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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.

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Keywords:	Obesity, Weight loss, Bariatric surgery, Neuroimaging, Cognition

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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
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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 **Rijnstate** and **Radboudumc neuroImaging** and
42 **Cognition in Obesity**) 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**

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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/m²). (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)

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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
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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)

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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,
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21 113 leukocyte cell-derived chemotaxin, fetuin B and hepassocin, which may indirectly affect brain
22
23 114 function and structure.(31, 32)

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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
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31 118 signalling to the brain. Gut microbiota may also affect the brain by producing (precursors of)
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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
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51 128 levels of CBF in the prefrontal cortex are associated with reduced performance on tests of executive
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53 129 function and episodic memory.(40, 41) Even in the prodromal stages of Alzheimer's disease, changes
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3 130 in CBF can be detected with perfusion MRI (arterial spin labelling; ASL), which may be used as a very
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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)

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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.

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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
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50 153 values and gut microbiota composition. Lastly, metabolic and histopathological parameters (at the
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52 154 time-point of the surgery) will be obtained to study whether associations or correlations exist
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54 155 between obesity-associated metabolic dysfunction of particular organs and brain function and
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3 156 structure. To our knowledge this is the first study in humans that investigates brain structure and
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5 157 function changes after BS-induced weight loss and possible linkage between adipose tissue, liver
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7 158 function and the gut microbiome. Additionally, this is the first study in bariatric research combining
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9 159 neuroimaging, cognition and extensive profiling of biological markers.
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13 161 The primary aim of the BARICO study (**BA**riatric surgery **Rijn**state and Radboudumc neuro**I**maging
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15 162 and **C**ognition in **O**besity) is to determine the long-term effect of weight loss after bariatric surgery
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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
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23 166 provide information that can be used to characterize the pathological state of organs, and eventually
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25 167 monitor this state via molecular signatures in the circulation. It will also provide information to
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27 168 stratify obese patients based on specific molecular signatures and pathways into risk groups
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29 169 regarding a particular organ dysfunction (mechanism-based subgroups). This study will therefore
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31 170 contribute to the development of better health campaigns, health care and preventatives to
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33 171 attenuate the impact of obesity. This paper describes the design and protocol of the BARICO study.
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38 173 **METHODS AND ANALYSIS**

39 174 **Study population**

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42 175 Patients who have already been screened and found eligible for BS based on the Fried guidelines will
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44 176 be asked to participate.(50) Totally, 150 patients will be included in the study. Study specific inclusion
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46 177 criteria are: (a) patients willing to perform neuropsychological tests and complete self-report
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48 178 questionnaires and sign an informed consent document; (b) age between 35 and 55 years; (c)
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50 179 patients must undergo Roux-en-Y gastric bypass (RYGB). Exclusion criteria for this study are: (a)
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52 180 previous or current neurological or severe psychiatric illness; (b) pregnancy; (c) treatment with any
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54 181 antibiotics, probiotics, or prebiotics three months before or during the study (excluding preoperative
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3 182 prophylaxis). A subgroup of 75 patients will be included in the MRI sub-study, extra inclusion criteria
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5 183 for this group are: (d) patients willing to undergo MRI scanning and perform tasks in the MRI scanner;
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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)
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11 186 nerve stimulators; (h) intracranial clips; (i) infraorbital or intraocular metallic fragments; (j) cochlear
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13 187 implants; (k) ferromagnetic implants; (l) lying circumference above the MRI space capacity; (m)
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15 188 colour blindness. The study has been approved by the medical research ethics committee CMO
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17 189 Region Arnhem-Nijmegen (NL63493.091.17) and is registered at the Netherlands Trial Register
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19 190 (trialregister.nl) 7288.
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23 192 **Study design**

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26 193 At different time points (4-8 weeks preoperative, 6, 24 months and 5, 10 years postoperative, figure
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28 194 1) several cognitive tests and questionnaires will be assessed. Furthermore, fasting blood and faecal
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30 195 matter will be collected in all patients (N=150) (blood at all time points, faeces 4-8 weeks
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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,
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36 198 length and weight will be assessed at each time point. A subgroup of patients (N=75) will additionally
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38 199 receive a (f)MRI scan 4-8 weeks preoperative and 2 years postoperative. During the whole study
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40 200 period (ten years) patients will be contacted by letter and via telephone at least once a year to assure
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42 201 the best follow up rate.
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46 203 **Recruitment procedures and consent**

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49 204 Patients are informed about the study by mail and telephone at least one week prior to their
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51 205 standard information visit (four to eight weeks before RYGB surgery). During this visit, patients will
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53 206 individually receive more information about this study and its objectives. Afterwards, the researchers
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55 207 will further clarify the study and the patients can ask for additional information. If they decide to
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3 208 participate and fulfil the inclusion criteria, informed consents will be signed. Although the obese
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5 209 population consists of more females than males, the aim is an equal sex distribution during the
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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

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13 213 **Outcome measures**

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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
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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
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31 222 information on the pathological state of liver, gut and adipose tissue and circulating mediators from
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33 223 corresponding plasma/serum samples obtained prior to and at surgery will provide insight into organ
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35 224 cross-talk and allow identification of biomarker signatures for metabolic health. Differences in
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37 225 metabolic health of the subjects may be associated with specific signalling molecule-profiles, which
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39 226 may be related to cognitive function.
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45 228 **(f)MRI**

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47 229 Subjects will be scanned in a 3T MAGNETOM Skyra MR scanner (Siemens AG, Healthcare Sector,
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49 230 Erlangen, Germany) using a 32-channel head coil. The MRI protocol included a T1-weighted 3D
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51 231 magnetization-prepared rapid gradient-echo (MPRAGE) sequence for anatomical reference and
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53 232 analysis (TR/TI/TE 2300/1100/3.03 ms; 8° flip angle; voxel size: 1.0 × 1.0 × 1.0 mm), a fluid-
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55 233 attenuated inversion recovery (FLAIR) sequence for white matter lesion visualization (TR/TI

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3 234 5000/1800 ms; voxel size: 1.0 × 1.0 × 1.0 mm), diffusion-weighted MRI scans using simultaneous
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5 235 Multi-slice echo planar imaging for probing microstructural properties (TR/TE 3275/91.4 ms; voxel
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7 236 size: 1.9 × 1.9 × 1.9 mm; 6x b=0 s/mm², 42x b=900 s/mm², 83x b=1800 s/mm²). To allow for offline
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9 237 distortion correction of the images, 7 more b=0 s/mm² volumes will be acquired using the exact same
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11 238 sequence parameters except for the inverted k-space read-out trajectory. An arterial-spin labelling
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13 239 sequence will be used for quantification of cerebral blood flow (TR/TE 2500/12 ms; voxel size: 4.0 × 4.0
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15 240 × 4.0 mm) and a multi-band, multi-echo planar imaging sequence will be used to measure blood
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17 241 oxygen level dependent (BOLD) contrast during the Stroop task (TR/TE 1500/12.4, 34.3, 56.2 ms; 75°
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19 242 flip angle; voxel size: 2.5 × 2.5 × 2.5 mm; field of view 210 mm; 51 transversal slices in interleaved
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21 243 order). The complete scanning protocol takes 45 minutes. For both time points, the same MR
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23 244 scanner, head coil and sequences will be used. Following the project MRI quality assurance is
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25 245 guaranteed by regular phantom measurements.
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30 247 **Cognitive assessment**

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32 248 Cognitive performance of all participants will be tested using an extensive neuropsychological test
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34 249 battery as detailed below. To assess general cognitive performance the Montreal Cognitive
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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
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40 252 attention between objects, since paying attention is not a static process. Working memory will be
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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
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50 257 letters within 60 seconds (for each letter). Episodic memory will be assessed via the immediate and
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52 258 delayed Story Recall subtest from the Rivermead Behavioural Memory Test (RBMT).(55) To control
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54 259 and correct for differences in premorbid intelligence between participants, verbal IQ will be
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3 260 estimated using the Dutch version of the National Adult Reading Test (NART) at baseline.(56) The
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5 261 MoCA, episodic memory test and COWAT have parallel versions, to avoid material-specific learning
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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
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13 265 episodic memory, attention, verbal fluency and executive function. Also, education level will be
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15 266 recorded in accordance with the Dutch education system using 7 categories (1 being the lowest level
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17 267 of education and 7 being the highest).(57)
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269 **Assessment of biological measurements**

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

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

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 molecular,
283 biochemical level. The different tissues collected will be subcutaneous, mesenteric and omental
284 adipose tissue, liver and jejunum. Tissue biopsies from these organs will be taken to assess potential
285 pathophysiological processes and to eventually define mechanism-based subgroups.

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

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

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9 314 **Sample size**

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11 315 The power calculation for the neuropsychological tasks is based on the results of the Digit Span
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13 316 subtest performed in a comparable study population.(17) With an expected standardized effect size
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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.

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34 326 **Analysis of primary outcome measures**

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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
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48 333 measures, the sequentially rejective multiple testing procedure described in Bretz *et al.* will be
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50 334 used.(66)

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55 336 **Analysis of secondary outcome measures**

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3 337 As secondary outcome measures, the metabolic and histopathological parameters (obtained analyses
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5 338 from tissues collected during the surgery) will be analysed cross-sectionally to examine correlations
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7 339 between these metabolic and histopathological parameters among each other and in relation to
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9 340 brain function and structure. Furthermore, potential mechanisms underlying the crosstalk along the
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11 341 gut-brain axis will be investigated by longitudinal analyses focusing on establishing correlations
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13 342 between brain structure/function changes and changes in circulation mediators or faecal microbiota
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15 343 composition. Pearson correlation analysis will be used to investigate potential correlations between
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17 344 variables.
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20 21 346 **Data monitoring**

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23 347 Every year, data monitoring and auditing will be conducted by an independent specialized monitor of
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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.
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30 31 351 **DISCUSSION**

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33 352 The BARICO study is a longitudinal, prospective study focusing on the effect of weight loss after BS on
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35 353 cognitive function and brain structure, measured with sensitive neuropsychological tests covering
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37 354 the most important domains, fMRI activation during the Stroop task, and several MRI techniques,
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39 355 such as DTI and ASL. To clarify the impact of metabolic dysfunction in obesity on brain function and
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41 356 structure, blood plasma and stool samples will be collected and analysed longitudinally as well as
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43 357 biopsies of key metabolic organs which will be collected during the RYGB and analysed cross-
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45 358 sectionally.
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48 359 Limited studies demonstrated improvement in several cognitive domains such as memory, attention
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50 360 and executive function after BS.(16, 17) Furthermore, obese individuals seem to have lower grey and
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52 361 white matter volumes and altered white matter densities. Several studies show rapid recovery of
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54 362 these brain structural abnormalities after BS(48, 49): for instance, Tuulari *et al.* showed a causal link
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3 363 between weight loss and brain tissue recovery.(48) Approximately 25-30% of the patients is expected
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5 364 not to reach sufficient weight loss (<50% excess weight loss) and thus it will be possible to study the
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7 365 effect of weight loss after BS on brain function and structure.

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

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19 371 Another strength of this study is the combination of neuroimaging and neuropsychological tests. In
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21 372 combination with the analysed metabolic and histopathological parameters (obtained in blood,
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23 373 organ biopsies and microbiota), the relation between multiple metabolic parameters can be
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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
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29 376 communication and may lead to beneficial alterations in adipose function, recovery of brain function
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31 377 and structure may be expected.(15, 67) Longitudinal analyses of the microbiota together with
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33 378 analysis of functional gut-derived metabolites in the circulation and cognitive outcomes may allow
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35 379 identification of mediators derived from the gut microflora that are relevant for cognition and
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37 380 prevention of cognitive decline.

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

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

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55 388 **ETHICS AND DISSEMINATION**

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3 389 The study protocol was authorized by the medical review ethics committee CMO Region Arnhem and
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5 390 Nijmegen (NL63493.091.17). All patients will sign informed consent on enrolment in the study. Study
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7 391 results of the study will be submitted for publication in peer-reviewed journals.
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4
5 393 Not applicable.

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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.

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19 400

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26
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30
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33 407

34
35 408 **Competing interests**

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

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40
41 411 **Patient consent**

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43 412 Obtained

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45 413

46
47 414 **Ethics approval**

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

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

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

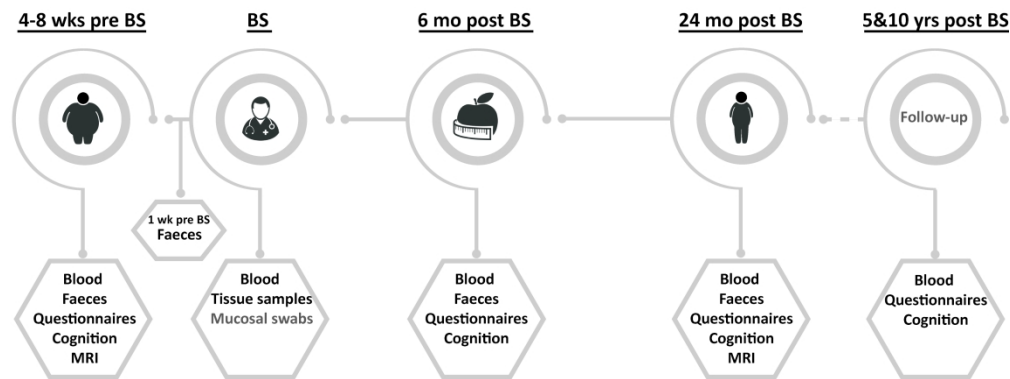


Figure 1. Overview of the study design. Blood samples are taken during regular blood withdrawal at 6 time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota analyses will be performed at set time points on the faeces collected at home by the patients (4-8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

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Secondary Subject Heading:	Research methods, Nutrition and metabolism, Radiology and imaging, Surgery
Keywords:	Obesity, Weight loss, Bariatric surgery, Neuroimaging, Cognition

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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.**

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8 4 Vreeken, D.^{1,2,3}, Wiesmann, M.³, Deden, L.N.^{1,2}, Arnoldussen, I.A.C.³, Aarts, E.⁴, Kessels, R.P.C.^{4,5,6},
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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 Radboudumc 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**

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3 57 This study has been approved by the medical review ethics committee CMO Region Arnhem and
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5 58 Nijmegen (NL63493.091.17). Research findings will be published in peer-reviewed journals and at
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7 59 conferences.
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12 61 **Trial registration**

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14 62 The Netherlands National Trial Register (trialregister.nl) 7288. Date registered: 29-June-2018.
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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/m²). (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

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3 100 cognitive decline and increase risk for developing dementia. However, mechanisms involved in this
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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
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9 103 organ releasing several hormones, proteins and cytokines, referred to as adipokines. Obesity is
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11 104 associated with dysfunctional white adipose tissue and therefore an imbalance in adipokines, such as
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13 105 increased levels of leptin and angiotensinogen, and low levels of adiponectin and omentin.(25, 26)
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15 106 Especially, visceral adipose tissue seems to produce unfavourable adipokines associated with more
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17 107 metabolic complications when compared to subcutaneous adipose tissue.(27-30) Importantly, the
18
19 108 distribution of fat tissue depots differs between sexes. Overall, men accumulate more abdominal and
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21 109 visceral fat than women.(30) Moreover, women have a higher level of adipokines such as leptin and
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23 110 adiponectin.(31, 32) This disbalance in adipokines may induce inflammation in several organs such as the
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25 111 liver, gut and vascular endothelium. The latter causing atherosclerosis, ultimately leading to changes in
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27 112 cerebral blood flow (CBF).(25)

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32 113 Secondly, signalling between, and within other organs, such as the liver, might be altered in obese
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34 114 patients. For example; the liver secretes hepatokines, such as insulin-like growth factor 1, selenoprotein
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36 115 P, leukocyte cell-derived chemotaxin, fetuin B and hepassocin, which may indirectly affect brain function
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38 116 and structure.(33, 34)

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40 117 Thirdly, the gut microbiota composition in obese people differs from that of non-obese individuals,
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42 118 affecting metabolic processes, weight and obesity-related comorbidities.(35, 36) Microbiota is involved
43
44 119 in adiposity and homeostasis but also influences energy balance via appetite and satiety signalling to the
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46 120 brain. Gut microbiota also affect the brain by producing (precursors of) neurotransmitters and short
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48 121 chain fatty acids, or through cytokines via the immune system.(37, 38) BS leads to a fast change in gut
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50 122 microbiota composition through changes in food intake, intestinal modifications due to the surgery itself,
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52 123 and metabolic improvements, eventually leading to changes in gut-brain communication.(15, 39, 40)

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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)
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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
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14 129 cortex are associated with reduced performance on executive function and episodic memory tests.(42,
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16 130 43) Even in the prodromal stages of Alzheimer's disease, changes in CBF can be detected with arterial
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18 131 spin labelling (ASL), which may be used as a very early biomarker for neurodegenerative
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20 132 disorders.(44) However, the technique requires further optimization and therefore several consortia are
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22 133 working on the implementation of ASL perfusion magnetic resonance imaging (MRI) for clinical
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24 134 applications to provide images of sufficient and diagnostic utility.(45)
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27 135 Furthermore, obesity is associated with changes in grey and white matter, which can be visualized using
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29 136 diffusion tensor imaging (DTI) and voxel-based morphometry analyses based on T1 weighted scans.(46,
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31 137 47) These structural changes are especially prominent in brain regions governing reward seeking,
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33 138 inhibitory control and appetite.(48, 49) There are indications that rapid recovery of structural
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35 139 abnormalities occur after BS, however long-term study data is lacking here. (50, 51)
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39 140 Additionally, impairment in attention span, executive function and memory are commonly reported in
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41 141 obese patients.(18, 19) Cognitive impairment revealed in obesity might be reversible and varies between
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43 142 cognitive domains however long-term follow-up studies are scarce. The Longitudinal Assessment of
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45 143 Bariatric Surgery (LABS) parent project is the most extensive longitudinal study to date focusing on
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47 144 cognitive changes in patients after BS. Investigators showed lasting improvements three years after
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49 145 surgery in the cognitive domains of attention, executive function and memory.(19)
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54 147 **Rationale**
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3 148 Cognitive benefits after BS are not equally exhibited across patients and cognitive domains. However,
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5 149 precise causes are still poorly understood, and underlying molecular mechanisms remain elusive. From
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7 150 the relatively short follow-up duration and small samples of BS patients in the studies reviewed, it is
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10 151 difficult to elaborate on the long-term consequences of obesity and its related diseases. In this study,
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12 152 underlying mechanisms of obesity-related cognitive disorders will be investigated by longitudinal studies
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14 153 correlating cognition to brain changes, blood serum and plasma values, and gut microbiota composition.
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16 154 Lastly, metabolic and histopathological parameters (at the time-point of surgery) will be obtained to
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18 155 study whether associations or correlations exist between obesity-associated metabolic dysfunctions of
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20 156 particular organs and brain function and structure. To our knowledge this is the first study in humans
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22 157 investigating changes in brain structure and function, and changes in adipose tissue, liver function and
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24 158 the gut microbiome, after BS-induced weight loss. Additionally, this is the first study in bariatric research
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26 159 combining neuroimaging, cognition and extensive profiling of biological markers.
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32 161 The primary aim of the BARICO study (**BA**riatric surgery **Rijn**state and **Rad**boudumc neuro**I**maging and
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34 162 **C**ognition in **O**besity) is to determine the long-term effect of weight loss on measures of brain function
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36 163 and structure after BS. The secondary aim is to provide mechanism-based rationales responsible for
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38 164 functional and structural decline in obese individuals. Therefore, the metabolic and inflammation status
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40 165 of organ biopsies will be determined together with molecular signatures via blood plasma/serum
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42 166 analyses. Furthermore, gut microbiota composition will be monitored over time to gain knowledge about
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44 167 the gut-brain axis.
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48 168 This study will contribute to the development of better health campaigns, healthcare and preventatives
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50 169 to attenuate the impact of obesity. This paper describes the design and protocol of the BARICO study.
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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.(52) 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.

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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)).

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3 196 Intraoperatively, several tissue biopsies will be collected and processed. Medical evaluation, including
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5 197 anthropometric measurements and information on comorbidities, will be assessed 4-8 weeks
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7 198 preoperative and during all postoperative time points. A schematic overview of the study is shown in
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10 199 figure 1. A subgroup of patients (N=75) will additionally receive a (f)MRI scan 4-8 weeks preoperative and
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12 200 24 months postoperative. During the whole study period (10 years) patients will be contacted by letter
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14 201 and via telephone at least once a year to ensure the best follow-up rate.
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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
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21 205 visit (4-8 weeks before RYGB surgery). During this visit, patients will individually receive more
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23 206 information about this study and its objectives. Afterwards, the researchers will further clarify the study
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25 207 and the patients can ask for additional information. If they decide to participate and fulfil the inclusion
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27 208 criteria, informed consents will be obtained. Although the obese population consists of more females
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29 209 than males, the aim is for an equal sex distribution during the recruitment period (i.e., a study population
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31 210 consisting of >30% men and >30% women).(1) Recruitment will take place between August 2018 and
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40 213 **Outcome measures**

41 214 The primary outcome measures are the neuropsychological tests scores, CBF values, hippocampal
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43 215 volume, mean diffusivity (MD) and fractional anisotropy (FA) (representing respectively grey and white
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45 216 matter integrity), and blood oxygen level dependent (BOLD) responses during the Stroop task.
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47 217 Combining neuroimaging and neuropsychological tests will give us more information on how and
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49 218 whether structural brain changes are related to functional brain changes. Secondary measures comprise
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51 219 of (histopathological and biochemical determined) health status of the collected tissue, gut microbiota
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3 220 composition changes (in jejunal mucosa and faeces) and the profiling of circulating mediators in blood
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5 221 (plasma and serum), as well as lifestyle and dietary habits in relation to cognitive function and brain
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7 222 structure. Combining information on the pathological state of liver, gut, adipose tissue and circulating
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9 223 mediators from corresponding plasma/serum samples, obtained prior to and at surgery, will provide
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11 224 insight into organ cross-talk and allow identification of biomarker signatures for metabolic health.
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14 225 Differences in metabolic health of the subjects may be associated with specific signalling molecule-
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16 226 profiles, which may be related to cognitive function.
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21 228 **(f)MRI**

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23 229 Subjects will be scanned in a 3T MAGNETOM Skyra MR scanner (Siemens AG, Healthcare Sector,
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25 230 Erlangen, Germany) using a 32-channel head coil. The MRI protocol included: a T1-weighted 3D
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27 231 magnetization-prepared rapid gradient-echo (MPRAGE) sequence for anatomical reference and analysis
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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
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31 233 recovery (FLAIR) sequence for white matter lesion visualization (TR/TI 5000/1800 ms; voxel size: 1.0 × 1.0
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33 234 × 1.0 mm), and diffusion-weighted MRI scans using simultaneous multi-slice echo planar imaging for
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35 235 probing microstructural properties (TR/TE 3275/91.4 ms; voxel size: 1.9 × 1.9 × 1.9 mm; 6x b=0 s/mm²,
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37 236 42x b=900 s/mm², 83x b=1800 s/mm²). To allow for offline distortion correction of the images, 7 more
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39 237 b=0 s/mm² volumes will be acquired using the exact same sequence parameters - except for the inverted
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41 238 k-space read-out trajectory. An ASL sequence will be used for quantification of CBF (TR/TE 2500/12 ms;
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43 239 voxel size: 4.0 × 4.0 × 4.0 mm) and a multi-band, multi-echo planar imaging sequence will be used to
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45 240 measure BOLD contrast during the Stroop task (TR/TE 1500/12.4, 34.3, 56.2 ms; 75° flip angle; voxel size:
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47 241 2.5 × 2.5 × 2.5 mm; field of view 210 mm; 51 transversal slices in interleaved order). The complete
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49 242 scanning protocol takes 45 minutes and for both time-points, the same: MR scanner, head coil, and
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3 243 sequences will be used. Following the project MRI quality assurance is guaranteed by regular phantom
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5 244 measurements.
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9 246 **Cognitive assessment**

10 247 Cognitive performance of all participants will be tested using an extensive neuropsychological test
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12 248 battery as detailed below. To assess general cognitive performance the Montreal Cognitive Assessment
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14 249 (MoCA) will be used.(53) To test attentional functions, the Flexibility subtest from the Tests of
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16 250 Attentional Performance (TAP 2.3) will be used.(54) This flexibility task focuses on shifting attention
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18 251 between objects. Working memory will be assessed via the Digit Span subtest from the Wechsler Adult
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20 252 Intelligence Scale Fourth Edition (WAIS-IV-NL).(55) Participants will have to repeat a series of digits in
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22 253 forward or backward order, or sort them numerically. The Controlled Oral Word Association Test
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24 254 (COWAT) will be used to determine verbal fluency.(56) Participants have to come up with as many words
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26 255 beginning with three designated letters within 60 seconds (for each letter). Episodic memory will be
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28 256 assessed via the immediate and delayed Story Recall subtest from the Rivermead Behavioural Memory
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30 257 Test (RBMT).(57) To control and correct for differences in premorbid intelligence between participants,
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32 258 verbal IQ will be estimated using the Dutch version of the National Adult Reading Test (NART) at
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34 259 baseline.(58) The MoCA, episodic memory test and COWAT have parallel versions, to avoid material-
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36 260 specific learning effects during the repeated testing. Additionally, the tests are standardized, have been
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38 261 validated for use across a wide age range and have good re-test reliability. Together these tests will
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40 262 provide a good overview on the overall cognitive performance of the patients, including aspects of
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42 263 working and episodic memory, attention, verbal fluency and executive function. Also, education level will
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44 264 be recorded in accordance with the Dutch education system using seven categories (one being the
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46 265 lowest level of education and seven being the highest).(59)
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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.

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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

291 and depressive symptoms will be assessed with the Beck Depression Inventory (BDI-II).(60, 61) To
292 estimate the participants' food/nutrient intake and eating behaviour 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 Short
294 Form 36 (SF-36).(62) Lastly, the results of BS will be evaluated via the Bariatric Analysis and Report
295 Outcome System (BAROS).(63)

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

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301 **Medical evaluation**

302 At several time points during the study (figure 1) a medical evaluation will take place where
303 anthropometric measurements such as: body weight, length, waist circumference and blood pressure
304 will be quantified. BMI will be calculated as weight divided by height in meters squared. Percentage
305 excess weight loss (%EWL) (defined as weight loss divided by preoperative excess weight, with excess
306 weight defined as the weight above a normal BMI of 25 kg/m²) will be calculated during the time points
307 after surgery, similar to percentage total body weight loss (%TBWL) (defined as weight loss divided by
308 preoperative weight). The success of BS in terms of weight loss will be defined as a sustained weight loss
309 larger than 50 %EWL.

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

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5 316 **Data management**
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7 317 Data management will be handled using Research Manager (RM, Cloud 9 Health Solutions©), an
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10 318 established software package and data management tool that follows Good Clinical Practice (GCP)
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12 319 guidelines.(68) Every change in the data is recorded in a log system and can be traced. Participants will
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14 320 be identified only by a study specific identification code. One researcher will keep a separate participant
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16 321 identification code list that matches the study-specific identifying codes with the participant's names.
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18 322 Documents will be maintained by the investigator in strict confidence.
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22 23 324 **Sample size**

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25 325 The power calculation for the neuropsychological tasks was based on the results of the Digit Span
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27 326 subtest performed in a comparable study population.(19) With an expected standardized effect size of at
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29 327 least 0.3 and a correlation of 0.7, a selected sample of 150 patients will be sufficient to reach 90% power.
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31 328 The power calculation for the MRI parameters is based on changes in the FA parameter studied by Zhang
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33 329 *et al.*(51) With an expected standardized effect size of at least 0.03 and a correlation of 0.5 including 75
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35 330 patients in the MRI group will be sufficient to reach 90% power. A significance level based on the
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37 331 sequentially rejective multiple testing procedure discussed by Bretz *et al.* (for the neuropsychological
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39 332 tests 3% and for the MRI parameters 2%) has been taken into account in the power calculation.(69) The
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41 333 inclusion of 150 patients with a subgroup of 75 for the MRI scan has been considered adequate to
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43 334 answer the research questions with sufficient power.
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50 336 **Analysis of primary outcome measures**

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52 337 As a primary outcome measure, baseline levels of the imaging parameters (such as MD and FA) will be
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54 338 compared with the results of the neuroimaging outcome 24 months after surgery (including %TBWL as a
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339 factor in the model). Next, the scores of the cognitive tests from five different time points will be
340 analysed and compared to %TBWL. Every dependent variable will be modelled in a separate linear mixed
341 model. %TBWL will be used as a factor. Different variables, such as: depression score, age, and gender,
342 will be (if appropriate) included in the model. For each model, we will decide which variables to include
343 as a factor to reduce the amount of unexplained variation. To correct for multiple outcome measures,
344 the sequentially rejective multiple testing procedure described in Bretz *et al.* will be used.(69) Data will
345 be analysed using SPSS (version 25 for Windows) and R (version 3.5.1 for Windows). For the cognitive
346 tests a p value of <0.03 and for the imaging parameters a p value of <0.02 will be considered as
347 statistically significant.

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

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

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3 363 **Patient and Public involvement**
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5 364 Patients and the public were not involved in the design of this study. Nevertheless, the results will be
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7 365 disseminated to the study participants via email, newsletters and social media platforms after the study
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9 366 results are published.
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14 368 **DISCUSSION**
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17 369 The BARICO study is a prospective study focusing on the effect of weight loss on cognitive function and
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19 370 brain structure after BS. This will be measured using sensitive neuropsychological tests covering the most
20
21 371 important domains, fMRI activation during the Stroop task, and several MRI techniques, such as DTI and
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23 372 ASL. To clarify the impact of metabolic dysfunction in obesity on brain function and structure, blood
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25 373 plasma and stool samples will be collected and analysed longitudinally, and biopsies of key metabolic
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27 374 organs will be collected during the RYGB and analysed cross-sectionally.
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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
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34 377 has been shown that obese individuals have lower grey and white matter volumes, and altered white
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36 378 matter densities, in comparison to healthy individuals with several studies showing a rapid recovery of
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38 379 these brain structural abnormalities after BS.(50, 51) For instance, Tuulari *et al.* showed a causal link
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40 380 between weight loss and brain tissue recovery.(50) Approximately 25-30% of the patients are not
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42 381 expected to reach sufficient weight loss (≤ 50 %EWL), and thus it will be possible to study the effect of
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44 382 weight loss after BS on brain function and structure.
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48 383 Perhaps the strength of this study is in the long follow-up duration after surgery: 24 months for the
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50 384 neuroimaging parameters, and 10 years for the neuropsychological tests. Furthermore, the strict
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52 385 inclusion criterion with respect to age range ensures a good representation of mid-life patients.
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3 386 Moreover, the majority of studies into BS patients are mostly composed of women but it is equally
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5 387 important to account for the variation in fat tissue distribution which is caused by differences in sex.(30)
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7 388 Another strength of this study is the combination of neuroimaging and neuropsychological tests.
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10 389 Alongside the analysis of metabolic and histopathological parameters (obtained in blood, organ biopsies
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12 390 and microbiota), meaning that the relation between multiple metabolic, neuroimaging and/or cognitive
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14 391 parameters can be investigated (e.g., adipokines, bioactive lipids (short-chain fatty acids) and organ
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16 392 dysfunction) in a comprehensive way. Since RYGB influences gut-brain communication, there may be
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18 393 beneficial alterations in adipose tissue functions, and/or recovery of brain function and structure
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20 394 following BS.(15, 70) Longitudinal analyses of the microbiota, together with analysis of functional gut-
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22 395 derived metabolites in the circulation and cognitive outcomes, may allow for the identification of
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24 396 mediators derived from gut microflora that are relevant to cognition and the prevention of cognitive
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28 397 decline.

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

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

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44 45 405 **ETHICS AND DISSEMINATION**

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48 406 The study protocol was authorized by the medical review ethics committee CMO Region Arnhem and
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50 407 Nijmegen (NL63493.091.17). All patients will sign informed consent forms upon enrolment in the study.

51
52 408 Study results will be submitted for publication in peer-reviewed journals.
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5 410 Not applicable.
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10 412 **Contributors**
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12 413 EOA and AJK conceived and designed the study. DV wrote the article and developed the protocol
13
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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.
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39 425 **Competing interests**
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41 426 The authors declare that they have no conflicts of interests.
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45 428 **Patient consent**
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47 429 Obtained
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52 431 **Ethics approval**
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54 432 Medical review ethics committee CMO Region Arnhem and Nijmegen (NL63493.091.17).
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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 plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia*. 2003;46(4):459-469.
5 494
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.
10 498 35. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest*. 2011;121(6):2126-2132.
11 499 36. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*.
12 500 2012;489(7415):242-249.
13 501 37. Torres-Fuentes C, Schellekens H, Dinan TG, *et al.* The microbiota-gut-brain axis in obesity. *Lancet Gastroenterol*
14 502 *Hepatol*. 2017;2(10):747-756.
15 503 38. Wang HX, Wang YP. Gut Microbiota-brain Axis. *Chin Med J (Engl)*. 2016;129(19):2373-2380.
16 504 39. Aron-Wisnewsky J, Dore J, Clement K. The importance of the gut microbiota after bariatric surgery. *Nat Rev*
17 505 *Gastroenterol Hepatol*. 2012;9(10):590-598.
18 506 40. Peat CM, Kleiman SC, Bulik CM, *et al.* The Intestinal Microbiome in Bariatric Surgery Patients. *Eur Eat Disord Rev*.
19 507 2015;23(6):496-503.
20 508 41. Cipolla MJ. Chapter 5: Control of Cerebral Blood Flow. *The Cerebral Circulation. Integrated Systems Physiology: From*
21 509 *Molecule to Function*. San Rafael (CA)2009. p. 29-36.
22 510 42. Willeumier KC, Taylor DV, Amen DG. Elevated BMI is associated with decreased blood flow in the prefrontal cortex
23 511 using SPECT imaging in healthy adults. *Obesity (Silver Spring)*. 2011;19(5):1095-1097.
24 512 43. Alosco ML, Spitznagel MB, Raz N, *et al.* Obesity interacts with cerebral hypoperfusion to exacerbate cognitive
25 513 impairment in older adults with heart failure. *Cerebrovasc Dis Extra*. 2012;2(1):88-98.
26 514 44. Wierenga CE, Hays CC, Zlatar ZZ. Cerebral blood flow measured by arterial spin labeling MRI as a preclinical marker of
27 515 Alzheimer's disease. *J Alzheimer's Dis*. 2014;42 (Suppl 4):S411-419.
28 516 45. Alsop DC, Detre JA, Golay X, *et al.* Recommended implementation of arterial spin-labeled perfusion MRI for clinical
29 517 applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. *Magn Reson*
30 518 *Med*. 2015;73(1):102-116.
31 519 46. Kullmann S, Callaghan MF, Heni M, *et al.* Specific white matter tissue microstructure changes associated with obesity.
32 520 *Neuroimage*. 2016;125:36-44.
33 521 47. Dobbie S, Wolf C, Lambert JC, *et al.* Abdominal obesity and lower gray matter volume: a Mendelian randomization
34 522 study. *Neurobiol Aging*. 2014;35(2):378-386.

- 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.
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3 564 **FIGURE LEGEND**
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7 566 **Figure 1.** Overview of the study design. Blood samples are taken during a regular blood withdrawal at six
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10 567 time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota
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12 568 analyses will be performed at set time points on the faeces (collected at home by the patients) (4-8 wks.
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14 569 pre BS, 1 wk. pre BS, 6 mo. post BS, 24 mo. post BS) and mucosal swabs (collected during surgery).
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16 570 Furthermore, biopsies of liver, jejunum and adipose tissue depots (subcutaneous, mesenteric and
17
18 571 omental) will be collected during surgery. Before surgery, (4-8 wks. pre BS) and at several time points
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20 572 after, (6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS) a medical evaluation will take place and
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22 573 all patients will complete questionnaires and neuropsychological measurements to test cognitive
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24 574 function. A subgroup of patients (N=75) will be examined with MRI (4-8 wks. pre BS and 24 mo. post BS).
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26 575 MRI; magnetic resonance imaging. BS; Bariatric surgery. Wks; weeks. Mo; months. Yrs; years.
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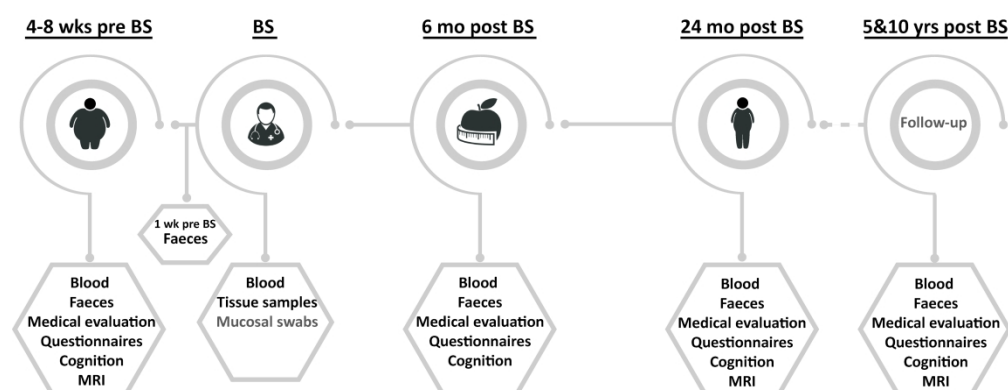


Figure 1. Overview of the study design. Blood samples are taken during a regular blood withdrawal at six time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota analyses will be performed at set time points on the faeces (collected at home by the patients)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

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Secondary Subject Heading:	Research methods, Nutrition and metabolism, Radiology and imaging, Surgery
Keywords:	Obesity, Weight loss, Bariatric surgery, Neuroimaging, Cognition

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Manuscripts

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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.**
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8 4 Vreeken, D.^{1,2,3}, Wiesmann, M.³, Deden, L.N.^{1,2}, Arnoldussen, I.A.C.³, Aarts, E.⁴, Kessels, R.P.C.^{4,5,6},
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39 29 **Short title: The BARICO study, effect of weight loss on brain function**

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43 32

44 33 **Keywords: obesity, weight loss, bariatric surgery, neuroimaging, cognition**
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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** neuro**I**maging and
42 **C**ognition in **O**besity) 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**

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3 59 This study has been approved by the medical review ethics committee CMO Region Arnhem and
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5 60 Nijmegen (NL63493.091.17). Research findings will be published in peer-reviewed journals and at
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7 61 conferences.
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12 63 **Trial registration**

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14 64 The Netherlands National Trial Register (trialregister.nl) 7288. Date registered: 29-June-2018.
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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/m²).(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

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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
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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)
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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)
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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)
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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
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50 126 in gut-brain communication.(15, 39, 40) Hence, metabolic organs, such as liver, gut and adipose
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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)
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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
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37 146 focusing on cognitive changes in patients after BS. Investigators showed lasting improvements three
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39 147 years after surgery in the cognitive domains of attention, executive function and memory.(19)
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149 Rationale

50 150 Cognitive benefits after BS are not equally exhibited across patients and cognitive domains. However,
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52 151 precise causes are still poorly understood, and underlying molecular mechanisms remain elusive.
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54 152 From the relatively short follow-up duration and small samples of BS patients in the studies
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56 153 reviewed, it is difficult to elaborate on the long-term consequences of obesity and its related
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58 154 diseases. In this study, underlying mechanisms of obesity-related cognitive disorders will be
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3 155 investigated by longitudinal studies correlating cognition to brain changes, blood serum and plasma
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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
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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,
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14 160 and changes in adipose tissue, liver function and the gut microbiome, after BS-induced weight loss.
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16 161 Additionally, this is the first study in bariatric research combining neuroimaging, cognition and
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18 162 extensive profiling of biological markers.
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22
23 164 The primary aim of the BARICO study (**BA**riatic surgery **Rijn**state and **Rad**boudumc **neuro**Imaging
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
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29 167 responsible for functional and structural decline in obese individuals. Therefore, the metabolic and
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31 168 inflammation status of organ biopsies will be determined together with molecular signatures via
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33 169 blood plasma/serum analyses. Furthermore, gut microbiota composition will be monitored over time
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35 170 to gain knowledge about the gut-brain axis.
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39 171 This study will contribute to the development of better health campaigns, healthcare and
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41 172 preventatives to attenuate the impact of obesity. This paper describes the design and protocol of the
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43 173 BARICO study.
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49 176 **METHODS AND ANALYSIS**

50 177 **Study population**

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52 178 Patients who have been screened and found eligible for BS based on the Fried guidelines will be
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54 179 asked to participate.⁽⁵²⁾ In total, 150 patients will be included in the study. Study specific inclusion
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56 180 criteria are: (a) patients willing to perform neuropsychological tests, complete self-report
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3 181 questionnaires and sign an informed consent document; (b) age between 35 and 55 years; (c)
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5 182 patients must undergo RYGB. A laparoscopic antecolic antegastric RYGB procedure will be performed
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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
11 186 preoperative prophylaxis). A subgroup of 75 patients will be included in the MRI sub-study, extra
12 187 inclusion criteria for this group are: (d) patients willing to undergo MRI scanning and perform tasks in
13 188 the MRI scanner; (e) right handed (more homogeneous sample and less variance). The standard
14 189 exclusion criteria for the MRI subgroup include: (d) claustrophobia; (e) epilepsy; (f) pacemakers and
15 190 defibrillators; (g) nerve stimulators; (h) intracranial clips; (i) infraorbital or intraocular metallic
16 191 fragments; (j) cochlear implants; (k) ferromagnetic implants; (l) circumference above the MRI space
17 192 capacity; (m) colour blindness. The study has been approved by the medical research ethics
18 193 committee CMO Region Arnhem-Nijmegen (NL63493.091.17) and is registered at the Netherlands
19 194 Trial Register (trialregister.nl) 7288.
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196 **Study design**

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39 197 At several time points (4-8 weeks preoperative, 6, 24 months and 5, 10 years postoperative (figure
40 198 1)) a number of cognitive tests and questionnaires will be performed, and their results assessed.
41 199 Furthermore, blood (after 8 hrs. period of fasting) and faecal matter will be collected from all
42 200 patients (N=150) (blood at all time points, faeces 4-8 weeks preoperative, 6 and 24 months
43 201 postoperative (figure 1)). Intraoperatively, several tissue biopsies will be collected and processed.
44 202 Medical evaluation, including anthropometric measurements and information on comorbidities, will
45 203 be assessed 4-8 weeks preoperative and during all postoperative time points. A schematic overview
46 204 of the study is shown in figure 1. A subgroup of patients (N=75) will additionally receive a (f)MRI scan
47 205 4-8 weeks preoperative and 24 months postoperative. During the whole study period (10 years)
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3 206 patients will be contacted by letter and via telephone at least once a year to ensure the best follow-
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5 207 up rate.
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10 209 **Recruitment procedures and consent**

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12 210 Patients are informed about the study by letter and telephone at least two weeks prior to their
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14 211 standard visit (4-8 weeks before RYGB surgery). During this visit, patients will individually receive
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16 212 more information about this study and its objectives. Afterwards, the researchers will further clarify
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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.,
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24 216 a study population consisting of >30% men and >30% women).(1) Recruitment will take place
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26 217 between August 2018 and August 2020.
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32 219 **Outcome measures**

33
34 220 The primary outcome measures are the neuropsychological tests scores, CBF values, hippocampal
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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.
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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
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44 225 comprise of (histopathological and biochemical determined) health status of the collected tissue, gut
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46 226 microbiota composition changes (in jejunal mucosa and faeces) and the profiling of circulating
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48 227 mediators in blood (plasma and serum), as well as lifestyle and dietary habits in relation to cognitive
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50 228 function and brain structure. Combining information on the pathological state of liver, gut, adipose
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52 229 tissue and circulating mediators from corresponding plasma/serum samples, obtained prior to and at
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54 230 surgery, will provide insight into organ cross-talk and allow identification of biomarker signatures for
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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/mm², 42x b=900 s/mm², 83x b=1800 s/mm²). To allow for offline
243 distortion correction of the images, 7 more b=0 s/mm² 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.⁽⁵³⁾ To test attentional functions, the Flexibility subtest from the
256 Tests of Attentional Performance (TAP 2.3) will be used.⁽⁵⁴⁾ This flexibility task focuses on shifting

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3 257 attention between objects. Working memory will be assessed via the Digit Span subtest from the
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5 258 Wechsler Adult Intelligence Scale Fourth Edition (WAIS-IV-NL).(55) Participants will have to repeat a
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7 259 series of digits in forward or backward order, or sort them numerically. The Controlled Oral Word
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10 260 Association Test (COWAT) will be used to determine verbal fluency.(56) Participants have to come up
11
12 261 with as many words beginning with three designated letters within 60 seconds (for each letter).
13
14 262 Episodic memory will be assessed via the immediate and delayed Story Recall subtest from the
15
16 263 Rivermead Behavioural Memory Test (RBMT).(57) To control and correct for differences in premorbid
17
18 264 intelligence between participants, verbal IQ will be estimated using the Dutch version of the National
19
20 265 Adult Reading Test (NART) at baseline.(58) The MoCA, episodic memory test and COWAT have
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22 266 parallel versions, to avoid material-specific learning effects during the repeated testing. Additionally,
23
24 267 the tests are standardized, have been validated for use across a wide age range and have good re-
25
26 268 test reliability. Together these tests will provide a good overview on the overall cognitive
27
28 269 performance of the patients, including aspects of working and episodic memory, attention, verbal
29
30 270 fluency and executive function. Also, education level will be recorded in accordance with the Dutch
31
32 271 education system using seven categories (one being the lowest level of education and seven being
33
34 272 the highest).(59)
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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)
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55 281 assessed by metabolomics, such as bile acids and bioactive (short chain) fatty acids, and other lipid
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57 282 species (untargeted lipidomics).
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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
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7 285 function readouts. Additionally, to gain insight into the microbiota in the intestinal mucosa, mucosal
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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.

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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
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22 292 processes and to eventually define mechanism-based subgroups.
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28 294 **Questionnaires**

29
30 295 At several time-points (figure 1) standardized questionnaires on lifestyle, education, success rate of
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32 296 the surgery and eating habits will be assessed. Most of the questionnaires are routine practice for
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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
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44 302 Bariatric Analysis and Report Outcome System (BAROS).(63)
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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)
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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/m²) 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 ≥ 7.0 mmol/L and HbA1c ≥ 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.

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.

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

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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
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17 342 been considered adequate to answer the research questions with sufficient power.
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22 23 344 **Analysis of primary outcome measures**

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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.
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51 52 357 **Analysis of secondary outcome measures**

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54 358 As secondary outcome measures, the metabolic and histopathological parameters (obtained analyses
55
56 359 from tissues collected during surgery) will be analysed cross-sectionally to examine correlations
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58 360 between and among each other, and in relation to brain function and structure. Furthermore,
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3 361 potential mechanisms underlying the crosstalk along the gut-brain axis will be investigated by
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5 362 longitudinal analyses focusing on establishing correlations between brain structure/function changes
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7 363 and changes in circulation mediators or faecal microbiota composition. Pearson correlation analysis
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9 364 will be used to investigate potential correlations between variables.
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13 14 366 **Data monitoring**

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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
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20 369 committee and the Netherlands Trial Register (trialregister.nl) 7288.
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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
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29 373 disseminated to the study participants via email, newsletters and social media platforms after the
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31 374 study results are published.
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35 36 376 **DISCUSSION**

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39 377 The BARICO study is a prospective study focusing on the effect of weight loss on cognitive function
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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
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47 381 structure, blood plasma and stool samples will be collected and analysed longitudinally, and biopsies
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49 382 of key metabolic organs will be collected during the RYGB and analysed cross-sectionally.
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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
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3 387 recovery of these brain structural abnormalities after BS.(50, 51) For instance, Tuulari *et al.* showed a
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5 388 causal link between weight loss and brain tissue recovery.(50) Approximately 25-30% of the patients
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7 389 are not expected to reach sufficient weight loss (≤ 50 %EWL), and thus it will be possible to study the
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9 390 effect of weight loss after BS on brain function and structure.

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

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25 397 Another strength of this study is the combination of neuroimaging and neuropsychological tests.
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27 398 Alongside the analysis of metabolic and histopathological parameters (obtained in blood, organ
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29 399 biopsies and microbiota), meaning that the relation between multiple metabolic, neuroimaging
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31 400 and/or cognitive parameters can be investigated (e.g., adipokines, bioactive lipids (short-chain fatty
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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
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37 403 brain function and structure following BS.(15, 70) Longitudinal analyses of the microbiota, together
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39 404 with analysis of functional gut-derived metabolites in the circulation and cognitive outcomes, may
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41 405 allow for the identification of mediators derived from gut microflora that are relevant to cognition
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43 406 and the prevention of cognitive decline.

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

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3 411 In conclusion, the BARICO study will reveal the relation and underlying mechanisms between obesity
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5 412 and brain function and structure. This information can be used to develop better health care as well
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7 413 as possible preventatives against obesity and associated disorders.
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11 415 **ETHICS AND DISSEMINATION**

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13
14 416 The study protocol was authorized by the medical review ethics committee CMO Region Arnhem and
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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.
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10 422 **Contributors**

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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*
11 509 *Hepatol*. 2017;2(10):747-756.
12
13 510 38. Wang HX, Wang YP. Gut Microbiota-brain Axis. *Chin Med J (Engl)*. 2016;129(19):2373-2380.
14
15 511 39. Aron-Wisnewsky J, Dore J, Clement K. The importance of the gut microbiota after bariatric surgery. *Nat Rev*
16 512 *Gastroenterol Hepatol*. 2012;9(10):590-598.
17
18 513 40. Peat CM, Kleiman SC, Bulik CM, *et al*. The Intestinal Microbiome in Bariatric Surgery Patients. *Eur Eat Disord Rev*.
19 514 2015;23(6):496-503.
20
21 515 41. Cipolla MJ. Chapter 5: Control of Cerebral Blood Flow. *The Cerebral Circulation. Integrated Systems Physiology:*
22 516 *From Molecule to Function*. San Rafael (CA)2009. p. 29-36.
23
24 517 42. Willeumier KC, Taylor DV, Amen DG. Elevated BMI is associated with decreased blood flow in the prefrontal cortex
25 518 using SPECT imaging in healthy adults. *Obesity (Silver Spring)*. 2011;19(5):1095-1097.
26
27 519 43. Alosco ML, Spitznagel MB, Raz N, *et al*. Obesity interacts with cerebral hypoperfusion to exacerbate cognitive
28 520 impairment in older adults with heart failure. *Cerebrovasc Dis Extra*. 2012;2(1):88-98.
29
30 521 44. Wierenga CE, Hays CC, Zlatar ZZ. Cerebral blood flow measured by arterial spin labeling MRI as a preclinical
31 522 marker of Alzheimer's disease. *J Alzheimer's Dis*. 2014;42 (Suppl 4):S411-419.
32
33 523 45. Alsop DC, Detre JA, Golay X, *et al*. Recommended implementation of arterial spin-labeled perfusion MRI for
34 524 clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia.
35 525 *Magn Reson Med*. 2015;73(1):102-116.
36
37 526 46. Kullmann S, Callaghan MF, Heni M, *et al*. Specific white matter tissue microstructure changes associated with
38 527 obesity. *Neuroimage*. 2016;125:36-44.
39
40 528 47. Debette S, Wolf C, Lambert JC, *et al*. Abdominal obesity and lower gray matter volume: a Mendelian
41 529 randomization study. *Neurobiol Aging*. 2014;35(2):378-386.
42
43 530 48. Karlsson HK, Tuulari JJ, Hirvonen J, *et al*. Obesity is associated with white matter atrophy: a combined diffusion
44 531 tensor imaging and voxel-based morphometric study. *Obesity (Silver Spring)*. 2013;21(12):2530-2537.
45
46 532 49. Arnoldussen IAC, Wiesmann M, Pelgrim CE, *et al*. Butyrate restores HFD-induced adaptations in brain function and
47 533 metabolism in mid-adult obese mice. *Int J Obes (Lond)*. 2017;41(6):935-944.
48
49 534 50. Tuulari JJ, Karlsson HK, Antikainen O, *et al*. Bariatric Surgery Induces White and Grey Matter Density Recovery in
50 535 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
4 537 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*
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
11 541 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*.
17 544 2008;22:498.
- 19 545 56. Schmand B, Groenink, S.C., van den Dungen, M. Letterfluency: psychometrische eigenschappen en Nederlandse
20 546 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;
24 548 1985.
- 26 549 58. Schmand B, Bakker D, Saan R, *et al.* The Dutch Reading Test for Adults: a measure of premorbid intelligence level.
28 550 *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
31 552 Gorcum; 1964.
- 34 553 60. Baecke JA, Burema, J., Frijters, J.E. A short questionnaire for the measurement of habitual physical activity in
35 554 epidemiological studies. *Am J Clin Nutr*. 1980;36(5):936-942.
- 37 555 61. Beck AT, Ward CH, Mendelson M, *et al.* An inventory for measuring depression. *Arch Gen Psychiatry*. 1961;4:561-
39 556 571.
- 41 557 62. Ware JE, Sherbourne, C.D. The MOS 36-Item Short-Form Health Survey (SF-36): I. Conceptual Framework and Item
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-
47 560 499.
- 48 561 64. Patton JH, Stanford MS, Barratt ES. Factor structure of the Barratt impulsiveness scale. *J Clin Psychol*.
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
53 564 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.
57 566 *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
11 572 70. Hoffstedt J, Andersson DP, Eriksson Hogling D, *et al*. Long-term Protective Changes in Adipose Tissue After Gastric
12 573 Bypass. *Diabetes Care*. 2017;40(1):77-84.
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3 575 **FIGURE LEGEND**
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7 577 **Figure 1.** Overview of the study design. Blood samples are taken during a regular blood withdrawal at
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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).
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11 579 Microbiota analyses will be performed at set time points on the faeces (collected at home by the
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13 580 patients) (4-8 wks. pre BS, 1 wk. pre BS, 6 mo. post BS, 24 mo. post BS) and mucosal swabs (collected
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15 581 during surgery). Furthermore, biopsies of liver, jejunum and adipose tissue depots (subcutaneous,
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17 582 mesenteric and omental) will be collected during surgery. Before surgery, (4-8 wks. pre BS) and at
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19 583 several time points after, (6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS) a medical
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21 584 evaluation will take place and all patients will complete questionnaires and neuropsychological
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23 585 measurements to test cognitive function. A subgroup of patients (N=75) will be examined with MRI
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25 586 (4-8 wks. pre BS and 24 mo. post BS). MRI; magnetic resonance imaging. BS; Bariatric surgery. Wks;
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27 587 weeks. Mo; months. Yrs; years.
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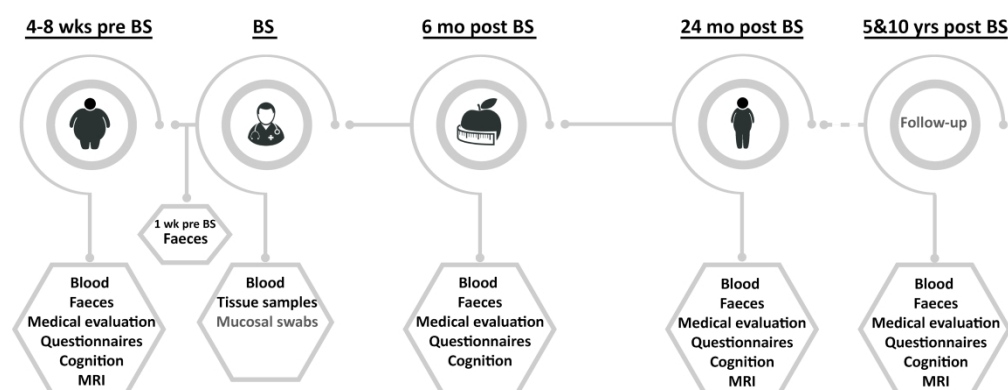
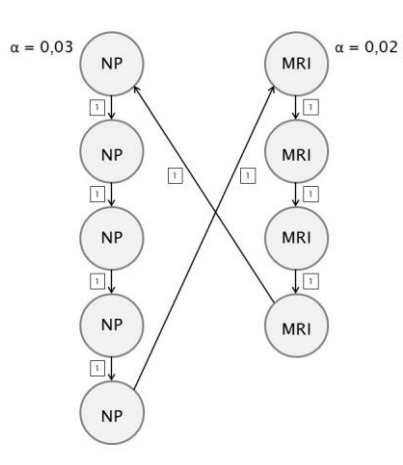


Figure 1. Overview of the study design. Blood samples are taken during a regular blood withdrawal at six time points (4-8 wks. pre BS, BS, 6 mo. post BS, 24 mo. post BS and 5 and 10 yrs. post BS). Microbiota analyses will be performed at set time points on the faeces (collected at home by the patients)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

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3 **1 SUPPLEMENTARY MATERIAL**
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8 3 Since multiple outcome measures will be studied, correction for this is applied using the sequentially
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10 4 rejective multiple testing procedure described in Bretz *et al.* (2008)(69). As we are highly interested
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12 5 in both the neuropsychological tests and the MRI parameters, the MRI parameters and the
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14 6 neuropsychological parameters are clustered. A significance level of 0.05 is used, and an alpha level
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16 7 of 0.03 is allocated to the neuropsychological tests and 0.02 to the MRI parameters. The
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18 8 neuropsychological tests and neuroimaging tests will be tested with a multiple testing procedure
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20 9 (supplementary figure 1). The neuropsychological tests will initially be tested at 3/5 of the overall
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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).
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24 11 Alpha will be reallocated when shown that the corresponding hypothesis is rejected. Based on the
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26 12 literature a specific hypothesis sequence will be tested (the sequence for the neuropsychological
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28 13 tests is: digit span, TAP flexibility task, story immediate/delayed recall, verbal fluency and MoCA; for
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30 14 the MRI parameters: DTI parameters, ASL measures, BOLD response of the Stroop test and grey and
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32 15 white matter volumes). Within each test separately correction for multiple testing will be included,
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34 16 for example for multiple brain areas analysed within a MRI parameter.
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21 FIGURE LEGEND

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

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