obesity, Weight loss, Bariatric surgery, Neuroimaging, Cognition

SCHOLARONE™  
Manuscripts

1  
2  
3  
4 1 **Study rationale and protocol of the BARICO study: a longitudinal, prospective, observational study to**  
5 2 **evaluate effects of weight loss on brain function and structure after bariatric surgery.**

6  
7 3  
8 4 Vreeken, D.<sup>1,2,3</sup>, Wiesmann, M.<sup>3</sup>, Deden, L.N.<sup>1,2</sup>, Arnoldussen, I.A.C.<sup>3</sup>, Aarts, E.<sup>4</sup>, Kessels, R.P.C.<sup>4,5,6</sup>,  
9 5 Kleemann, R.<sup>7</sup>, Hazebroek, E.J.<sup>1,2</sup>, Aarts, E.O.<sup>1,2\*</sup>, Kiliaan, A.J.<sup>3\*\*</sup>

10 6  
11  
12  
13 7 <sup>1</sup> *Department of Surgery, Rijnstate Hospital, Arnhem, the Netherlands.*

14 8 <sup>2</sup> *Vitalys Clinic, Velp, the Netherlands.*

15 9 <sup>3</sup> *Department of Anatomy, Donders Institute for Brain, Cognition and Behaviour, Radboud university*  
16 10 *medical center, Nijmegen, the Netherlands.*

17 11 <sup>4</sup> *Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, the Netherlands.*

18 12 <sup>5</sup> *Department of Medical Psychology, Radboud University Medical Center, Nijmegen, the Netherlands.*

19 13 <sup>6</sup> *Vincent van Gogh Institute for Psychiatry, Venray, the Netherlands.*

20 14 <sup>7</sup> *Department of Metabolic Health Research, Netherlands Organization for Applied Scientific Research*  
21 15 *(TNO), Leiden, the Netherlands.*

22 16  
23 17 \*These authors contributed equally.

24 18 † **Corresponding author:**

25 19 Amanda J. Kiliaan, PhD

26 20 Donders Institute for Brain, Cognition, and Behaviour

27 21 Radboud university medical center

28 22 Department of Anatomy (109)

29 23 Geert Grooteplein 21N

30 24 6525 EZ Nijmegen, the Netherlands

31 25 Phone: +31 24 361 4378

32 26 Email: [amanda.kiliaan@radboudumc.nl](mailto:amanda.kiliaan@radboudumc.nl)

33 27  
34 28 **Short title: The BARICO study, effect of weight loss on brain function**

35 29 Words count (excluding title page, abstract, references and figure): 4130

36 30 Words count abstract: 300

37 31  
38 32 **Keywords: obesity, weight loss, bariatric surgery, neuroimaging, cognition**

## 33 **ABSTRACT**

### 34 **Introduction**

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

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

### 47 **Methods and analysis**

48 Data from 150 Dutch BS patients (age between 35 and 55, men and women) will be collected at various  
49 time points between 2 months before and up to 10 years after surgery. Neuropsychological tests,  
50 questionnaires, blood, faeces and tissue samples will be collected before, during and after surgery to  
51 measure changes in cognition, microbiota, metabolic activity and inflammation over time. A subgroup of  
52 75 participants will undergo (f)MRI in relation to executive functioning (determined by the Stroop task),  
53 grey and white matter volumes, and cerebral blood flow. Regression analyses will be used to explore  
54 associations between weight loss and outcome measures.

### 56 **Ethics and dissemination**

1  
2  
3 57 This study has been approved by the medical review ethics committee CMO Region Arnhem and  
4  
5 58 Nijmegen (NL63493.091.17). Research findings will be published in peer-reviewed journals and at  
6  
7 59 conferences.  
8  
9

10 60

11  
12 61 **Trial registration**

13  
14 62 The Netherlands National Trial Register (trialregister.nl) 7288. Date registered: 29-June-2018.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## 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.



## 76 INTRODUCTION

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

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

1  
2  
3 100 cognitive decline and increase risk for developing dementia. However, mechanisms involved in this  
4  
5 101 organ-organ crosstalk are poorly understood.(4, 21-24) Firstly, one proposed mechanism is the altered  
6  
7 102 signalling of visceral and abdominal adipose tissue; adipose tissue acts as an independent endocrine  
8  
9 103 organ releasing several hormones, proteins and cytokines, referred to as adipokines. Obesity is  
10  
11 104 associated with dysfunctional white adipose tissue and therefore an imbalance in adipokines, such as  
12  
13  
14 105 increased levels of leptin and angiotensinogen, and low levels of adiponectin and omentin.(25, 26)  
15  
16 106 Especially, visceral adipose tissue seems to produce unfavourable adipokines associated with more  
17  
18 107 metabolic complications when compared to subcutaneous adipose tissue.(27-30) Importantly, the  
19  
20 108 distribution of fat tissue depots differs between sexes. Overall, men accumulate more abdominal and  
21  
22 109 visceral fat than women.(30) Moreover, women have a higher level of adipokines such as leptin and  
23  
24 110 adiponectin.(31, 32) This disbalance in adipokines may induce inflammation in several organs such as the  
25  
26 111 liver, gut and vascular endothelium. The latter causing atherosclerosis, ultimately leading to changes in  
27  
28 112 cerebral blood flow (CBF).(25)  
29  
30  
31  
32 113 Secondly, signalling between, and within other organs, such as the liver, might be altered in obese  
33  
34 114 patients. For example; the liver secretes hepatokines, such as insulin-like growth factor 1, selenoprotein  
35  
36 115 P, leukocyte cell-derived chemotaxin, fetuin B and hepcidin, which may indirectly affect brain function  
37  
38 116 and structure.(33, 34)  
39  
40  
41 117 Thirdly, the gut microbiota composition in obese people differs from that of non-obese individuals,  
42  
43 118 affecting metabolic processes, weight and obesity-related comorbidities.(35, 36) Microbiota is involved  
44  
45 119 in adiposity and homeostasis but also influences energy balance via appetite and satiety signalling to the  
46  
47 120 brain. Gut microbiota also affect the brain by producing (precursors of) neurotransmitters and short  
48  
49 121 chain fatty acids, or through cytokines via the immune system.(37, 38) BS leads to a fast change in gut  
50  
51 122 microbiota composition through changes in food intake, intestinal modifications due to the surgery itself,  
52  
53 123 and metabolic improvements, eventually leading to changes in gut-brain communication.(15, 39, 40)  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 124 Hence, metabolic organs, such as liver, gut and adipose tissue and gut microbiota may constitute new  
4  
5 125 therapeutic targets. Although long-term results are not yet clear, the gut microbiota has already become  
6  
7 126 a target for anti-obesity treatments.(37)

9  
10 127 Obesity is associated with impaired CBF, which may lead to inadequate oxygen and energy supply in the  
11  
12 128 brain and eventually loss of white and grey matter integrity.(41, 42) Lower levels of CBF in the prefrontal  
13  
14 129 cortex are associated with reduced performance on executive function and episodic memory tests.(42,  
15  
16 130 43) Even in the prodromal stages of Alzheimer's disease, changes in CBF can be detected with arterial  
17  
18 131 spin labelling (ASL), which may be used as a very early biomarker for neurodegenerative  
19  
20 132 disorders.(44) However, the technique requires further optimization and therefore several consortia are  
21  
22 133 working on the implementation of ASL perfusion magnetic resonance imaging (MRI) for clinical  
23  
24 134 applications to provide images of sufficient and diagnostic utility.(45)

25  
26  
27 135 Furthermore, obesity is associated with changes in grey and white matter, which can be visualized using  
28  
29 136 diffusion tensor imaging (DTI) and voxel-based morphometry analyses based on T1 weighted scans.(46,  
30  
31 137 47) These structural changes are especially prominent in brain regions governing reward seeking,  
32  
33 138 inhibitory control and appetite.(48, 49) There are indications that rapid recovery of structural  
34  
35 139 abnormalities occur after BS, however long-term study data is lacking here. (50, 51)

36  
37  
38 140 Additionally, impairment in attention span, executive function and memory are commonly reported in  
39  
40 141 obese patients.(18, 19) Cognitive impairment revealed in obesity might be reversible and varies between  
41  
42 142 cognitive domains however long-term follow-up studies are scarce. The Longitudinal Assessment of  
43  
44 143 Bariatric Surgery (LABS) parent project is the most extensive longitudinal study to date focusing on  
45  
46 144 cognitive changes in patients after BS. Investigators showed lasting improvements three years after  
47  
48 145 surgery in the cognitive domains of attention, executive function and memory.(19)

49  
50  
51  
52 146

53  
54 147 **Rationale**

1  
2  
3 148 Cognitive benefits after BS are not equally exhibited across patients and cognitive domains. However,  
4  
5 149 precise causes are still poorly understood, and underlying molecular mechanisms remain elusive. From  
6  
7 150 the relatively short follow-up duration and small samples of BS patients in the studies reviewed, it is  
8  
9  
10 151 difficult to elaborate on the long-term consequences of obesity and its related diseases. In this study,  
11  
12 152 underlying mechanisms of obesity-related cognitive disorders will be investigated by longitudinal studies  
13  
14 153 correlating cognition to brain changes, blood serum and plasma values, and gut microbiota composition.  
15  
16 154 Lastly, metabolic and histopathological parameters (at the time-point of surgery) will be obtained to  
17  
18 155 study whether associations or correlations exist between obesity-associated metabolic dysfunctions of  
19  
20 156 particular organs and brain function and structure. To our knowledge this is the first study in humans  
21  
22 157 investigating changes in brain structure and function, and changes in adipose tissue, liver function and  
23  
24 158 the gut microbiome, after BS-induced weight loss. Additionally, this is the first study in bariatric research  
25  
26 159 combining neuroimaging, cognition and extensive profiling of biological markers.  
27  
28  
29

30 160  
31  
32 161 The primary aim of the BARICO study (**BA**riatric surgery **Rijn**state and **Rad**boudumc neuro**I**maging and  
33  
34 162 **C**ognition in **O**besity) is to determine the long-term effect of weight loss on measures of brain function  
35  
36 163 and structure after BS. The secondary aim is to provide mechanism-based rationales responsible for  
37  
38 164 functional and structural decline in obese individuals. Therefore, the metabolic and inflammation status  
39  
40 165 of organ biopsies will be determined together with molecular signatures via blood plasma/serum  
41  
42 166 analyses. Furthermore, gut microbiota composition will be monitored over time to gain knowledge about  
43  
44 167 the gut-brain axis.  
45  
46

47  
48 168 This study will contribute to the development of better health campaigns, healthcare and preventatives  
49  
50 169 to attenuate the impact of obesity. This paper describes the design and protocol of the BARICO study.  
51

52 170  
53

54 171  
55  
56  
57  
58  
59  
60

## 172 **METHODS AND ANALYSIS**

### 173 **Study population**

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

190

### 191 **Study design**

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

1  
2  
3 196 Intraoperatively, several tissue biopsies will be collected and processed. Medical evaluation, including  
4  
5 197 anthropometric measurements and information on comorbidities, will be assessed 4-8 weeks  
6  
7 198 preoperative and during all postoperative time points. A schematic overview of the study is shown in  
8  
9  
10 199 figure 1. A subgroup of patients (N=75) will additionally receive a (f)MRI scan 4-8 weeks preoperative and  
11  
12 200 24 months postoperative. During the whole study period (10 years) patients will be contacted by letter  
13  
14 201 and via telephone at least once a year to ensure the best follow-up rate.  
15  
16  
17 202

### 18 203 **Recruitment procedures and consent**

19 204 Patients are informed about the study by letter and telephone at least two weeks prior to their standard  
20  
21 205 visit (4-8 weeks before RYGB surgery). During this visit, patients will individually receive more  
22  
23 206 information about this study and its objectives. Afterwards, the researchers will further clarify the study  
24  
25 207 and the patients can ask for additional information. If they decide to participate and fulfil the inclusion  
26  
27 208 criteria, informed consents will be obtained. Although the obese population consists of more females  
28  
29 209 than males, the aim is for an equal sex distribution during the recruitment period (i.e., a study population  
30  
31 210 consisting of >30% men and >30% women).(1) Recruitment will take place between August 2018 and  
32  
33 211 August 2020.  
34  
35  
36  
37  
38  
39 212

### 40 213 **Outcome measures**

41 214 The primary outcome measures are the neuropsychological tests scores, CBF values, hippocampal  
42  
43 215 volume, mean diffusivity (MD) and fractional anisotropy (FA) (representing respectively grey and white  
44  
45 216 matter integrity), and blood oxygen level dependent (BOLD) responses during the Stroop task.  
46  
47 217 Combining neuroimaging and neuropsychological tests will give us more information on how and  
48  
49 218 whether structural brain changes are related to functional brain changes. Secondary measures comprise  
50  
51 219 of (histopathological and biochemical determined) health status of the collected tissue, gut microbiota  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 220 composition changes (in jejunal mucosa and faeces) and the profiling of circulating mediators in blood  
4  
5 221 (plasma and serum), as well as lifestyle and dietary habits in relation to cognitive function and brain  
6  
7 222 structure. Combining information on the pathological state of liver, gut, adipose tissue and circulating  
8  
9 223 mediators from corresponding plasma/serum samples, obtained prior to and at surgery, will provide  
10  
11 224 insight into organ cross-talk and allow identification of biomarker signatures for metabolic health.  
12  
13  
14 225 Differences in metabolic health of the subjects may be associated with specific signalling molecule-  
15  
16 226 profiles, which may be related to cognitive function.  
17  
18  
19 227  
20

## 21 228 **(f)MRI**

22  
23 229 Subjects will be scanned in a 3T MAGNETOM Skyra MR scanner (Siemens AG, Healthcare Sector,  
24  
25 230 Erlangen, Germany) using a 32-channel head coil. The MRI protocol included: a T1-weighted 3D  
26  
27 231 magnetization-prepared rapid gradient-echo (MPRAGE) sequence for anatomical reference and analysis  
28  
29 232 (TR/TI/TE 2300/1100/3.03 ms; 8° flip angle; voxel size: 1.0 × 1.0 × 1.0 mm), a fluid-attenuated inversion  
30  
31 233 recovery (FLAIR) sequence for white matter lesion visualization (TR/TI 5000/1800 ms; voxel size: 1.0 × 1.0  
32  
33 234 × 1.0 mm), and diffusion-weighted MRI scans using simultaneous multi-slice echo planar imaging for  
34  
35 235 probing microstructural properties (TR/TE 3275/91.4 ms; voxel size: 1.9 × 1.9 × 1.9 mm; 6x b=0 s/mm<sup>2</sup>,  
36  
37 236 42x b=900 s/mm<sup>2</sup>, 83x b=1800 s/mm<sup>2</sup>). To allow for offline distortion correction of the images, 7 more  
38  
39 237 b=0 s/mm<sup>2</sup> volumes will be acquired using the exact same sequence parameters - except for the inverted  
40  
41 238 k-space read-out trajectory. An ASL sequence will be used for quantification of CBF (TR/TE 2500/12 ms;  
42  
43 239 voxel size: 4.0 × 4.0 × 4.0 mm) and a multi-band, multi-echo planar imaging sequence will be used to  
44  
45 240 measure BOLD contrast during the Stroop task (TR/TE 1500/12.4, 34.3, 56.2 ms; 75° flip angle; voxel size:  
46  
47 241 2.5 × 2.5 × 2.5 mm; field of view 210 mm; 51 transversal slices in interleaved order). The complete  
48  
49 242 scanning protocol takes 45 minutes and for both time-points, the same: MR scanner, head coil, and  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 243 sequences will be used. Following the project MRI quality assurance is guaranteed by regular phantom  
4  
5 244 measurements.

6  
7 245

8  
9  
10 246 **Cognitive assessment**

11  
12 247 Cognitive performance of all participants will be tested using an extensive neuropsychological test  
13  
14 248 battery as detailed below. To assess general cognitive performance the Montreal Cognitive Assessment  
15  
16 249 (MoCA) will be used.(53) To test attentional functions, the Flexibility subtest from the Tests of  
17  
18 250 Attentional Performance (TAP 2.3) will be used.(54) This flexibility task focuses on shifting attention  
19  
20 251 between objects. Working memory will be assessed via the Digit Span subtest from the Wechsler Adult  
21  
22 252 Intelligence Scale Fourth Edition (WAIS-IV-NL).(55) Participants will have to repeat a series of digits in  
23  
24 253 forward or backward order, or sort them numerically. The Controlled Oral Word Association Test  
25  
26 254 (COWAT) will be used to determine verbal fluency.(56) Participants have to come up with as many words  
27  
28 255 beginning with three designated letters within 60 seconds (for each letter). Episodic memory will be  
29  
30 256 assessed via the immediate and delayed Story Recall subtest from the Rivermead Behavioural Memory  
31  
32 257 Test (RBMT).(57) To control and correct for differences in premorbid intelligence between participants,  
33  
34 258 verbal IQ will be estimated using the Dutch version of the National Adult Reading Test (NART) at  
35  
36 259 baseline.(58) The MoCA, episodic memory test and COWAT have parallel versions, to avoid material-  
37  
38 260 specific learning effects during the repeated testing. Additionally, the tests are standardized, have been  
39  
40 261 validated for use across a wide age range and have good re-test reliability. Together these tests will  
41  
42 262 provide a good overview on the overall cognitive performance of the patients, including aspects of  
43  
44 263 working and episodic memory, attention, verbal fluency and executive function. Also, education level will  
45  
46 264 be recorded in accordance with the Dutch education system using seven categories (one being the  
47  
48 265 lowest level of education and seven being the highest).(59)

49  
50  
51  
52  
53  
54 266



### 267 **Assessment of biological measurements**

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

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

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

### 287 **Questionnaires**

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

1  
2  
3 291 and depressive symptoms will be assessed with the Beck Depression Inventory (BDI-II).(60, 61) To  
4  
5 292 estimate the participants' food/nutrient intake and eating behaviour patients will be asked to fill out an  
6  
7 293 eating diary of two days (a weekday and a weekend day). Quality of Life will be evaluated with the Short  
8  
9  
10 294 Form 36 (SF-36).(62) Lastly, the results of BS will be evaluated via the Bariatric Analysis and Report  
11  
12 295 Outcome System (BAROS).(63)  
13  
14 296 More specifically: the Barratt impulsivity scale (BIS-11)(64) and Behavioural inhibition/activation system  
15  
16 297 (BIS/BAS)(65) questionnaires on impulsivity and reward sensitivity are included as reward sensitivity and  
17  
18 298 impulsivity have both previously been suggested to contribute to overeating.(66) Indeed, some facets of  
19  
20 299 impulsivity and reward sensitivity have shown to be relevant in eating- and weight regulation.(67)  
21  
22  
23 300

### 301 **Medical evaluation**

27  
28 302 At several time points during the study (figure 1) a medical evaluation will take place where  
29  
30 303 anthropometric measurements such as: body weight, length, waist circumference and blood pressure  
31  
32 304 will be quantified. BMI will be calculated as weight divided by height in meters squared. Percentage  
33  
34 305 excess weight loss (%EWL) (defined as weight loss divided by preoperative excess weight, with excess  
35  
36 306 weight defined as the weight above a normal BMI of 25 kg/m<sup>2</sup>) will be calculated during the time points  
37  
38 307 after surgery, similar to percentage total body weight loss (%TBWL) (defined as weight loss divided by  
39  
40 308 preoperative weight). The success of BS in terms of weight loss will be defined as a sustained weight loss  
41  
42 309 larger than 50 %EWL.  
43  
44

45 310 Furthermore, data on comorbidities like T2DM, HT and DL and associated medication will be collected  
46  
47 311 before the surgery and at all time-points after surgery. Comorbidities will be defined using following  
48  
49 312 criteria: for T2DM a fasting plasma glucose of  $\geq 7.0$  mmol/L and HbA1c  $\geq 48$  mmol/mol (HbA1c  $\geq 6.5\%$ ) or  
50  
51 313 the use of oral antidiabetic or insulin medication; for HT the use of antihypertensive drug treatment; for  
52  
53 314 DL the use of statins.  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 315  
4  
5 316 **Data management**  
6  
7 317 Data management will be handled using Research Manager (RM, Cloud 9 Health Solutions©), an  
8  
9  
10 318 established software package and data management tool that follows Good Clinical Practice (GCP)  
11  
12 319 guidelines.(68) Every change in the data is recorded in a log system and can be traced. Participants will  
13  
14 320 be identified only by a study specific identification code. One researcher will keep a separate participant  
15  
16 321 identification code list that matches the study-specific identifying codes with the participant's names.  
17  
18 322 Documents will be maintained by the investigator in strict confidence.  
19  
20  
21 323

### 22 23 324 **Sample size**

24  
25 325 The power calculation for the neuropsychological tasks was based on the results of the Digit Span  
26  
27 326 subtest performed in a comparable study population.(19) With an expected standardized effect size of at  
28  
29 327 least 0.3 and a correlation of 0.7, a selected sample of 150 patients will be sufficient to reach 90% power.  
30  
31 328 The power calculation for the MRI parameters is based on changes in the FA parameter studied by Zhang  
32  
33 329 *et al.*(51) With an expected standardized effect size of at least 0.03 and a correlation of 0.5 including 75  
34  
35 330 patients in the MRI group will be sufficient to reach 90% power. A significance level based on the  
36  
37 331 sequentially rejective multiple testing procedure discussed by Bretz *et al.* (for the neuropsychological  
38  
39 332 tests 3% and for the MRI parameters 2%) has been taken into account in the power calculation.(69) The  
40  
41 333 inclusion of 150 patients with a subgroup of 75 for the MRI scan has been considered adequate to  
42  
43 334 answer the research questions with sufficient power.  
44  
45  
46  
47  
48  
49

### 50 336 **Analysis of primary outcome measures**

51  
52 337 As a primary outcome measure, baseline levels of the imaging parameters (such as MD and FA) will be  
53  
54 338 compared with the results of the neuroimaging outcome 24 months after surgery (including %TBWL as a  
55  
56  
57  
58  
59  
60

1  
2  
3 339 factor in the model). Next, the scores of the cognitive tests from five different time points will be  
4  
5 340 analysed and compared to %TBWL. Every dependent variable will be modelled in a separate linear mixed  
6  
7 341 model. %TBWL will be used as a factor. Different variables, such as: depression score, age, and gender,  
8  
9 342 will be (if appropriate) included in the model. For each model, we will decide which variables to include  
10  
11 343 as a factor to reduce the amount of unexplained variation. To correct for multiple outcome measures,  
12  
13 344 the sequentially rejective multiple testing procedure described in Bretz *et al.* will be used.(69) Data will  
14  
15 345 be analysed using SPSS (version 25 for Windows) and R (version 3.5.1 for Windows). For the cognitive  
16  
17 346 tests a  $p$  value of  $<0.03$  and for the imaging parameters a  $p$  value of  $<0.02$  will be considered as  
18  
19 347 statistically significant.  
20  
21  
22  
23  
24

#### 25 349 **Analysis of secondary outcome measures**

26  
27 350 As secondary outcome measures, the metabolic and histopathological parameters (obtained analyses  
28  
29 351 from tissues collected during surgery) will be analysed cross-sectionally to examine correlations between  
30  
31 352 and among each other, and in relation to brain function and structure. Furthermore, potential  
32  
33 353 mechanisms underlying the crosstalk along the gut-brain axis will be investigated by longitudinal  
34  
35 354 analyses focusing on establishing correlations between brain structure/function changes and changes in  
36  
37 355 circulation mediators or faecal microbiota composition. Pearson correlation analysis will be used to  
38  
39 356 investigate potential correlations between variables.  
40  
41  
42  
43  
44

#### 45 358 **Data monitoring**

46  
47 359 Every year, data monitoring and auditing will be conducted by an independent specialised monitor from  
48  
49 360 the Rijnstate Hospital. Yearly, a summary of the progress will be submitted to the ethical committee and  
50  
51 361 the Netherlands Trial Register (trialregister.nl) 7288.  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 363 **Patient and Public involvement**  
4

5 364 Patients and the public were not involved in the design of this study. Nevertheless, the results will be  
6  
7 365 disseminated to the study participants via email, newsletters and social media platforms after the study  
8  
9 366 results are published.  
10

11  
12 367

13  
14 368 **DISCUSSION**  
15

16 369 The BARICO study is a prospective study focusing on the effect of weight loss on cognitive function and  
17  
18 370 brain structure after BS. This will be measured using sensitive neuropsychological tests covering the most  
19  
20 371 important domains, fMRI activation during the Stroop task, and several MRI techniques, such as DTI and  
21  
22 372 ASL. To clarify the impact of metabolic dysfunction in obesity on brain function and structure, blood  
23  
24 373 plasma and stool samples will be collected and analysed longitudinally, and biopsies of key metabolic  
25  
26 374 organs will be collected during the RYGB and analysed cross-sectionally.  
27  
28

29  
30 375 After BS, there have only been a limited number of long-term studies demonstrating improvement in  
31  
32 376 several cognitive domains, including memory, attention and executive function.(18, 19) Furthermore, it  
33  
34 377 has been shown that obese individuals have lower grey and white matter volumes, and altered white  
35  
36 378 matter densities, in comparison to healthy individuals with several studies showing a rapid recovery of  
37  
38 379 these brain structural abnormalities after BS.(50, 51) For instance, Tuulari *et al.* showed a causal link  
39  
40 380 between weight loss and brain tissue recovery.(50) Approximately 25-30% of the patients are not  
41  
42 381 expected to reach sufficient weight loss ( $\leq 50$  %EWL), and thus it will be possible to study the effect of  
43  
44 382 weight loss after BS on brain function and structure.  
45  
46

47  
48 383 Perhaps the strength of this study is in the long follow-up duration after surgery: 24 months for the  
49  
50 384 neuroimaging parameters, and 10 years for the neuropsychological tests. Furthermore, the strict  
51  
52 385 inclusion criterion with respect to age range ensures a good representation of mid-life patients.  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 386 Moreover, the majority of studies into BS patients are mostly composed of women but it is equally  
4  
5 387 important to account for the variation in fat tissue distribution which is caused by differences in sex.(30)  
6  
7 388 Another strength of this study is the combination of neuroimaging and neuropsychological tests.  
8  
9  
10 389 Alongside the analysis of metabolic and histopathological parameters (obtained in blood, organ biopsies  
11  
12 390 and microbiota), meaning that the relation between multiple metabolic, neuroimaging and/or cognitive  
13  
14 391 parameters can be investigated (e.g., adipokines, bioactive lipids (short-chain fatty acids) and organ  
15  
16 392 dysfunction) in a comprehensive way. Since RYGB influences gut-brain communication, there may be  
17  
18 393 beneficial alterations in adipose tissue functions, and/or recovery of brain function and structure  
19  
20 394 following BS.(15, 70) Longitudinal analyses of the microbiota, together with analysis of functional gut-  
21  
22 395 derived metabolites in the circulation and cognitive outcomes, may allow for the identification of  
23  
24 396 mediators derived from gut microflora that are relevant to cognition and the prevention of cognitive  
25  
26  
27  
28 397 decline.

29  
30 398 The BARICO study has the potential to be the first to demonstrate interactions between the periphery  
31  
32 399 and central nervous system after weight loss in humans, in particular it will question the roles and  
33  
34 400 involvement of the brain, and adipose tissue, liver and gut microbiota, after weight loss caused by BS.

35  
36 401 In conclusion, the BARICO study will reveal the relation and underlying mechanisms between obesity and  
37  
38 402 brain function and structure. This information can be used to develop better health care as well as  
39  
40 403 possible preventatives against obesity and associated disorders.

41  
42  
43 404

#### 44 45 405 **ETHICS AND DISSEMINATION**

46  
47 406 The study protocol was authorized by the medical review ethics committee CMO Region Arnhem and  
48  
49 407 Nijmegen (NL63493.091.17). All patients will sign informed consent forms upon enrolment in the study.  
50  
51 408 Study results will be submitted for publication in peer-reviewed journals.  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 409 **Acknowledgements**  
4

5 410 Not applicable.  
6  
7 411  
8  
9

10 412 **Contributors**

11  
12 413 EOA and AJK conceived and designed the study. DV wrote the article and developed the protocol  
13  
14 414 together with EOA, AJK, EJH, and RK. EJH, EOA and AJK are the principal investigators and DV is the main  
15  
16 415 investigator. MW, LND, IAA, EA, RK and RPCK are co- investigators in the participating centres. All  
17  
18 416 authors critically reviewed the content and approved the final manuscript.  
19  
20

21 417  
22  
23 418 **Funding**  
24

25 419 This work is supported by a grant of the Rijnstate-Radboudumc promotion fund. The histopathological  
26  
27 420 and biochemical analyses will be performed in collaboration with the Netherlands Organisation for  
28  
29 421 Applied Scientific Research (TNO) Metabolic Health Research (Leiden, the Netherlands) with support  
30  
31 422 from TNO's Research program Biomedical Health and the Shared Research Program GLoBAL, an initiative  
32  
33 423 of Radboudumc, Rijnstate and TNO.  
34  
35  
36  
37 424

38  
39 425 **Competing interests**  
40

41 426 The authors declare that they have no conflicts of interests.  
42  
43 427  
44

45 428 **Patient consent**

46  
47  
48 429 Obtained  
49  
50 430

51  
52 431 **Ethics approval**  
53

54 432 Medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17).  
55  
56  
57  
58  
59  
60

433 **REFERENCES**

- 434
- 435 1. WHO. Obesity and overweight; Fact sheet 2018.
- 436 2. Espeland MA, Erickson K, Neiberg RH, *et al.* Brain and white matter hyperintensity volumes after 10 years of random  
437 assignment to lifestyle intervention. *Diabetes care.* 2016;39(5):764-771.
- 438 3. Anstey K, Cherbuin N, Budge M, *et al.* Body mass index in midlife and late-life as a risk factor for dementia: a  
439 meta-analysis of prospective studies. *Obes Rev.* 2011;12(5):426-437.
- 440 4. Emmerzaal TL, Kiliaan AJ, Gustafson DR. 2003-2013: a decade of body mass index, Alzheimer's disease, and dementia. *J*  
441 *Alzheimer's Dis.* 2015;43(3):739-755.
- 442 5. Maayan L, Hoogendoorn C, Sweat V, *et al.* Disinhibited eating in obese adolescents is associated with orbitofrontal  
443 volume reductions and executive dysfunction. *Obesity (Silver Spring).* 2011;19(7):1382-1387.
- 444 6. Cournot M, Marquie J, Ansiau D, *et al.* Relation between body mass index and cognitive function in healthy middle-  
445 aged men and women. *Neurology.* 2006;67(7):1208-1214.
- 446 7. Gunstad J, Lhotsky A, Wendell CR, *et al.* Longitudinal examination of obesity and cognitive function: results from the  
447 Baltimore longitudinal study of aging. *Neuroepidemiology.* 2010;34(4):222-229.
- 448 8. Prickett C, Brennan L, Stolwyk R. Examining the relationship between obesity and cognitive function: a systematic  
449 literature review. *Obes Res Clin Pract.* 2015;9(2):93-113.
- 450 9. Bastard J-P, Maachi M, Lagathu C, *et al.* Recent advances in the relationship between obesity, inflammation, and  
451 insulin resistance. *Eur Cytokine Netw.* 2006;17(1):4-12.
- 452 10. Gloy VL, Briel M, Bhatt DL, *et al.* Bariatric surgery versus non-surgical treatment for obesity: a systematic review and  
453 meta-analysis of randomised controlled trials. *BMJ.* 2013;347:f5934.
- 454 11. Europe W. Body mass index - BMI 2018. Available from: [http://www.euro.who.int/en/health-topics/disease-](http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi)  
455 [prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi.](http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi)
- 456 12. Gisella Carranza-Leon B, Puzziferri N, Adams-Huet B, *et al.* Metabolic response 4years after gastric bypass in a  
457 complete cohort with type 2 diabetes mellitus. *Diabetes Res Clin Pract.* 2018;137:224-230.
- 458 13. Dogan K, Betzel B, Homan J, *et al.* Long-term effects of laparoscopic Roux-en-Y gastric bypass on diabetes mellitus,  
459 hypertension and dyslipidaemia in morbidly obese patients. *Obes Surg.* 2014;24(11):1835-1842.
- 460 14. Kim KS, Sandoval DA. Endocrine Function after Bariatric Surgery. *Compr Physiol.* 2017;7(3):783-798.
- 461 15. Ballsmider LA, Vaughn AC, David M, *et al.* Sleeve gastrectomy and Roux-en-Y gastric bypass alter the gut-brain  
462 communication. *Neural Plast.* 2015;2015:601985.



- 1  
2  
3 463 16. Murphy R, Tsai P, Jullig M, *et al.* Differential Changes in Gut Microbiota After Gastric Bypass and Sleeve Gastrectomy  
4  
5 464 Bariatric Surgery Vary According to Diabetes Remission. *Obes Surg.* 2017;27(4):917-925.  
6  
7 465 17. Zhang H, DiBaise JK, Zuccolo A, *et al.* Human gut microbiota in obesity and after gastric bypass. *PNAS.*  
8  
9 466 2009;106(7):2365-2370.  
10  
11 467 18. Handley JD, Williams DM, Caplin S, *et al.* Changes in cognitive function following bariatric surgery: a systematic review.  
12  
13 468 *Obes Surg.* 2016;26(10):2530-2537.  
14  
15 469 19. Alosco ML, Galioto R, Spitznagel MB, *et al.* Cognitive function after bariatric surgery: evidence for improvement 3 years  
16  
17 470 after surgery. *Am J Surg.* 2014;207(6):870-876.  
18  
19 471 20. Tuulari JJ. Effects of Obesity and Weight Loss Following Bariatric Surgery on Brain Function, Structural Integrity and  
20  
21 472 Metabolism. 2015.  
22  
23 473 21. Kivipelto M, Ngandu T, Fratiglioni L, *et al.* Obesity and vascular risk factors at midlife and the risk of dementia and  
24  
25 474 Alzheimer disease. *Arch Neurol.* 2005;62(10):1556-1560.  
26  
27 475 22. Whitmer R, Gustafson D, Barrett-Connor E, *et al.* Central obesity and increased risk of dementia more than three  
28  
29 476 decades later. *Neurology.* 2008;71(14):1057-1064.  
30  
31 477 23. Whitmer RA, Gunderson EP, Barrett-Connor E, *et al.* Obesity in middle age and future risk of dementia: a 27 year  
32  
33 478 longitudinal population based study. *BMJ.* 2005;330(7504):1360.  
34  
35 479 24. Whitmer RA, Gunderson EP, Quesenberry CP, *et al.* Body mass index in midlife and risk of Alzheimer disease and  
36  
37 480 vascular dementia. *Curr Alzheimer Res.* 2007;4(2):103-109.  
38  
39 481 25. Arnoldussen IA, Kiliaan AJ, Gustafson DR. Obesity and dementia: adipokines interact with the brain. *Eur*  
40  
41 482 *Neuropsychopharmacol.* 2014;24(12):1982-1999.  
42  
43 483 26. Jaganathan R, Ravindran R, Dhanasekaran S. Emerging Role of Adipocytokines in Type 2 Diabetes as Mediators of  
44  
45 484 Insulin Resistance and Cardiovascular Disease. *Can J Diabetes.* 2017.  
46  
47 485 27. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. *Trends Endocrinol Metab.* 2000;11(8):327-332.  
48  
49 486 28. Arner P. Not all fat is alike. *The Lancet.* 1998;351(9112):1301-1302.  
50  
51 487 29. Foster MT, Pagliassotti MJ. Metabolic alterations following visceral fat removal and expansion: Beyond anatomic  
52  
53 488 location. *Adipocyte.* 2012;1(4):192-199.  
54  
55 489 30. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity  
56  
57 490 complications. *Mol Aspects Med.* 2013;34(1):1-11.  
58  
59 491 31. Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese  
60  
492 humans. *N Engl J Med.* 1996;334(5):292-295.

- 1  
2  
3 493 32. Cnop M, Havel PJ, Utzschneider KM, *et al.* Relationship of adiponectin to body fat distribution, insulin sensitivity and  
4 494 plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia*. 2003;46(4):459-469.
- 5  
6 495 33. Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. *Nat Rev Endocrinol*.  
7 496 2017;13(9):509-520.
- 8  
9 497 34. Stefan N, Haring H-U. The role of hepatokines in metabolism. *Nat Rev Endocrinol*. 2013;9(3):144-152.
- 10  
11 498 35. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest*. 2011;121(6):2126-2132.
- 12  
13 499 36. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*.  
14 500 2012;489(7415):242-249.
- 15  
16 501 37. Torres-Fuentes C, Schellekens H, Dinan TG, *et al.* The microbiota-gut-brain axis in obesity. *Lancet Gastroenterol*  
17 502 *Hepatol*. 2017;2(10):747-756.
- 18  
19 503 38. Wang HX, Wang YP. Gut Microbiota-brain Axis. *Chin Med J (Engl)*. 2016;129(19):2373-2380.
- 20  
21 504 39. Aron-Wisnewsky J, Dore J, Clement K. The importance of the gut microbiota after bariatric surgery. *Nat Rev*  
22 505 *Gastroenterol Hepatol*. 2012;9(10):590-598.
- 23  
24 506 40. Peat CM, Kleiman SC, Bulik CM, *et al.* The Intestinal Microbiome in Bariatric Surgery Patients. *Eur Eat Disord Rev*.  
25 507 2015;23(6):496-503.
- 26  
27 508 41. Cipolla MJ. Chapter 5: Control of Cerebral Blood Flow. *The Cerebral Circulation. Integrated Systems Physiology: From*  
28 509 *Molecule to Function*. San Rafael (CA)2009. p. 29-36.
- 29  
30 510 42. Willeumier KC, Taylor DV, Amen DG. Elevated BMI is associated with decreased blood flow in the prefrontal cortex  
31 511 using SPECT imaging in healthy adults. *Obesity (Silver Spring)*. 2011;19(5):1095-1097.
- 32  
33 512 43. Alosco ML, Spitznagel MB, Raz N, *et al.* Obesity interacts with cerebral hypoperfusion to exacerbate cognitive  
34 513 impairment in older adults with heart failure. *Cerebrovasc Dis Extra*. 2012;2(1):88-98.
- 35  
36 514 44. Wierenga CE, Hays CC, Zlatar ZZ. Cerebral blood flow measured by arterial spin labeling MRI as a preclinical marker of  
37 515 Alzheimer's disease. *J Alzheimer's Dis*. 2014;42 (Suppl 4):S411-419.
- 38  
39 516 45. Alsop DC, Detre JA, Golay X, *et al.* Recommended implementation of arterial spin-labeled perfusion MRI for clinical  
40 517 applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. *Magn Reson*  
41 518 *Med*. 2015;73(1):102-116.
- 42  
43 519 46. Kullmann S, Callaghan MF, Heni M, *et al.* Specific white matter tissue microstructure changes associated with obesity.  
44 520 *Neuroimage*. 2016;125:36-44.
- 45  
46 521 47. Dobbie S, Wolf C, Lambert JC, *et al.* Abdominal obesity and lower gray matter volume: a Mendelian randomization  
47 522 study. *Neurobiol Aging*. 2014;35(2):378-386.
- 48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 523 48. Karlsson HK, Tuulari JJ, Hirvonen J, *et al.* Obesity is associated with white matter atrophy: a combined diffusion tensor  
4 524 imaging and voxel-based morphometric study. *Obesity (Silver Spring)*. 2013;21(12):2530-2537.
- 5 525 49. Arnoldussen IAC, Wiesmann M, Pelgrim CE, *et al.* Butyrate restores HFD-induced adaptations in brain function and  
6 526 metabolism in mid-adult obese mice. *Int J Obes (Lond)*. 2017;41(6):935-944.
- 7 527 50. Tuulari JJ, Karlsson HK, Antikainen O, *et al.* Bariatric Surgery Induces White and Grey Matter Density Recovery in the  
8 528 Morbidly Obese: A Voxel-Based Morphometric Study. *Hum Brain Mapp*. 2016;37(11):3745-3756.
- 9 529 51. Zhang Y, Ji G, Xu M, *et al.* Recovery of brain structural abnormalities in morbidly obese patients after bariatric surgery.  
10 530 *Int J Obes (Lond)*. 2016;40(10):1558-1565.
- 11 531 52. Fried M, Hainer V, Basdevant A, *et al.* Interdisciplinary European Guidelines on Surgery of Severe Obesity. *Obes Facts*.  
12 532 2008;1(1):52-59.
- 13 533 53. Nasreddine Z, Philips NA, Bédirian V, *et al.* The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild  
14 534 Cognitive Impairment. *J Am Geriatr Soc*. 2005;53(4):695-699.
- 15 535 54. Zimmerman P, Fimm B. Test for Attentional Performance (TAP), Manual. *Würselen, Germany: Psytest*. 1994.
- 16 536 55. Wechsler D. Wechsler adult intelligence scale-Fourth Edition (WAIS-IV). *San Antonio, TX: NCS Pearson*. 2008;22:498.
- 17 537 56. Schmand B, Groenink, S.C., van den Dungen, M. Letterfluency: psychometrische eigenschappen en Nederlandse  
18 538 normen. *Tijdschr Gerontol Geriatr*. 2008;39(2):64-76.
- 19 539 57. Wilson B, Cockburn J, Baddeley A. Rivermead Behavioural Memory Test. London: Thames Valley Test Company; 1985.
- 20 540 58. Schmand B, Bakker D, Saan R, *et al.* The Dutch Reading Test for Adults: a measure of premorbid intelligence level.  
21 541 *Tijdschr Gerontol Geriatr*. 1991;22(1):15-19.
- 22 542 59. Verhage F. Intelligentie en leeftijd: Onderzoek bij Nederlanders van twaalf tot zevenenzeventig jaar. Assen: Van  
23 543 Gorcum; 1964.
- 24 544 60. Baecke JA, Burema, J., Frijters, J.E. A short questionnaire for the measurement of habitual physical activity in  
25 545 epidemiological studies. *Am J Clin Nutr*. 1980;36(5):936-942.
- 26 546 61. Beck AT, Ward CH, Mendelson M, *et al.* An inventory for measuring depression. *Arch Gen Psychiatry*. 1961;4:561-571.
- 27 547 62. Ware JE, Sherbourne, C.D. The MOS 36-Item Short-Form Health Survey (SF-36): I. Conceptual Framework and Item  
28 548 Selection. *Medical Care*. 1992;30(6):473-483.
- 29 549 63. Oria HE, Moorehead M.K. . Bariatric analysis and reporting outcome system (BAROS). *Obes Surg*. 1998;8(5):487-499.
- 30 550 64. Patton JH, Stanford MS, Barratt ES. Factor structure of the Barratt impulsiveness scale. *J Clin Psychol*. 1995;51(6):768-  
31 551 774.

- 1  
2  
3 552 65. Carver CS, White T.L. . Behavioral Inhibition, Behavioral Activation, and Affective Responses to Impending Reward and  
4  
5 553 Punishment: The BIS/BAS Scales. *J Pers Soc Psychol*. 1994;67(2):319-333.  
6  
7 554 66. Michaud A, Vainik U, Garcia-Garcia I, *et al*. Overlapping Neural Endophenotypes in Addiction and Obesity. *Frontiers in*  
8  
9 555 *endocrinology*. 2017;8:127.  
10  
11 556 67. Meule A, Hofmann J, Weghuber D, *et al*. Impulsivity, perceived self-regulatory success in dieting, and body mass in  
12  
13 557 children and adolescents: A moderated mediation model. *Appetite*. 2016;107:15-20.  
14  
15 558 68. ICH harmonised tripartite guideline for good clinical practice: Brookwood Medical Publications Ltd; 1996.  
16  
17 559 69. Bretz F, Maurer W, Brannath W, *et al*. A graphical approach to sequentially rejective multiple test procedures. *Stat*  
18  
19 560 *Med*. 2009;28(4):586-604.  
20  
21 561 70. Hoffstedt J, Andersson DP, Eriksson Hogling D, *et al*. Long-term Protective Changes in Adipose Tissue After Gastric  
22  
23 562 Bypass. *Diabetes Care*. 2017;40(1):77-84.  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 564 **FIGURE LEGEND**  
4  
5  
6 565

7 566 **Figure 1.** Overview of the study design. Blood samples are taken during a regular blood withdrawal at six  
8  
9 567 time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota  
10  
11 568 analyses will be performed at set time points on the faeces (collected at home by the patients) (4-8 wks.  
12  
13 569 pre BS, 1 wk. pre BS, 6 mo. post BS, 24 mo. post BS) and mucosal swabs (collected during surgery).  
14  
15 570 Furthermore, biopsies of liver, jejunum and adipose tissue depots (subcutaneous, mesenteric and  
16  
17 571 omental) will be collected during surgery. Before surgery, (4-8 wks. pre BS) and at several time points  
18  
19 572 after, (6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS) a medical evaluation will take place and  
20  
21 573 all patients will complete questionnaires and neuropsychological measurements to test cognitive  
22  
23 574 function. A subgroup of patients (N=75) will be examined with MRI (4-8 wks. pre BS and 24 mo. post BS).  
24  
25 575 MRI; magnetic resonance imaging. BS; Bariatric surgery. Wks; weeks. Mo; months. Yrs; years.  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

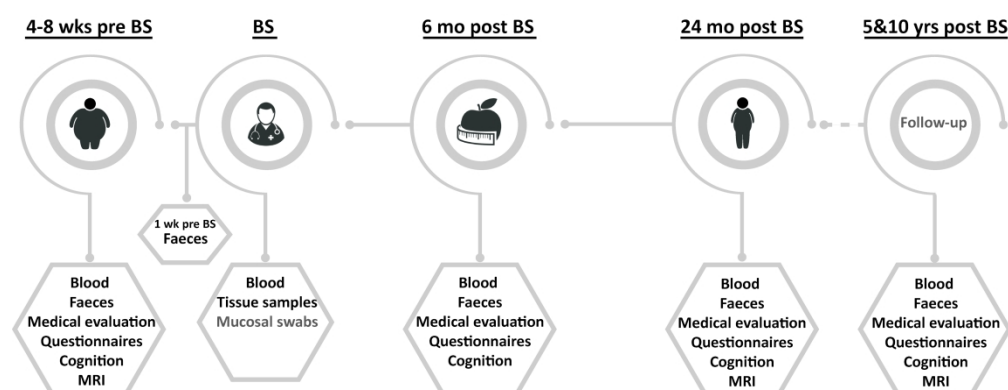


Figure 1. Overview of the study design. Blood samples are taken during a regular blood withdrawal at six time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota analyses will be performed at set time points on the faeces (collected at home by the patients) (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.

# BMJ Open

## 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.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025464.R2
Article Type:	Protocol
Date Submitted by the Author:	23-Nov-2018
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
<b>Primary Subject Heading</b>:	Neurology
Secondary Subject Heading:	Research methods, Nutrition and metabolism, Radiology and imaging, Surgery
Keywords:	Obesity, Weight loss, Bariatric surgery, Neuroimaging, Cognition

SCHOLARONE™  
Manuscripts

1  
2  
3  
4 **1 Study rationale and protocol of the BARICO study: a longitudinal, prospective, observational study**  
5 **2 to evaluate effects of weight loss on brain function and structure after bariatric surgery.**  
6  
7

8 **4** Vreeken, D.<sup>1,2,3</sup>, Wiesmann, M.<sup>3</sup>, Deden, L.N.<sup>1,2</sup>, Arnoldussen, I.A.C.<sup>3</sup>, Aarts, E.<sup>4</sup>, Kessels, R.P.C.<sup>4,5,6</sup>,  
9 Kleemann, R.<sup>7</sup>, Hazebroek, E.J.<sup>1,2</sup>, Aarts, E.O.<sup>1,2\*</sup>, Kiliaan, A.J.<sup>3\*\*</sup>  
10  
11

12  
13  
14 <sup>1</sup> *Department of Surgery, Rijnstate Hospital, Arnhem, the Netherlands.*

15 <sup>2</sup> *Vitalys Clinic, Velp, the Netherlands.*

16 <sup>3</sup> *Department of Anatomy, Donders Institute for Brain, Cognition and Behaviour, Radboud university*  
17 *medical center, Nijmegen, the Netherlands.*

18 <sup>4</sup> *Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, the*  
19 *Netherlands.*

20 <sup>5</sup> *Department of Medical Psychology, Radboud University Medical Center, Nijmegen, the Netherlands.*

21 <sup>6</sup> *Vincent van Gogh Institute for Psychiatry, Venray, the Netherlands.*

22 <sup>7</sup> *Department of Metabolic Health Research, Netherlands Organization for Applied Scientific Research*  
23 *(TNO), Leiden, the Netherlands.*  
24  
25

26  
27  
28  
29  
30  
31  
32 \*These authors contributed equally.

33 **† Corresponding author:**

34 Amanda J. Kiliaan, PhD

35 Donders Institute for Brain, Cognition, and Behaviour

36 Radboud university medical center

37 Department of Anatomy (109)

38 Geert Grooteplein 21N

39 6525 EZ Nijmegen, the Netherlands

40 Phone: +31 24 361 4378

41 Email: [amanda.kiliaan@radboudumc.nl](mailto:amanda.kiliaan@radboudumc.nl)  
42  
43  
44  
45  
46  
47  
48  
49

50 **29 Short title: The BARICO study, effect of weight loss on brain function**

51 Words count (excluding title page, abstract, references and figure): 4136

52 Words count abstract: 300  
53  
54  
55  
56

57 **33 Keywords: obesity, weight loss, bariatric surgery, neuroimaging, cognition**  
58  
59  
60



## 34 **ABSTRACT**

### 35 **Introduction**

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

41 The aim of the BARICO study (**BA**riatic surgery **Rijnstate** and **Radboudumc neuroImaging** and  
42 **Cognition in Obesity**) is to determine the long-term effect of weight loss after BS on brain function  
43 and structure, using sensitive neuropsychological tests and (functional) magnetic resonance imaging  
44 ((f)MRI). Secondary study endpoints are associated with changes in metabolic and inflammation  
45 status of adipose tissue, liver and gut, in relation to brain structure and function. Also, the possible  
46 correlation between weight loss, gut microbiota composition change and neuropsychological  
47 outcomes will be investigated.

### 49 **Methods and analysis**

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

### 58 **Ethics and dissemination**

1  
2  
3 59 This study has been approved by the medical review ethics committee CMO Region Arnhem and  
4  
5 60 Nijmegen (NL63493.091.17). Research findings will be published in peer-reviewed journals and at  
6  
7 61 conferences.  
8  
9

10 62

11  
12 63 **Trial registration**

13  
14 64 The Netherlands National Trial Register (trialregister.nl) 7288. Date registered: 29-June-2018.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## 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.

## 78 INTRODUCTION

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

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

1  
2  
3 104 mechanism is the altered signalling of visceral and abdominal adipose tissue; adipose tissue acts as  
4  
5 105 an independent endocrine organ releasing several hormones, proteins and cytokines, referred to as  
6  
7 106 adipokines. Obesity is associated with dysfunctional white adipose tissue and therefore an imbalance  
8  
9  
10 107 in adipokines, such as increased levels of leptin and angiotensinogen, and low levels of adiponectin  
11  
12 108 and omentin.(25, 26) Especially, visceral adipose tissue seems to produce unfavourable adipokines  
13  
14 109 associated with more metabolic complications when compared to subcutaneous adipose tissue.(27-  
15  
16 110 30) Importantly, the distribution of fat tissue depots differs between sexes. Overall, men accumulate  
17  
18 111 more abdominal and visceral fat than women.(30) Moreover, women have a higher level of  
19  
20 112 adipokines such as leptin and adiponectin.(31, 32) This disbalance in adipokines may induce  
21  
22 113 inflammation in several organs such as the liver, gut and vascular endothelium. The latter causing  
23  
24 114 atherosclerosis, ultimately leading to changes in cerebral blood flow (CBF).(25)  
25  
26  
27 115 Secondly, signalling between, and within other organs, such as the liver, might be altered in obese  
28  
29 116 patients. For example; the liver secretes hepatokines, such as insulin-like growth factor 1,  
30  
31 117 selenoprotein P, leukocyte cell-derived chemotaxin, fetuin B and hepassocin, which may indirectly  
32  
33 118 affect brain function and structure.(33, 34)  
34  
35  
36 119 Thirdly, the gut microbiota composition in obese people differs from that of non-obese individuals,  
37  
38 120 affecting metabolic processes, weight and obesity-related comorbidities.(35, 36) Microbiota is  
39  
40 121 involved in adiposity and homeostasis but also influences energy balance via appetite and satiety  
41  
42 122 signalling to the brain. Gut microbiota also affect the brain by producing (precursors of)  
43  
44 123 neurotransmitters and short chain fatty acids, or through cytokines via the immune system.(37, 38)  
45  
46 124 BS leads to a fast change in gut microbiota composition through changes in food intake, intestinal  
47  
48 125 modifications due to the surgery itself, and metabolic improvements, eventually leading to changes  
49  
50 126 in gut-brain communication.(15, 39, 40) Hence, metabolic organs, such as liver, gut and adipose  
51  
52 127 tissue and gut microbiota may constitute new therapeutic targets. Although long-term results are not  
53  
54 128 yet clear, the gut microbiota has already become a target for anti-obesity treatments.(37)  
55  
56  
57  
58  
59  
60

1  
2  
3 129 Obesity is associated with impaired CBF, which may lead to inadequate oxygen and energy supply in  
4  
5 130 the brain and eventually loss of white and grey matter integrity.(41, 42) Lower levels of CBF in the  
6  
7 131 prefrontal cortex are associated with reduced performance on executive function and episodic  
8  
9 132 memory tests.(42, 43) Even in the prodromal stages of Alzheimer's disease, changes in CBF can be  
10  
11 133 detected with arterial spin labelling (ASL), which may be used as a very early biomarker for  
12  
13 134 neurodegenerative disorders.(44) However, the technique requires further optimization and  
14  
15 135 therefore several consortia are working on the implementation of ASL perfusion magnetic resonance  
16  
17 136 imaging (MRI) for clinical applications to provide images of sufficient and diagnostic utility.(45)  
18  
19 137 Furthermore, obesity is associated with changes in grey and white matter, which can be visualized  
20  
21 138 using diffusion tensor imaging (DTI) and voxel-based morphometry analyses based on T1 weighted  
22  
23 139 scans.(46, 47) These structural changes are especially prominent in brain regions governing reward  
24  
25 140 seeking, inhibitory control and appetite.(48, 49) There are indications that rapid recovery of  
26  
27 141 structural abnormalities occur after BS, however long-term study data is lacking here. (50, 51)  
28  
29 142 Additionally, impairment in attention span, executive function and memory are commonly reported  
30  
31 143 in obese patients.(18, 19) Cognitive impairment revealed in obesity might be reversible and varies  
32  
33 144 between cognitive domains however long-term follow-up studies are scarce. The Longitudinal  
34  
35 145 Assessment of Bariatric Surgery (LABS) parent project is the most extensive longitudinal study to date  
36  
37 146 focusing on cognitive changes in patients after BS. Investigators showed lasting improvements three  
38  
39 147 years after surgery in the cognitive domains of attention, executive function and memory.(19)  
40  
41  
42  
43  
44  
45  
46  
47

## 149 **Rationale**

50 150 Cognitive benefits after BS are not equally exhibited across patients and cognitive domains. However,  
51  
52 151 precise causes are still poorly understood, and underlying molecular mechanisms remain elusive.  
53  
54 152 From the relatively short follow-up duration and small samples of BS patients in the studies  
55  
56 153 reviewed, it is difficult to elaborate on the long-term consequences of obesity and its related  
57  
58 154 diseases. In this study, underlying mechanisms of obesity-related cognitive disorders will be  
59  
60

1  
2  
3 155 investigated by longitudinal studies correlating cognition to brain changes, blood serum and plasma  
4  
5 156 values, and gut microbiota composition. Lastly, metabolic and histopathological parameters (at the  
6  
7 157 time-point of surgery) will be obtained to study whether associations or correlations exist between  
8  
9  
10 158 obesity-associated metabolic dysfunctions of particular organs and brain function and structure. To  
11  
12 159 our knowledge this is the first study in humans investigating changes in brain structure and function,  
13  
14 160 and changes in adipose tissue, liver function and the gut microbiome, after BS-induced weight loss.  
15  
16 161 Additionally, this is the first study in bariatric research combining neuroimaging, cognition and  
17  
18 162 extensive profiling of biological markers.  
19  
20  
21 163

22  
23 164 The primary aim of the BARICO study (**BA**riatic surgery **Rijn**state and **Radboud**umc **neuroIm**aging  
24  
25 165 and **C**ognition in **O**besity) is to determine the long-term effect of weight loss on measures of brain  
26  
27 166 function and structure after BS. The secondary aim is to provide mechanism-based rationales  
28  
29 167 responsible for functional and structural decline in obese individuals. Therefore, the metabolic and  
30  
31 168 inflammation status of organ biopsies will be determined together with molecular signatures via  
32  
33 169 blood plasma/serum analyses. Furthermore, gut microbiota composition will be monitored over time  
34  
35 170 to gain knowledge about the gut-brain axis.  
36  
37  
38

39 171 This study will contribute to the development of better health campaigns, healthcare and  
40  
41 172 preventatives to attenuate the impact of obesity. This paper describes the design and protocol of the  
42  
43 173 BARICO study.  
44  
45  
46 174

## 47 175

### 48 176 **METHODS AND ANALYSIS**

#### 49 177 **Study population**

50  
51  
52 178 Patients who have been screened and found eligible for BS based on the Fried guidelines will be  
53  
54 179 asked to participate.<sup>(52)</sup> In total, 150 patients will be included in the study. Study specific inclusion  
55  
56  
57  
58  
59 180 criteria are: (a) patients willing to perform neuropsychological tests, complete self-report  
60

1  
2  
3 181 questionnaires and sign an informed consent document; (b) age between 35 and 55 years; (c)  
4  
5 182 patients must undergo RYGB. A laparoscopic antecolic antegastric RYGB procedure will be performed  
6  
7 183 (biliopancreatic limb of 150 cm, alimentary limb of 100 cm). Exclusion criteria for this study are: (a)  
8  
9 184 previous or current neurological or severe psychiatric illness; (b) pregnancy; (c) treatment with any  
10 185 antibiotics, probiotics, or prebiotics three months before or at any point during the study (excluding  
11  
12 186 preoperative prophylaxis). A subgroup of 75 patients will be included in the MRI sub-study, extra  
13  
14 187 inclusion criteria for this group are: (d) patients willing to undergo MRI scanning and perform tasks in  
15  
16 188 the MRI scanner; (e) right handed (more homogeneous sample and less variance). The standard  
17  
18 189 exclusion criteria for the MRI subgroup include: (d) claustrophobia; (e) epilepsy; (f) pacemakers and  
19  
20 190 defibrillators; (g) nerve stimulators; (h) intracranial clips; (i) infraorbital or intraocular metallic  
21  
22 191 fragments; (j) cochlear implants; (k) ferromagnetic implants; (l) circumference above the MRI space  
23  
24 192 capacity; (m) colour blindness. The study has been approved by the medical research ethics  
25  
26 193 committee CMO Region Arnhem-Nijmegen (NL63493.091.17) and is registered at the Netherlands  
27  
28 194 Trial Register (trialregister.nl) 7288.  
29  
30  
31  
32  
33  
34  
35

### 36 196 **Study design**

37  
38  
39 197 At several time points (4-8 weeks preoperative, 6, 24 months and 5, 10 years postoperative (figure  
40  
41 198 1)) a number of cognitive tests and questionnaires will be performed, and their results assessed.  
42  
43 199 Furthermore, blood (after 8 hrs. period of fasting) and faecal matter will be collected from all  
44  
45 200 patients (N=150) (blood at all time points, faeces 4-8 weeks preoperative, 6 and 24 months  
46  
47 201 postoperative (figure 1)). Intraoperatively, several tissue biopsies will be collected and processed.  
48  
49 202 Medical evaluation, including anthropometric measurements and information on comorbidities, will  
50  
51 203 be assessed 4-8 weeks preoperative and during all postoperative time points. A schematic overview  
52  
53 204 of the study is shown in figure 1. A subgroup of patients (N=75) will additionally receive a (f)MRI scan  
54  
55 205 4-8 weeks preoperative and 24 months postoperative. During the whole study period (10 years)  
56  
57  
58  
59  
60



1  
2  
3 206 patients will be contacted by letter and via telephone at least once a year to ensure the best follow-  
4  
5 207 up rate.  
6

7  
8 208

9  
10 209 **Recruitment procedures and consent**

11  
12 210 Patients are informed about the study by letter and telephone at least two weeks prior to their  
13  
14 211 standard visit (4-8 weeks before RYGB surgery). During this visit, patients will individually receive  
15  
16 212 more information about this study and its objectives. Afterwards, the researchers will further clarify  
17  
18 213 the study and the patients can ask for additional information. If they decide to participate and fulfil  
19  
20 214 the inclusion criteria, informed consents will be obtained. Although the obese population consists of  
21  
22 215 more females than males, the aim is for an equal sex distribution during the recruitment period (i.e.,  
23  
24 216 a study population consisting of >30% men and >30% women).(1) Recruitment will take place  
25  
26 217 between August 2018 and August 2020.  
27  
28 218

29  
30 219

31  
32 219 **Outcome measures**

33  
34 220 The primary outcome measures are the neuropsychological tests scores, CBF values, hippocampal  
35  
36 221 volume, mean diffusivity (MD) and fractional anisotropy (FA) (representing respectively grey and  
37  
38 222 white matter integrity), and blood oxygen level dependent (BOLD) responses during the Stroop task.  
39  
40 223 Combining neuroimaging and neuropsychological tests will give us more information on how and  
41  
42 224 whether structural brain changes are related to functional brain changes. Secondary measures  
43  
44 225 comprise of (histopathological and biochemical determined) health status of the collected tissue, gut  
45  
46 226 microbiota composition changes (in jejunal mucosa and faeces) and the profiling of circulating  
47  
48 227 mediators in blood (plasma and serum), as well as lifestyle and dietary habits in relation to cognitive  
49  
50 228 function and brain structure. Combining information on the pathological state of liver, gut, adipose  
51  
52 229 tissue and circulating mediators from corresponding plasma/serum samples, obtained prior to and at  
53  
54 230 surgery, will provide insight into organ cross-talk and allow identification of biomarker signatures for  
55  
56  
57  
58  
59  
60

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

233

#### 234 **(f)MRI**

235 Subjects will be scanned in a 3T MAGNETOM Skyra MR scanner (Siemens AG, Healthcare Sector,  
236 Erlangen, Germany) using a 32-channel head coil. The MRI protocol included: a T1-weighted 3D  
237 magnetization-prepared rapid gradient-echo (MPRAGE) sequence for anatomical reference and  
238 analysis (TR/TI/TE 2300/1100/3.03 ms; 8° flip angle; voxel size: 1.0 × 1.0 × 1.0 mm), a fluid-  
239 attenuated inversion recovery (FLAIR) sequence for white matter lesion visualization (TR/TI  
240 5000/1800 ms; voxel size: 1.0 × 1.0 × 1.0 mm), and diffusion-weighted MRI scans using simultaneous  
241 multi-slice echo planar imaging for probing microstructural properties (TR/TE 3275/91.4 ms; voxel  
242 size: 1.9 × 1.9 × 1.9 mm; 6x b=0 s/mm<sup>2</sup>, 42x b=900 s/mm<sup>2</sup>, 83x b=1800 s/mm<sup>2</sup>). To allow for offline  
243 distortion correction of the images, 7 more b=0 s/mm<sup>2</sup> volumes will be acquired using the exact same  
244 sequence parameters - except for the inverted k-space read-out trajectory. An ASL sequence will  
245 used for quantification of CBF (TR/TE 2500/12 ms; voxel size: 4.0 × 4.0 × 4.0 mm) and a multi-band,  
246 multi-echo planar imaging sequence will be used to measure BOLD contrast during the Stroop task  
247 (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;  
248 51 transversal slices in interleaved order). The complete scanning protocol takes 45 minutes and for  
249 both time-points, the same: MR scanner, head coil, and sequences will be used. Following the project  
250 MRI quality assurance is guaranteed by regular phantom measurements.

251

#### 252 **Cognitive assessment**

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

1  
2  
3 257 attention between objects. Working memory will be assessed via the Digit Span subtest from the  
4  
5 258 Wechsler Adult Intelligence Scale Fourth Edition (WAIS-IV-NL).(55) Participants will have to repeat a  
6  
7 259 series of digits in forward or backward order, or sort them numerically. The Controlled Oral Word  
8  
9 260 Association Test (COWAT) will be used to determine verbal fluency.(56) Participants have to come up  
10  
11 261 with as many words beginning with three designated letters within 60 seconds (for each letter).  
12  
13 262 Episodic memory will be assessed via the immediate and delayed Story Recall subtest from the  
14  
15 263 Rivermead Behavioural Memory Test (RBMT).(57) To control and correct for differences in premorbid  
16  
17 264 intelligence between participants, verbal IQ will be estimated using the Dutch version of the National  
18  
19 265 Adult Reading Test (NART) at baseline.(58) The MoCA, episodic memory test and COWAT have  
20  
21 266 parallel versions, to avoid material-specific learning effects during the repeated testing. Additionally,  
22  
23 267 the tests are standardized, have been validated for use across a wide age range and have good re-  
24  
25 268 test reliability. Together these tests will provide a good overview on the overall cognitive  
26  
27 269 performance of the patients, including aspects of working and episodic memory, attention, verbal  
28  
29 270 fluency and executive function. Also, education level will be recorded in accordance with the Dutch  
30  
31 271 education system using seven categories (one being the lowest level of education and seven being  
32  
33 272 the highest).(59)  
34  
35  
36  
37  
38  
39  
40

#### 41 274 **Assessment of biological measurements**

42  
43 275 At several time points (figure 1) fasting (at least 8 hrs.) blood samples from the participants will be  
44  
45 276 collected. As standard procedure classical parameters, such as several vitamins (vitamin B12, D and  
46  
47 277 folic acid) and lipids (triglycerides and cholesterol) will be measured. Special interest is taken on  
48  
49 278 circulating mediators of organ cross-talk, such as: cytokines, oxylipids, adipokines, hormones and  
50  
51 279 inflammation markers (e.g., C-reactive protein, serum amyloid A, vascular cell adhesion molecule 1,  
52  
53 280 transforming growth factor beta), as well as metabolites (derived from organs or microbiota)  
54  
55 281 assessed by metabolomics, such as bile acids and bioactive (short chain) fatty acids, and other lipid  
56  
57 282 species (untargeted lipidomics).  
58  
59  
60

1  
2  
3 283 Besides blood samples, faeces will be collected (figure 1) using “faeces collection kits for at home” in  
4  
5 284 order to monitor gut-microbiota changes and relate them to cognition and brain structure and  
6  
7 285 function readouts. Additionally, to gain insight into the microbiota in the intestinal mucosa, mucosal  
8  
9  
10 286 swabs will be collected during surgery within the jejunum (two places; 150 and 250cm from Treitz  
11  
12 287 ligament) and stomach pouch.

13  
14 288 As metabolically active organs such as the liver and adipose tissue interact directly and indirectly with  
15  
16 289 the brain, biopsies of these organs will be collected and analysed on histopathological, and  
17  
18 290 biochemical level. Tissue biopsies from subcutaneous, mesenteric and omental adipose tissue, liver  
19  
20 291 and jejunum. Tissue biopsies from these organs will be taken to assess potential pathophysiological  
21  
22 292 processes and to eventually define mechanism-based subgroups.  
23  
24  
25  
26  
27

## 28 294 **Questionnaires**

29  
30 295 At several time-points (figure 1) standardized questionnaires on lifestyle, education, success rate of  
31  
32 296 the surgery and eating habits will be assessed. Most of the questionnaires are routine practice for  
33  
34 297 patients undergoing BS at the Rijnstate hospital. Physical activity will be assessed via the Baecke  
35  
36 298 questionnaire and depressive symptoms will be assessed with the Beck Depression Inventory (BDI-  
37  
38 299 II).(60, 61) To estimate the participants’ food/nutrient intake and eating behaviour patients will be  
39  
40 300 asked to fill out an eating diary of two days (a weekday and a weekend day). Quality of Life will be  
41  
42 301 evaluated with the Short Form 36 (SF-36).(62) Lastly, the results of BS will be evaluated via the  
43  
44 302 Bariatric Analysis and Report Outcome System (BAROS).(63)  
45  
46  
47

48 303 More specifically: the Barratt impulsivity scale (BIS-11)(64) and Behavioural inhibition/activation  
49  
50 304 system (BIS/BAS)(65) questionnaires on impulsivity and reward sensitivity are included as reward  
51  
52 305 sensitivity and impulsivity have both previously been suggested to contribute to overeating.(66)  
53  
54 306 Indeed, some facets of impulsivity and reward sensitivity have shown to be relevant in eating- and  
55  
56 307 weight regulation.(67)  
57  
58  
59  
60

### 309 **Medical evaluation**

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

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

323

### 324 **Data management**

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

331

### 332 **Sample size**

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

1  
2  
3 335 of at least 0.3 and a correlation of 0.7, a selected sample of 150 patients will be sufficient to reach  
4  
5 336 90% power. The power calculation for the MRI parameters is based on changes in the FA parameter  
6  
7 337 studied by Zhang *et al.*(51) With an expected standardized effect size of at least 0.03 and a  
8  
9 338 correlation of 0.5 including 75 patients in the MRI group will be sufficient to reach 90% power. A  
10  
11 339 significance level based on the sequentially rejective multiple testing procedure discussed by Bretz *et*  
12  
13 340 *al.* (for the neuropsychological tests 3% and for the MRI parameters 2%) has been taken into account  
14  
15 341 in the power calculation.(69) The inclusion of 150 patients with a subgroup of 75 for the MRI scan has  
16  
17 342 been considered adequate to answer the research questions with sufficient power.  
18  
19 343

20  
21 344

#### 22 23 344 **Analysis of primary outcome measures**

24  
25 345 As a primary outcome measure, baseline levels of the imaging parameters (such as MD and FA) will  
26  
27 346 be compared with the results of the neuroimaging outcome 24 months after surgery (including  
28  
29 347 %TBWL as a factor in the model). Next, the scores of the cognitive tests from five different time  
30  
31 348 points will be analysed and compared to %TBWL. Every dependent variable will be modelled in a  
32  
33 349 separate linear mixed model. %TBWL will be used as a factor. Different variables, such as: depression  
34  
35 350 score, age, and gender, will be (if appropriate) included in the model. For each model, we will decide  
36  
37 351 which variables to include as a factor to reduce the amount of unexplained variation. To correct for  
38  
39 352 multiple outcome measures, the sequentially rejective multiple testing procedure described in Bretz  
40  
41 353 *et al.* will be used (more information in the supplementary material).(69) Data will be analysed using  
42  
43 354 SPSS (version 25 for Windows) and R (version 3.5.1 for Windows). For the cognitive tests a *p* value of  
44  
45 355 <0.03 and for the imaging parameters a *p* value of <0.02 will be considered as statistically significant.  
46  
47 356

48  
49 357

#### 50 51 357 **Analysis of secondary outcome measures**

52  
53 358 As secondary outcome measures, the metabolic and histopathological parameters (obtained analyses  
54  
55 359 from tissues collected during surgery) will be analysed cross-sectionally to examine correlations  
56  
57 360 between and among each other, and in relation to brain function and structure. Furthermore,  
58  
59  
60

1  
2  
3 361 potential mechanisms underlying the crosstalk along the gut-brain axis will be investigated by  
4  
5 362 longitudinal analyses focusing on establishing correlations between brain structure/function changes  
6  
7 363 and changes in circulation mediators or faecal microbiota composition. Pearson correlation analysis  
8  
9 364 will be used to investigate potential correlations between variables.  
10  
11  
12 365

#### 13 14 366 **Data monitoring**

15  
16 367 Every year, data monitoring and auditing will be conducted by an independent specialised monitor  
17  
18 368 from the Rijnstate Hospital. Yearly, a summary of the progress will be submitted to the ethical  
19  
20 369 committee and the Netherlands Trial Register (trialregister.nl) 7288.  
21  
22

23 370

#### 24 25 371 **Patient and Public involvement**

26  
27 372 Patients and the public were not involved in the design of this study. Nevertheless, the results will be  
28  
29 373 disseminated to the study participants via email, newsletters and social media platforms after the  
30  
31 374 study results are published.  
32  
33

34 375

#### 35 36 376 **DISCUSSION**

37  
38  
39 377 The BARICO study is a prospective study focusing on the effect of weight loss on cognitive function  
40  
41 378 and brain structure after BS. This will be measured using sensitive neuropsychological tests covering  
42  
43 379 the most important domains, fMRI activation during the Stroop task, and several MRI techniques,  
44  
45 380 such as DTI and ASL. To clarify the impact of metabolic dysfunction in obesity on brain function and  
46  
47 381 structure, blood plasma and stool samples will be collected and analysed longitudinally, and biopsies  
48  
49 382 of key metabolic organs will be collected during the RYGB and analysed cross-sectionally.  
50  
51

52 383 After BS, there have only been a limited number of long-term studies demonstrating improvement in  
53  
54 384 several cognitive domains, including memory, attention and executive function.(18, 19) Furthermore,  
55  
56 385 it has been shown that obese individuals have lower grey and white matter volumes, and altered  
57  
58 386 white matter densities, in comparison to healthy individuals with several studies showing a rapid  
59  
60

1  
2  
3 387 recovery of these brain structural abnormalities after BS.(50, 51) For instance, Tuulari *et al.* showed a  
4  
5 388 causal link between weight loss and brain tissue recovery.(50) Approximately 25-30% of the patients  
6  
7 389 are not expected to reach sufficient weight loss ( $\leq 50$  %EWL), and thus it will be possible to study the  
8  
9 390 effect of weight loss after BS on brain function and structure.

10  
11  
12 391 Perhaps the strength of this study is in the long follow-up duration after surgery: 24 months for the  
13  
14 392 neuroimaging parameters, and 10 years for the neuropsychological tests. Furthermore, the strict  
15  
16 393 inclusion criterion with respect to age range ensures a good representation of mid-life patients.  
17  
18 394 Moreover, the majority of studies into BS patients are mostly composed of women but it is equally  
19  
20 395 important to account for the variation in fat tissue distribution which is caused by differences in  
21  
22 396 sex.(30)

23  
24  
25 397 Another strength of this study is the combination of neuroimaging and neuropsychological tests.  
26  
27 398 Alongside the analysis of metabolic and histopathological parameters (obtained in blood, organ  
28  
29 399 biopsies and microbiota), meaning that the relation between multiple metabolic, neuroimaging  
30  
31 400 and/or cognitive parameters can be investigated (e.g., adipokines, bioactive lipids (short-chain fatty  
32  
33 401 acids) and organ dysfunction) in a comprehensive way. Since RYGB influences gut-brain  
34  
35 402 communication, there may be beneficial alterations in adipose tissue functions, and/or recovery of  
36  
37 403 brain function and structure following BS.(15, 70) Longitudinal analyses of the microbiota, together  
38  
39 404 with analysis of functional gut-derived metabolites in the circulation and cognitive outcomes, may  
40  
41 405 allow for the identification of mediators derived from gut microflora that are relevant to cognition  
42  
43 406 and the prevention of cognitive decline.

44  
45  
46 407 The BARICO study has the potential to be the first to demonstrate interactions between the  
47  
48 408 periphery and central nervous system after weight loss in humans, in particular it will question the  
49  
50 409 roles and involvement of the brain, and adipose tissue, liver and gut microbiota, after weight loss  
51  
52 410 caused by BS.



1  
2  
3 411 In conclusion, the BARICO study will reveal the relation and underlying mechanisms between obesity  
4  
5 412 and brain function and structure. This information can be used to develop better health care as well  
6  
7 413 as possible preventatives against obesity and associated disorders.  
8  
9

10 414

#### 11 415 **ETHICS AND DISSEMINATION**

12  
13  
14 416 The study protocol was authorized by the medical review ethics committee CMO Region Arnhem and  
15  
16 417 Nijmegen (NL63493.091.17). All patients will sign informed consent forms upon enrolment in the  
17  
18 418 study. Study results will be submitted for publication in peer-reviewed journals.  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 419 **Acknowledgements**  
4

5 420 Not applicable.  
6  
7 421  
8  
9

10 422 **Contributors**

11  
12 423 EOA and AJK conceived and designed the study. DV wrote the article and developed the protocol  
13  
14 424 together with EOA, AJK, EJH, and RK. EJH, EOA and AJK are the principal investigators and DV is the  
15  
16 425 main investigator. MW, LND, IAA, EA, RK and RPKC are co- investigators in the participating centres.  
17  
18 426 All authors critically reviewed the content and approved the final manuscript.  
19  
20  
21 427  
22

23 428 **Funding**

24  
25 429 This work is supported by a grant of the Rijnstate-Radboudumc promotion fund. The  
26  
27 430 histopathological and biochemical analyses will be performed in collaboration with the Netherlands  
28  
29 431 Organisation for Applied Scientific Research (TNO) Metabolic Health Research (Leiden, the  
30  
31 432 Netherlands) with support from TNO's Research program Biomedical Health and the Shared Research  
32  
33 433 Program GLoBAL, an initiative of Radboudumc, Rijnstate and TNO.  
34  
35  
36  
37 434  
38

39 435 **Competing interests**

40  
41 436 The authors declare that they have no conflicts of interests.  
42  
43 437  
44

45 438 **Ethics approval**

46  
47 439 Medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17).  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

440 **REFERENCES**

- 441
- 442 1. WHO. Obesity and overweight; Fact sheet 2018.
- 443 2. Espeland MA, Erickson K, Neiberg RH, *et al.* Brain and white matter hyperintensity volumes after 10 years of  
444 random assignment to lifestyle intervention. *Diabetes care.* 2016;39(5):764-771.
- 445 3. Anstey K, Cherbuin N, Budge M, *et al.* Body mass index in midlife and late-life as a risk factor for dementia: a  
446 meta-analysis of prospective studies. *Obes Rev.* 2011;12(5):426-437.
- 447 4. Emmerzaal TL, Kiliaan AJ, Gustafson DR. 2003-2013: a decade of body mass index, Alzheimer's disease, and  
448 dementia. *J Alzheimer's Dis.* 2015;43(3):739-755.
- 449 5. Maayan L, Hoogendoorn C, Sweat V, *et al.* Disinhibited eating in obese adolescents is associated with orbitofrontal  
450 volume reductions and executive dysfunction. *Obesity (Silver Spring).* 2011;19(7):1382-1387.
- 451 6. Cournot M, Marquie J, Ansiau D, *et al.* Relation between body mass index and cognitive function in healthy  
452 middle-aged men and women. *Neurology.* 2006;67(7):1208-1214.
- 453 7. Gunstad J, Lhotsky A, Wendell CR, *et al.* Longitudinal examination of obesity and cognitive function: results from  
454 the Baltimore longitudinal study of aging. *Neuroepidemiology.* 2010;34(4):222-229.
- 455 8. Prickett C, Brennan L, Stolwyk R. Examining the relationship between obesity and cognitive function: a systematic  
456 literature review. *Obes Res Clin Pract.* 2015;9(2):93-113.
- 457 9. Bastard J-P, Maachi M, Lagathu C, *et al.* Recent advances in the relationship between obesity, inflammation, and  
458 insulin resistance. *Eur Cytokine Netw.* 2006;17(1):4-12.
- 459 10. Gloy VL, Briel M, Bhatt DL, *et al.* Bariatric surgery versus non-surgical treatment for obesity: a systematic review  
460 and meta-analysis of randomised controlled trials. *BMJ.* 2013;347:f5934.
- 461 11. Europe W. Body mass index - BMI 2018. Available from: [http://www.euro.who.int/en/health-topics/disease-](http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi)  
462 [prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi.](http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi)
- 463 12. Gisella Carranza-Leon B, Puzifferri N, Adams-Huet B, *et al.* Metabolic response 4years after gastric bypass in a  
464 complete cohort with type 2 diabetes mellitus. *Diabetes Res Clin Pract.* 2018;137:224-230.
- 465 13. Dogan K, Betzel B, Homan J, *et al.* Long-term effects of laparoscopic Roux-en-Y gastric bypass on diabetes mellitus,  
466 hypertension and dyslipidaemia in morbidly obese patients. *Obes Surg.* 2014;24(11):1835-1842.
- 467 14. Kim KS, Sandoval DA. Endocrine Function after Bariatric Surgery. *Compr Physiol.* 2017;7(3):783-798.
- 468 15. Ballsmider LA, Vaughn AC, David M, *et al.* Sleeve gastrectomy and Roux-en-Y gastric bypass alter the gut-brain  
469 communication. *Neural Plast.* 2015;2015:601985.
- 470 16. Murphy R, Tsai P, Jullig M, *et al.* Differential Changes in Gut Microbiota After Gastric Bypass and Sleeve  
471 Gastrectomy Bariatric Surgery Vary According to Diabetes Remission. *Obes Surg.* 2017;27(4):917-925.

- 1  
2  
3 472 17. Zhang H, DiBaise JK, Zuccolo A, *et al.* Human gut microbiota in obesity and after gastric bypass. *PNAS*.  
4 473 2009;106(7):2365-2370.  
5  
6 474 18. Handley JD, Williams DM, Caplin S, *et al.* Changes in cognitive function following bariatric surgery: a systematic  
7 475 review. *Obes Surg*. 2016;26(10):2530-2537.  
8  
9 476 19. Alosco ML, Galioto R, Spitznagel MB, *et al.* Cognitive function after bariatric surgery: evidence for improvement 3  
10 477 years after surgery. *Am J Surg*. 2014;207(6):870-876.  
11  
12 478 20. Tuulari JJ. Effects of Obesity and Weight Loss Following Bariatric Surgery on Brain Function, Structural Integrity  
13 479 and Metabolism. 2015.  
14  
15 480 21. Kivipelto M, Ngandu T, Fratiglioni L, *et al.* Obesity and vascular risk factors at midlife and the risk of dementia and  
16 481 Alzheimer disease. *Arch Neurol*. 2005;62(10):1556-1560.  
17  
18 482 22. Whitmer R, Gustafson D, Barrett-Connor E, *et al.* Central obesity and increased risk of dementia more than three  
19 483 decades later. *Neurology*. 2008;71(14):1057-1064.  
20  
21 484 23. Whitmer RA, Gunderson EP, Barrett-Connor E, *et al.* Obesity in middle age and future risk of dementia: a 27 year  
22 485 longitudinal population based study. *BMJ*. 2005;330(7504):1360.  
23  
24 486 24. Whitmer RA, Gunderson EP, Quesenberry CP, *et al.* Body mass index in midlife and risk of Alzheimer disease and  
25 487 vascular dementia. *Curr Alzheimer Res*. 2007;4(2):103-109.  
26  
27 488 25. Arnoldussen IA, Kiliaan AJ, Gustafson DR. Obesity and dementia: adipokines interact with the brain. *Eur*  
28 489 *Neuropsychopharmacol*. 2014;24(12):1982-1999.  
29  
30 490 26. Jaganathan R, Ravindran R, Dhanasekaran S. Emerging Role of Adipocytokines in Type 2 Diabetes as Mediators of  
31 491 Insulin Resistance and Cardiovascular Disease. *Can J Diabetes*. 2017.  
32  
33 492 27. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. *Trends Endocrinol Metab*. 2000;11(8):327-332.  
34  
35 493 28. Arner P. Not all fat is alike. *The Lancet*. 1998;351(9112):1301-1302.  
36  
37 494 29. Foster MT, Pagliassotti MJ. Metabolic alterations following visceral fat removal and expansion: Beyond anatomic  
38 495 location. *Adipocyte*. 2012;1(4):192-199.  
39  
40 496 30. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for  
41 497 obesity complications. *Mol Aspects Med*. 2013;34(1):1-11.  
42  
43 498 31. Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal-weight and  
44 499 obese humans. *N Engl J Med*. 1996;334(5):292-295.  
45  
46 500 32. Cnop M, Havel PJ, Utzschneider KM, *et al.* Relationship of adiponectin to body fat distribution, insulin sensitivity  
47 501 and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia*. 2003;46(4):459-469.  
48  
49 502 33. Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. *Nat Rev*  
50 503 *Endocrinol*. 2017;13(9):509-520.

- 1  
2  
3 504 34. Stefan N, Haring H-U. The role of hepatokines in metabolism. *Nat Rev Endocrinol*. 2013;9(3):144-152.  
4  
5 505 35. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest*. 2011;121(6):2126-2132.  
6  
7 506 36. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*.  
8 507 2012;489(7415):242-249.  
9  
10 508 37. Torres-Fuentes C, Schellekens H, Dinan TG, *et al*. The microbiota-gut-brain axis in obesity. *Lancet Gastroenterol*  
11 *Hepatol*. 2017;2(10):747-756.  
12 509  
13  
14 510 38. Wang HX, Wang YP. Gut Microbiota-brain Axis. *Chin Med J (Engl)*. 2016;129(19):2373-2380.  
15  
16 511 39. Aron-Wisnewsky J, Dore J, Clement K. The importance of the gut microbiota after bariatric surgery. *Nat Rev*  
17 *Gastroenterol Hepatol*. 2012;9(10):590-598.  
18 512  
19 513 40. Peat CM, Kleiman SC, Bulik CM, *et al*. The Intestinal Microbiome in Bariatric Surgery Patients. *Eur Eat Disord Rev*.  
20 514 2015;23(6):496-503.  
21  
22  
23 515 41. Cipolla MJ. Chapter 5: Control of Cerebral Blood Flow. *The Cerebral Circulation. Integrated Systems Physiology:*  
24 *From Molecule to Function*. San Rafael (CA)2009. p. 29-36.  
25 516  
26  
27 517 42. Willeumier KC, Taylor DV, Amen DG. Elevated BMI is associated with decreased blood flow in the prefrontal cortex  
28 518 using SPECT imaging in healthy adults. *Obesity (Silver Spring)*. 2011;19(5):1095-1097.  
29  
30 519 43. Alosco ML, Spitznagel MB, Raz N, *et al*. Obesity interacts with cerebral hypoperfusion to exacerbate cognitive  
31 520 impairment in older adults with heart failure. *Cerebrovasc Dis Extra*. 2012;2(1):88-98.  
32  
33  
34 521 44. Wierenga CE, Hays CC, Zlatar ZZ. Cerebral blood flow measured by arterial spin labeling MRI as a preclinical  
35 522 marker of Alzheimer's disease. *J Alzheimer's Dis*. 2014;42 (Suppl 4):S411-419.  
36  
37  
38 523 45. Alsop DC, Detre JA, Golay X, *et al*. Recommended implementation of arterial spin-labeled perfusion MRI for  
39 524 clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia.  
40 525 *Magn Reson Med*. 2015;73(1):102-116.  
41  
42  
43 526 46. Kullmann S, Callaghan MF, Heni M, *et al*. Specific white matter tissue microstructure changes associated with  
44 527 obesity. *Neuroimage*. 2016;125:36-44.  
45  
46  
47 528 47. Debette S, Wolf C, Lambert JC, *et al*. Abdominal obesity and lower gray matter volume: a Mendelian  
48 529 randomization study. *Neurobiol Aging*. 2014;35(2):378-386.  
49  
50 530 48. Karlsson HK, Tuulari JJ, Hirvonen J, *et al*. Obesity is associated with white matter atrophy: a combined diffusion  
51 531 tensor imaging and voxel-based morphometric study. *Obesity (Silver Spring)*. 2013;21(12):2530-2537.  
52  
53  
54 532 49. Arnoldussen IAC, Wiesmann M, Pelgrim CE, *et al*. Butyrate restores HFD-induced adaptations in brain function and  
55 533 metabolism in mid-adult obese mice. *Int J Obes (Lond)*. 2017;41(6):935-944.  
56  
57  
58 534 50. Tuulari JJ, Karlsson HK, Antikainen O, *et al*. Bariatric Surgery Induces White and Grey Matter Density Recovery in  
59 535 the Morbidly Obese: A Voxel-Based Morphometric Study. *Hum Brain Mapp*. 2016;37(11):3745-3756.

- 1  
2  
3 536 51. Zhang Y, Ji G, Xu M, *et al.* Recovery of brain structural abnormalities in morbidly obese patients after bariatric  
4  
5 537 surgery. *Int J Obes (Lond)*. 2016;40(10):1558-1565.
- 6 538 52. Fried M, Hainer V, Basdevant A, *et al.* Interdisciplinary European Guidelines on Surgery of Severe Obesity. *Obes*  
7  
8 539 *Facts*. 2008;1(1):52-59.
- 10 540 53. Nasreddine Z, Philips NA, Bédirian V, *et al.* The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For  
11  
12 541 Mild Cognitive Impairment. *J Am Geriatr Soc*. 2005;53(4):695-699.
- 14 542 54. Zimmerman P, Fimm B. Test for Attentional Performance (TAP), Manual. *Würselen, Germany: Psytest*. 1994.
- 16 543 55. Wechsler D. Wechsler adult intelligence scale-Fourth Edition (WAIS-IV). *San Antonio, TX: NCS Pearson*.  
17  
18 544 2008;22:498.
- 19 545 56. Schmand B, Groenink, S.C., van den Dungen, M. Letterfluency: psychometrische eigenschappen en Nederlandse  
20  
21 546 normen. *Tijdschr Gerontol Geriatr*. 2008;39(2):64-76.
- 23 547 57. Wilson B, Cockburn J, Baddeley A. Rivermead Behavioural Memory Test. London: Thames Valley Test Company;  
24  
25 548 1985.
- 27 549 58. Schmand B, Bakker D, Saan R, *et al.* The Dutch Reading Test for Adults: a measure of premorbid intelligence level.  
28  
29 550 *Tijdschr Gerontol Geriatr*. 1991;22(1):15-19.
- 30 551 59. Verhage F. Intelligentie en leeftijd: Onderzoek bij Nederlanders van twaalf tot zevenenzeventig jaar. Assen: Van  
31  
32 552 Gorcum; 1964.
- 34 553 60. Baecke JA, Burema, J., Frijters, J.E. A short questionnaire for the measurement of habitual physical activity in  
35  
36 554 epidemiological studies. *Am J Clin Nutr*. 1980;36(5):936-942.
- 38 555 61. Beck AT, Ward CH, Mendelson M, *et al.* An inventory for measuring depression. *Arch Gen Psychiatry*. 1961;4:561-  
39  
40 556 571.
- 41 557 62. Ware JE, Sherbourne, C.D. The MOS 36-Item Short-Form Health Survey (SF-36): I. Conceptual Framework and Item  
42  
43 558 Selection. *Medical Care*. 1992;30(6):473-483.
- 45 559 63. Oria HE, Moorehead M.K. . Bariatric analysis and reporting outcome system (BAROS). *Obes Surg*. 1998;8(5):487-  
46  
47 560 499.
- 48 561 64. Patton JH, Stanford MS, Barratt ES. Factor structure of the Barratt impulsiveness scale. *J Clin Psychol*.  
49  
50 562 1995;51(6):768-774.
- 52 563 65. Carver CS, White T.L. . Behavioral Inhibition, Behavioral Activation, and Affective Responses to Impending Reward  
53  
54 564 and Punishment: The BIS/BAS Scales. *J Pers Soc Psychol*. 1994;67(2):319-333.
- 56 565 66. Michaud A, Vainik U, Garcia-Garcia I, *et al.* Overlapping Neural Endophenotypes in Addiction and Obesity.  
57  
58 566 *Frontiers in endocrinology*. 2017;8:127.

- 1  
2  
3 567 67. Meule A, Hofmann J, Weghuber D, *et al*. Impulsivity, perceived self-regulatory success in dieting, and body mass in  
4 568 children and adolescents: A moderated mediation model. *Appetite*. 2016;107:15-20.  
5  
6 569 68. ICH harmonised tripartite guideline for good clinical practice: Brookwood Medical Publications Ltd; 1996.  
7  
8 570 69. Bretz F, Maurer W, Brannath W, *et al*. A graphical approach to sequentially rejective multiple test procedures. *Stat*  
9 571 *Med*. 2009;28(4):586-604.  
10  
11  
12 572 70. Hoffstedt J, Andersson DP, Eriksson Hogling D, *et al*. Long-term Protective Changes in Adipose Tissue After Gastric  
13 Bypass. *Diabetes Care*. 2017;40(1):77-84.  
14 573  
15  
16 574

For peer review only

1  
2  
3 575 **FIGURE LEGEND**  
4

5 576

6  
7 577 **Figure 1.** Overview of the study design. Blood samples are taken during a regular blood withdrawal at  
8  
9 578 six time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS).  
10  
11 579 Microbiota analyses will be performed at set time points on the faeces (collected at home by the  
12  
13 580 patients) (4-8 wks. pre BS, 1 wk. pre BS, 6 mo. post BS, 24 mo. post BS) and mucosal swabs (collected  
14  
15 581 during surgery). Furthermore, biopsies of liver, jejunum and adipose tissue depots (subcutaneous,  
16  
17 582 mesenteric and omental) will be collected during surgery. Before surgery, (4-8 wks. pre BS) and at  
18  
19 583 several time points after, (6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS) a medical  
20  
21 584 evaluation will take place and all patients will complete questionnaires and neuropsychological  
22  
23 585 measurements to test cognitive function. A subgroup of patients (N=75) will be examined with MRI  
24  
25 586 (4-8 wks. pre BS and 24 mo. post BS). MRI; magnetic resonance imaging. BS; Bariatric surgery. Wks;  
26  
27 587 weeks. Mo; months. Yrs; years.  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



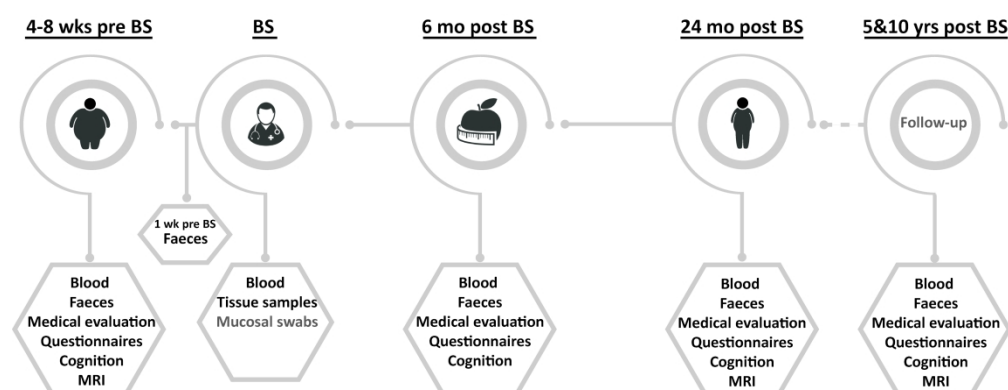
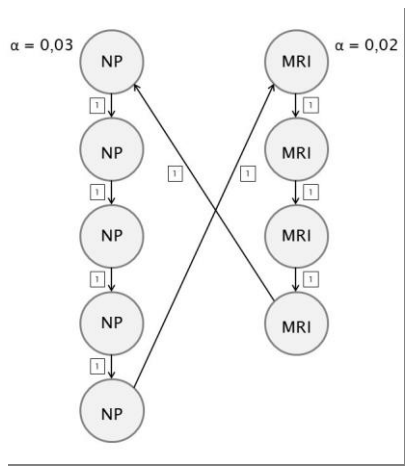


Figure 1. Overview of the study design. Blood samples are taken during a regular blood withdrawal at six time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota analyses will be performed at set time points on the faeces (collected at home by the patients) (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.

1  
2  
3 1 **SUPPLEMENTARY MATERIAL**  
4  
5  
6 2  
7

8 3 Since multiple outcome measures will be studied, correction for this is applied using the sequentially  
9  
10 4 rejective multiple testing procedure described in Bretz *et al.* (2008)(69). As we are highly interested  
11  
12 5 in both the neuropsychological tests and the MRI parameters, the MRI parameters and the  
13  
14 6 neuropsychological parameters are clustered. A significance level of 0.05 is used, and an alpha level  
15  
16 7 of 0.03 is allocated to the neuropsychological tests and 0.02 to the MRI parameters. The  
17  
18 8 neuropsychological tests and neuroimaging tests will be tested with a multiple testing procedure  
19  
20 9 (supplementary figure 1). The neuropsychological tests will initially be tested at 3/5 of the overall  
21  
22 10 type I error rate (i.e. 0.03 two-sided) and neuroimaging parameters at 2/5 of it (i.e. 0.02 two-sided).  
23  
24 11 Alpha will be reallocated when shown that the corresponding hypothesis is rejected. Based on the  
25  
26 12 literature a specific hypothesis sequence will be tested (the sequence for the neuropsychological  
27  
28 13 tests is: digit span, TAP flexibility task, story immediate/delayed recall, verbal fluency and MoCA; for  
29  
30 14 the MRI parameters: DTI parameters, ASL measures, BOLD response of the Stroop test and grey and  
31  
32 15 white matter volumes). Within each test separately correction for multiple testing will be included,  
33  
34 16 for example for multiple brain areas analysed within a MRI parameter.  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

18



19

20

21 **FIGURE LEGEND**

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

24