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Title: Medium term post-bariatric surgery deficit of vitamin B12 is predicted by deficit at time of surgery

Running title: Predictors of B12 deficit after bariatric surgery

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The authors declare no conflict of interest.

Abbreviations:

ALT, Alanine Aminotransferase

AST, Aspartate Aminotransferase

APRI, Aspartate Aminotransferase-to-Platelet Ratio Index

BS, Bariatric Surgery

BMI, Body Mass Index

HCY, Homocysteine

HOMA-IR, Homeostasis Model Assessment of Insulin Resistance

IF, Intrinsic Factor

LGB, Laparoscopic Gastric Bypass

LSG, Laparoscopic Sleeve Gastrectomy

MMA, Methyl Malonic Acid

NAFLD, Non-alcoholic Fatty Liver Disease

T2D, Type 2 Diabetes

1 **Abstract**

2

3 **Background:** Patients with morbid obesity have a high risk of deficits in micronutrients, after
4 bariatric surgery. The reasons why systematic use of multivitamin and trace element supplements
5 cannot prevent all deficits are complex and should deserve more attention. Little is known about
6 the influence of micronutrient deficits at surgery.

7 **Aim:** This present study aimed to explore the deficit in vitamin B12 *vs* other micronutrients
8 during the follow-up of a French cohort of cases with bariatric surgery under systematic
9 multivitamin/trace elements supplementation and to determine whether it was influenced by
10 clinical, metabolic characteristics at surgery.

11 **Methods:** We prospectively enrolled obese patients with bariatric surgery (laparoscopic gastric
12 bypass or laparoscopic sleeve gastrectomy) between 2013 and 2018 (OBESEPI/ALDEPI Cohort,
13 NCT02663388). They received a daily multivitamin/micronutrients supplement. Follow-up
14 data at 4 visits, 2, 12, 18 and 24 months after surgery, were collected.

15 **Results:** The highest rate of deficits was observed at visit 1 for vitamin D (35.7%), iron (21.9%)
16 and folate (10.2%). Except B12, the deficits of all micronutrients decreased in later visits. In
17 contrast, cases with vitamin B12 deficit decreased from 13.5% at surgery to 2.0% at visit 1, and
18 increased in later visits, with a maximum of 12.0% at visit 3. Vitamin B12 concentration at
19 surgery was the single predictor of B12 deficit at visit 3. It was also associated with age, and
20 APRI score, an index of nonalcoholic fatty liver disease (NAFLD), in multivariate analysis.

21 **Conclusions:** The failure of systematic supplementation with multivitamin/trace elements tablets
22 to prevent specific deficits illustrates the need for adapted specific supplementations, in some
23 cases. The worsening of B12 deficit rate in the 18-24 months follow-up depends in part to low
24 B12 at time of surgery. A special consideration should be devoted to this subset of patients.

25

26 The cohort study was registered at clinicaltrials.gov as NCT02663388

27 Keywords: obesity, B12, bariatric surgery

28

29

30

31 **Introduction**

32

33 In the last decades, obesity has become a global health issue that affects 600 million people
34 worldwide [1]. In France, the prevalence of obesity is estimated at 17% [2]. To date, bariatric
35 surgery (BS) represents the gold standard in treating patients with morbid obesity when lifestyle
36 interventions such as diet, physical activity, behavior therapy and pharmacotherapy fail [3]. The
37 most commonly performed bariatric procedures worldwide are gastric bypass and vertical sleeve
38 gastrectomy [4]. Gastric bypass is a restrictive and malabsorptive technique, with a small tubular
39 pouch created in the upper part of the stomach anastomosed to the distal end of a transected
40 small bowel limb, allowing the food to bypass the duodenum, and the proximal jejunum and
41 leading to macro- and micronutrients defective absorption. Vertical sleeve gastrectomy removes
42 80% of the stomach along the great curvature, including fundus needed for acid and intrinsic
43 factor secretion, leading to early satiety [4].

44 Patients with morbid obesity who have a BMI > 40kg/m² have a higher risk of nutritional
45 deficiencies after bariatric surgery [5-7] and develop micronutrient deficiencies in 25-vitamin D,
46 folate, zinc, iron, selenium and vitamin B12 [8, 9]. They are related to malabsorption and
47 decreased availability and to decreased dietary intake [5-7]. Moreover, they are prone to
48 micronutrient deficits not only after but also prior to bariatric surgery[10]. The systematic use of
49 multivitamin and trace element supplements is recommended. However the composition of most
50 of these supplements is not adapted to prevent deficits in all micronutrients after surgery. In
51 some cases, the additional intake of one or more micronutrients is needed [11]. This is
52 particularly the case for vitamin B12.

53 Of note, vitamin B12, or cobalamin, is a water-soluble vitamin, the metabolic role of which is
54 crucial for normal blood formation, mucosal regeneration, neuronal function and energy
55 metabolism [12-14]. Its dietary sources include meat, eggs, liver, fish and dairy products [12,
56 14]. Bariatric surgeries such as Roux-en-Y gastric bypass and sleeve gastrectomy compromise
57 vitamin B12 absorption [9, 13], but a high rate of B12 deficit is also observed in morbid obesity
58 cases prior surgery [15]. Nevertheless, little is known about the determinants at base line of
59 vitamin B12 deficiency post-surgery regardless of a methodical vitamin B12 supplementation.
60 This present study aimed to explore the deficit in vitamin B12 compared to other micronutrients
61 during the follow-up of a French cohort of cases undergoing bariatric surgery and subjected to

62 systematic multivitamin supplementation. We found that vitamin B12 deficit at baseline
63 influenced the post surgery increase of B12 deficit despite systematic supplementation.

64

65 **Materials and Methods**

66

67 This longitudinal prospective study enrolled obese patients (body mass index – BMI>35 kg/m²)
68 who underwent bariatric surgery between years 2013 and 2018 at the bariatric surgery center of
69 the university hospital in Nancy, France, NCT02663388. All patients signed specific informed
70 consents. Investigations conformed to the principles outlined in the Declaration of Helsinki and
71 received approval from the local ethical committee. Anthropometric and biochemical data were
72 collected at preoperative and postoperative follow-up times.

73 **Surgical procedure**

74 All surgeries were laparotomic performed by the same team of surgeons. Two surgical technical
75 were performed: laparoscopic gastric bypass (LGB) or laparoscopic sleeve gastrectomy (LSG) as
76 describe elsewhere [16]

77 **Follow-up**

78 Follow-up data are collected over a 2-years period for patients who underwent any type of
79 bariatric surgery. The follow-up visit was assessed by analyzes of medical records at two,
80 twelve, eighteen, twenty-four months after surgery. Following surgery, all-patients received
81 standard dietary and lifestyle recommendations [17]. The patients received 200 µg folic acid
82 every day, 1000 µg B12 every week, 100 000 UI vitamin D monthly and a daily
83 multivitamin/micronutrients supplement Azinc (AZINC®, Arkopharma, Carro, France). The
84 composition of the tablet is given in supplementary data **Table S1**.

85 **Anthropometric measurements**

86 Anthropometric evaluation was performed with weight, height, BMI, percentage of excess
87 weight loss (%EWL) and weight regain.

88 Anthropometric measurements for weight and height were measured on a digital medical-scale
89 (Seca, Hamburg, Germany) and BMI was calculated accordingly. Excess weight loss (EWL)
90 percentage was calculated as follows: [(pre-operation weight - post-operation
91 weight)/(preoperation weight ideal body weight)] *100. Ideal body weight was considered as the

92 weight for BMI at 25 kg/m² [14]. Percentage of total weight loss (TWL) was calculated as
93 follows: $([\text{pre-operation weight} - \text{post-operation weight}] / [\text{pre-operation weight}]) * 100$ [18].
94 Vitamin B12 and folate serum concentrations were determined by the SNB SimulTRAC-box for
95 radio-immunoassay with [⁵⁷Co]-vitamin B12 / [¹²⁵I]-folate as tracers (MP Biomedicals Germany
96 GmbH, Eschwege, Germany). Red blood cell folate (RBC folate) was determined using the same
97 radio-immunoassay method after total blood extract hemolysis in presence of ascorbic acid.
98 Homocysteine and methylmalonic acid (MMA) were determined by UPLC-MS/MS, by using an
99 ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 mm × 50 mm, Waters Corporation, Guyancourt,
100 France). Vitamin D (25-OH) was determined by Access 25(OH) Vitamin D Total Immunoassay
101 kit (Dxl 800, Brea, California, USA) and selenium, zinc and magnesium by Atomic Absorption
102 (AA) Spectroscopy (PinAAcle 900T, Perkin Elmer Inc., Rodgau, Germany). Other biochemical
103 parameters, including fasting plasma concentrations of iron, saturation coefficient of transferrin,
104 ferritin, glucose, cholesterol, triglycerides, HDL-cholesterol, ASAT and ALAT were determined
105 by color-turbidimetry on an AU 2700 Olympus apparatus (Beckman Coulter, Brea, California,
106 USA). LDL-cholesterol values were calculated using the Friedwald formula. The aspartate
107 aminotransferase to platelets ratio index (APRI score) was used to assess the presence of
108 nonalcoholic fatty liver disease (NAFLD)[19].

109

110 **Statistical Analyses**

111 Continuous data were reported as mean ± standard deviation. Incidence of nominal variables was
112 reported as percentage. Non-parametric tests were used when data distribution was not normal.
113 Repeated measures ANOVA was used to analyze correlated plasma samples from blood samples
114 baseline and postoperatively follow-up. P-values < 0.05 were considered as significant.
115 Statistical Analyses were performed with Stata/SE 12.1 (Texas, USA) and Prism 8 software
116 (California, USA).

117

118 **Results**

119 **General characteristics at base-line**

120 A total of three hundred ninety-nine (399) bariatric surgical patients were enrolled in the present
121 study. At baseline, 352 patients underwent LGB and 47 underwent LSG. Patient demographics
122 according to surgery are displayed in **Table 1**. LSG patients had a body mass index more

123 important than LGB patients ($p < 0.0001$) while age, vitamin B12 and other biochemical
124 parameters were comparable. Mean pre-operative B12 in the LGB and LSG groups were similar
125 (**Table 1**). Determinants of B12 at baseline were age (Rho 0.1714, $p=0.0001$), folate (Rho
126 0.1769, $p=0.0023$), ASAT (Rho 0.2187, $p=0.0002$), ALAT (Rho 0.2477, $p<0.0001$) and C-
127 reactive protein (Rho 0.1241, $p=0.0332$). A total of 56 patients were deficient for B12 (B12
128 lower than 150 pmol/L) (13.1%) (**Table 2**). Patients with B12 deficit were 5-year younger than
129 those without deficit (**Table 2**, $p = 0.0022$), and as expected, they had a slight increase of the
130 blood concentration of homocysteine and MMA. They have also a lower concentration of folate
131 (**Table 2**, $p = 0.0076$). A total of 19 patients had a combined deficit in folate and B12. We
132 observed no difference in anthropometric data and biochemical parameters between patients with
133 and without B12 deficit.

134 **Comparative status in B12 and other micronutrients during follow-up**

135 The number of cases examined during follow-up decreased from 399 at surgery to 370 (92.7%),
136 310 (77.7%), 251 (62.9%) and 205 (51.4%) at visits 1, 2, 3 and 4 respectively. The weight
137 reduction was observed at all visits (**Table 3**). We reported the concentration and frequency of
138 deficits in B12, folate, iron, 25OH-vitamin D, selenium, zinc and magnesium at each visit of
139 follow-up (**Table 4**). The highest rates of deficits were observed at visit 1 for vitamin D (35.7%)
140 and iron (21.9%) and they decreased at visits 2 and 3. We observed a more dramatic effect with
141 folate. The 10% rate of folate deficit at visit 1 decreased down to 6.1% in later visits. The lowest
142 rates of deficit were observed for vitamin B12, selenium, zinc and magnesium (**Table 4**). Patients
143 with B12 deficit at surgery maintain a lower concentration of vitamin B12 throughout the follow-
144 up visits and a higher risk of recurrence of B12 deficit after visit (**Figure 1a**). Even though
145 patients had a dramatic reduction in the percentage of subjects with vitamin B12 deficit at visit 1,
146 compared to baseline, with a percentage that decreased from 13.5% to 2.0% (**Figure 1b**). In
147 contrast to those of other micronutrients, the rate of B12 deficits increased during the 6-24
148 months interval, despite supplementation, raising 12.0% at visit 3. This worsening of B12 deficit
149 post surgery was not related to global malabsorption of micronutrients and/or to a bad
150 compliance to supplement intake, since only one case had a combined deficit in B12, folate and
151 vitamin D. The B12 deficit was associated neither to weight loss nor to type of surgery.
152 Metformin had a very limited influence on the rate of B12 deficit as only two cases with deficit
153 received this treatment during follow-up. We investigated therefore whether the worsening of

154 B12 deficit in the post surgical period could be due, at least in part, to predisposing factors at
155 baseline, e.g. at time of surgery.

156 **Predictors at time of surgery of B12 status at 18 months after surgery.**

157 We compared the clinical and biological characteristics of patients at surgery according to the
158 presence or absence of B12 deficit at visit 3. Among all clinical and biological parameters
159 recorded at surgery, vitamin B12 was the single parameter that was associated with a higher risk
160 of specific deficit in the post surgery period (**Table 5**). The B12 deficit was neither due to the
161 type of surgery (**Table S2**) nor to renal failure, as the blood concentration of creatinine was
162 lower than 15 mg/L at surgery and in the post surgery follow-up of all patients. It was not due to
163 treatment with metformin, as previously reported [15]. Univariate analysis showed that age,
164 folates, homocysteine, ALAT, ASAT and APRI score were significant determinants of vitamin
165 B12 concentration at surgery (**Table 6**). The concentration of B12 was associated with age, and
166 APRI score in multivariate analysis (**Table 6**, $p=0.012$ and $p<0.001$, respectively).

167

168 **Discussion**

169

170 This prospective study relies on a cohort of 399 bariatric surgery cases with reporting of clinical
171 data and biological analyses at surgery and during a 2-years follow-up. The bariatric surgery can
172 produce deficits of micronutrients or intensify previous deficiencies by limiting food intake
173 and/or micronutrient absorption, with the need to treat cases with micronutrient supplements
174 [11]. In our series, we observed a very low rate of multiple deficits in micronutrients and a
175 relatively high prevalence of specific deficits, in particular in iron and vitamin D, despite the
176 systematic supplementation given to all cases during follow-up. This is in agreement with
177 previous studies [11, 20, 21] and illustrates the need for a systematic use of multi-micronutrient
178 supplements and in the cases in which this treatment is insufficient, the use of additional adapted
179 specific supplementations. The deficit in most micronutrients decreased during times of follow-
180 up, in our cohort, in particular that of folate. Vitamin B12 behaved distinctly. The B12 deficit
181 was highly prevalent at surgery (13.1%), as previously observed [10, 15, 22] and decreased
182 dramatically to 2% at visit 1, demonstrating the short-term efficiency of the supplementation. We
183 found a raise of vitamin B12 deficit in the 12-24 months interval following bariatric surgery,
184 with a maximum of 12% at 18 months. However, this increase of B12 deficit was lower than that

185 reported in previous studies, probably because of the partial efficacy of the systematic
186 supplementation [23, 24]. It is consistent with a recent study, which observed a decrease in
187 plasma B12 after 6 months follow-up and an earlier decrease in plasma holoTC and increase in
188 MMA at 2 months post surgery in relation with decreased absorption capacity of B12. All
189 patients received a weekly supplementation equivalent to 150 µg B12 per day after surgery, a
190 dose, which could be insufficient to restore a normal status in all patients, in regard to the
191 conclusions of a recent systematic study [25]. The type of vitamin B12 used for supplementation
192 has also to be considered. Administration of cyanocobalamin results in a significant increase in
193 holoTC in comparison with hydroxocobalamin, in healthy adults with low and normal cobalamin
194 status [26].

195 The deficit could result from limitation in intake of micronutrient supplements and limited B12
196 absorption [14]. Bariatric surgery decreases the dietary absorption of vitamin B12 through
197 diverse mechanisms that include gastric achlorhydria, decreased secretion of intrinsic factor and
198 bacterial overgrowth in the intestinal ileum [14]. These mechanisms lead also to malabsorption
199 of iron and folate, but they had a partial influence to explain the post surgery deficit of B12 of
200 most of our cases as we reported only one case with a combined deficit in the 3 micronutrients.
201 We observed no difference in vitamin B12 status according to type of bariatric surgery. Whether
202 the type of surgery influence the secretion of intrinsic factor remains unknown. Gastrointestinal
203 transcriptomic response following Roux-in-Y gastric bypass shows a non-significant decreased
204 expression of intrinsic factor gene in the mucosa of remnant stomach [27]. In contrast to type of
205 bariatric surgery, the low B12 status at surgery was a strong predictor of the worsening of B12
206 status in the 18-24 months delay after surgery, despite B12 supplementation. The association of
207 B12 with APRI score of NAFLD is consistent with the association between elevated blood
208 concentration of B12 and cirrhosis in a previous study [26]. This suggests that patients with low
209 B12 at surgery have altered enterohepatic circulation that could reduced body stores of B12 and
210 increase the risk to have a deficit later than the 2 months post surgery. The enterohepatic cycle
211 plays an important role in the homeostasis of vitamin B12, with daily excretion of vitamin B12
212 and analogues estimated at 2.5 micrograms, to be compared to the dietary requirements
213 estimated at 2.4 micrograms/day [27]. Vitamin B12 is excreted in bile as a complex with
214 haptocorrin, its carrier protein, which is degraded by pancreatic enzymes in the lumen of the
215 duodenum to allow the transfer of B12 to gastric intrinsic factor [28]. In case of gastric bypass,

216 haptocorrin is degraded in the lumen of the duodenal loop in absence of intrinsic factor. The
217 released vitamin B12 is then captured by bacteria [4, 14, 28]. In case of vertical sleeve
218 gastrectomy, the resection of fundus produces a collapse of intrinsic factor secretion, which
219 interrupts the B12 enterohepatic cycle [4, 14, 27, 28]. Therefore, in case of bariatric surgery,
220 vitamin B12 excreted in bile is not recycled. Previous work suggested that NASH and cirrhosis
221 decrease the bile excretion of vitamin B12 bound to haptocorrin [26]. Consequently, NASH
222 reflected by high APRI score attenuates the loss of biliary vitamin B12, in case of bariatric
223 surgery. In contrast, in absence of steatohepatitis objectified by lower APRI score, the biliary
224 excretion of B12 is optimal and represents a permanent loss of "endogenous" vitamin B12,
225 which is not compensated if daily intakes by supplements are not sufficient.

226 Our study has limitations and strengths. We did not assess whether vitamin B12 deficit was
227 associated with increased metabolic markers such as homocysteine and MMA and with
228 holotranscobalamin during follow-up, as suggested very recently in a much smaller size follow-
229 up study of cases after bariatric surgery [9]. The compliance to follow-up visits was lower than
230 our initial expectation, with 205 cases (51%) examined at visit 4. Strengths of our study are the
231 important size of our cohort, the systematic supplementation and follow-up of our study
232 population and the comparison of clinical and biological data at time of surgery and during the
233 medium-term follow-up. Additionally, the study considered the status of most micronutrients
234 with higher prevalence of deficits after bariatric surgery.

235 In conclusion, our study showed that a supplementation with a multivitamin marked formulation
236 associated with a weekly intake of vitamin B12 was partially efficient to prevent micronutrient
237 deficits in the 2-year post bariatric surgery period of most cases. The cases in which this
238 treatment was insufficient had specific deficits, which can be treated by adapted specific
239 supplementations. B12 produced a distinct situation, with a worsening of the deficit frequency in
240 the 18-24 months following surgery through determinants that included low B12 at time of
241 surgery. A special consideration should be therefore devoted to this subset of patients, who
242 should be followed carefully and treated with adapted doses of vitamin B12.

243

244 **Author Contributions**

245 RMGR and JLG conducted research, analyzed data, performed statistical analysis, wrote
246 manuscript and had primary responsibility for final content. DA conducted research, performed

247 analyses, analyzed data, revised paper; ZL conducted research, performed analyses; AM
248 conducted research; DQ, MAS and LB conducted research, analyzed data, revised paper; DM
249 analyzed databases and revised manuscript.

250

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255

256

257 **Conflict of Interests**

258 The authors declare no conflicts of interests and the results herein have not yet been published
259 previously.

260

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341 **Tables**342 **Table 1** Baseline demographics of patients according with type of surgery

Variable	LGB Mean \pm SD	LSG Mean \pm SD	LGB vs LSG p-value
Age (years)	45.3 \pm 11.3	43.8 \pm 13.1	0.4544
Weight (kg)	123.4 \pm 18.8	157.3 \pm 25.6	<0.0001
BMI (kg/m ²)	45.1 \pm 5.6	56.4 \pm 8.5	<0.0001
Plasma folate (nmol/L)	16.5 \pm 12.7	16.3 \pm 9.3	0.5073
Plasma B12 (pmol/L)	268.0 \pm 318.7	257.0 \pm 153.3	0.6273
Glucose (g/L)	1.1 \pm 0.5	1.8 \pm 0.4	0.1157
Glucose mmol/L (UI/l)	5.8 \pm 1.9	6.1 \pm 1.9	0.1157
Insulin (μ mol/L)	44.8 \pm 51.4	38.5 \pm 33.7	0.3454
Total Cholesterol (g/L)	1.8 \pm 0.4	1.7 \pm 0.4	0.0023
TG (g/L)	1.7 \pm 0.8	1.5 \pm 0.5	0.8771
HDL (g/L)	0.41 \pm 0.09	0.37 \pm 0.07	0.0371
LDL (g/L)	1.17 \pm 0.31	1.07 \pm 0.29	0.0529
AST (UI/l)	34.9 \pm 27.8	43.9 \pm 31.8	0.0123
ALT (UI/l)	33.5 \pm 36.5	39.5 \pm 70.6	0.7808
CRP (mg/L)	18.3 \pm 26.5	21.8 \pm 25.9	0.0072
Platelet (Giga/ μ l)	281 \pm 64	297 \pm 71	0.1025
Hcy (μ mol/L)	136 \pm 4.7	13.2 \pm 3.7	0.6693
MMA (μ mol/L)	0.15 \pm 0.07	0.15 \pm 0.06	0.9533
APRI Score	0.38 \pm 0.33	0.45 \pm 0.35	0.0740
HOMA-IR	6.0 \pm 4.6	6.8 \pm 2.9	0.1299

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344 *ALT alanine aminotransferase, AST aspartate aminotransferase, BMI body mass index, Hcy*
345 *homocysteine, HDL high density lipoprotein, LDL low density lipoprotein, LGB laparoscopic*
346 *gastric bypass, LSG laparoscopic sleeve gastrectomy, HOMA-IR homeostasis model assessment*
347 *of insulin resistance, MMA methyl malonic acid, SD standard deviation, TG triglycerides*

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355 **Table 2** Clinical and biological characteristics of subjects according to B12 status at base-line

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Variable	B12 Non-Deficit at surgery (≥ 150 pmol/L) N=343		B12 Deficit at surgery (<150 pmol/L) N=56		p-value
	N	Mean \pm SD	N	Mean \pm SD	
Age (years)	343	45.8 \pm 11.4	56	40.8 \pm 11.2	0.0022
Weight (kg)	343	128.8 \pm 22.6	56	127.7 \pm 22.7	0.6285
BMI (kg/m ²)	343	46.6 \pm 7.2	56	45.8 \pm 6.5	0.5622
Plasma folate (nmol/L)	343	16.8 \pm 12.4	56	13.6 \pm 10.7	0.0076
Plasma B12 (pmol/L)	343	290.0 \pm 316.6	56	117.4 \pm 27.6	<0.0001
Glucose (g/L)	333	1.1 \pm 0.5	54	1.1 \pm 0.4	0.5506
Insulin (μ mol/L)	343	46.0 \pm 51.8	56	36.1 \pm 50.4	0.0213
Total Cholesterol (g/L)	343	1.8 \pm 0.3	55	1.8 \pm 0.4	0.2835
TG (g/L)	343	1.6 \pm 0.8	55	1.4 \pm 0.5	0.3953
HDL (g/L)	343	0.4 \pm 0.1	55	0.4 \pm 0.1	0.9824
LDL (g/L)	343	1.1 \pm 0.3	55	1.1 \pm 0.3	0.4744
AST (UI/l)	343	37.8 \pm 29.7	55	23.4 \pm 9.1	<0.0001
ALT (UI/l)	343	35.9 \pm 43.9	55	20. \pm 14.1	0.0086
CRP (mg/L)	341	19.3 \pm 27.2	55	15.7 \pm 19.9	0.5214
Platelet (Giga/ μ l)	335	281.6 \pm 65.7	55	288.3 \pm 58.7	0.3677
Hcy (μ mol/L)	241	13.4 \pm 4.4	37	15.6 \pm 6.7	0.0393
MMA (μ mol/L)	241	0.15 \pm 0.07	37	0.17 \pm 0.07	0.0234
APRI Score	333	0.41 \pm 0.35	54	0.24 \pm 0.11	<0.0001

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359 *ALT alanine aminotransferase, AST aspartate aminotransferase, BMI body mass index, Hcy*360 *homocysteine, HDL high density lipoprotein, LDL low density lipoprotein, HOMA-IR*361 *homeostasis model assessment of insulin resistance, MMA methyl malonic acid, SD standard*362 *deviation, TG triglycerides*

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372 **Table 3** Evolution of anthropometric indicators at and after bariatric surgery, visits 1, 2, 3, and 4.

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Visit	TWL			%TWL			%EBMI			Delta BMI		
	LGB	LSG	P-value	LGB	LSG	P-value	LSG	LSG	P-value	LGB	LSG	P-value
1	11.4±6.1	11.8±5.9	0.669	26.4±15.7	26.5±16.6	0.066	27±16.1	22.7±12.8	0.066	5.1±2.7	6.6±3.4	0.005
2	46.7±20.4	79.1±25.3	<0.000	52.8±20.9	55.8±24.7	0.004	54.7±21.3	44.5±19.0	0.004	10.4±3.7	12.9±5.0	0.918
3	53±20.0	84.3±28.9	<0.000	68.9±23.0	69.7±23.1	<0.000	71.2±22.4	55.7±20.3	<0.000	13.5±3.8	16±5.4	0.07
4	55.6±20.5	89.4±28.8	<0.000	84.9±52.7	83	0.001	77.7±30.1	59.8±34.8	0.0011	14.9±4.0	18±12.4	0.945

374 *BMI body mass index, %EBMI Percent excess BMI Loss, LGB laparoscopic gastric bypass, LSG*375 *laparoscopic sleeve gastrectomy, % TWL Percent of total weight loss.*

376 **Table 4** Evolution of concentration of micronutrients and percent of deficiency at the preoperative (B12 only) and after bariatric
 377 surgery, visits 1, 2, 3, and 4.

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	B12		Folates		Iron		25OH vitamin D		Selenium		Zinc		Magnesium	
	pmol/L	<150	nmol/L	<7	mg/L		ng/mL	<20	µg/L	<40	mg/L	<0.45	mg/L	<15
	Mean ± SD	% def	Mean ± SD	% def	Mean ± SD	% def	Mean ± SD	% def	Mean ± SD	% def	Mean ± SD	% def	Mean ± SD	% def
Base line	266.1±299.9	13.9	16.3±12.3											
Visit 1	416.0±276.1	1.9	16.5±7.8	10.2	0.75±0.30	21.9	22.7±8.5	35.7	66.3±15.9	5.6	0.81±0.91	1.6	19.9±1.9	2.1
Visit 2	391.8±240.8	7.0	15.7±8.3	12.7	0.77±0.29	15.8	30.6±10.4	12.0	68.7±16.9	3.8	0.88±1.53	1.3	18.9±3.5	5.7
Visit 3	294.1±162.7	12.0	18.3±10.5	12.2	0.89±0.38	15.7	27.1±9.5	20.8	73.9±16.3	2.4	0.72±0.12	1.1	20.4±2.1	1.9
Visit 4	337.5±214.5	10.5	19.9±10.6	6.1	0.91±0.33	12.9	26.3±9.2	23.2	71.9±16.9	3.5	0.72±0.14	1.3	20.8±4.3	0.6

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380 Table 5. Clinical and biological characteristics at time of surgery according with low B12 at visit
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Variable	B12 Non Deficit at V3		B12 Deficit at V3		p-value
	N	Mean±SD	N	Mean±SD	
Age (years)	117	45.4±10.3	16	44.3±12.1	0.5919
Weight (kg)	117	126.7±20.7	16	126.0±25.5	0.9311
BMI (kg/m ²)	117	45.8±6.7	16	44.8±6.6	0.6040
Plasma folate (nmol/L)	116	13.4±8.41	16	13.1±8.1	0.9250
Plasma B12 (pmol/L)	116	253.7±96.9	16	168.4±59.4	0.0004
Glucose (g/L)	113	1.1±0.5	16	1.1±0.5	0.8108
Glucose mmol/L (UI/l)	94	5.8±2.3	12	5.4±1.2	0.6214
Insulin (µmol/L)	116	42.2±33.6	16	35.3±19.0	0.7617
Total Cholesterol (g/L)	114	1.7±0.4	16	1.9±0.3	0.5580
TG (g/L)	114	1.5±0.6	16	1.6±0.6	0.2926
HDL (g/L)	114	0.4±0.1	16	0.4±0.1	0.9717
LDL (g/L)	114	1.8±0.3	16	1.2±0.3	0.5144
AST (UI/l)	114	35.4±27.9	16	32.2±20.7	0.9943
ALT (UI/l)	114	40.9±51.4	16	28.9±19.5	0.5999
CRP (mg/L)	114	19.9±32.4	16	16.1±25.2	0.5469
Platelet (Giga/µl)	113	293.1±68.4	16	285.1±67.7	0.7723
Hcy (µmol/L)	94	13.1±4.1	12	15.3±9.6	0.5799
MMA (µmol/L)	94	0.14±0.07	12	0.14±0.04	0.8730
APRI Score	110	0.37±0.33	16	0.33±0.17	0.8617
HOMA-IR	112	12.3±13.9	16	9.3±5.3	0.7241

383
 384 *ALT alanine aminotransferase, AST aspartate aminotransferase, BMI body mass index, Hcy*
 385 *homocysteine, HDL high density lipoprotein, LDL low density lipoprotein, HOMA-IR*
 386 *homeostasis model assessment of insulin resistance, MMA methyl malonic acid, SD standard*
 387 *deviation, TG triglycerides*

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1 **Table 6** Determinants of B12 in univariate and multivariate analysis

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	Univariate		Multivariate	
	Rho	p-value	Z	p-value
Age	0.17	<0.001	2.52	0.012
Folates	0.19	0.001		
ASAT	0.23	<0.001		
ALAT	0.24	<0.001		
APRI score	0.22	<0.001	4.18	<0.001
Homocysteine	-0.15	0.012		

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4 *ALT alanine aminotransferase, AST aspartate aminotransferase*

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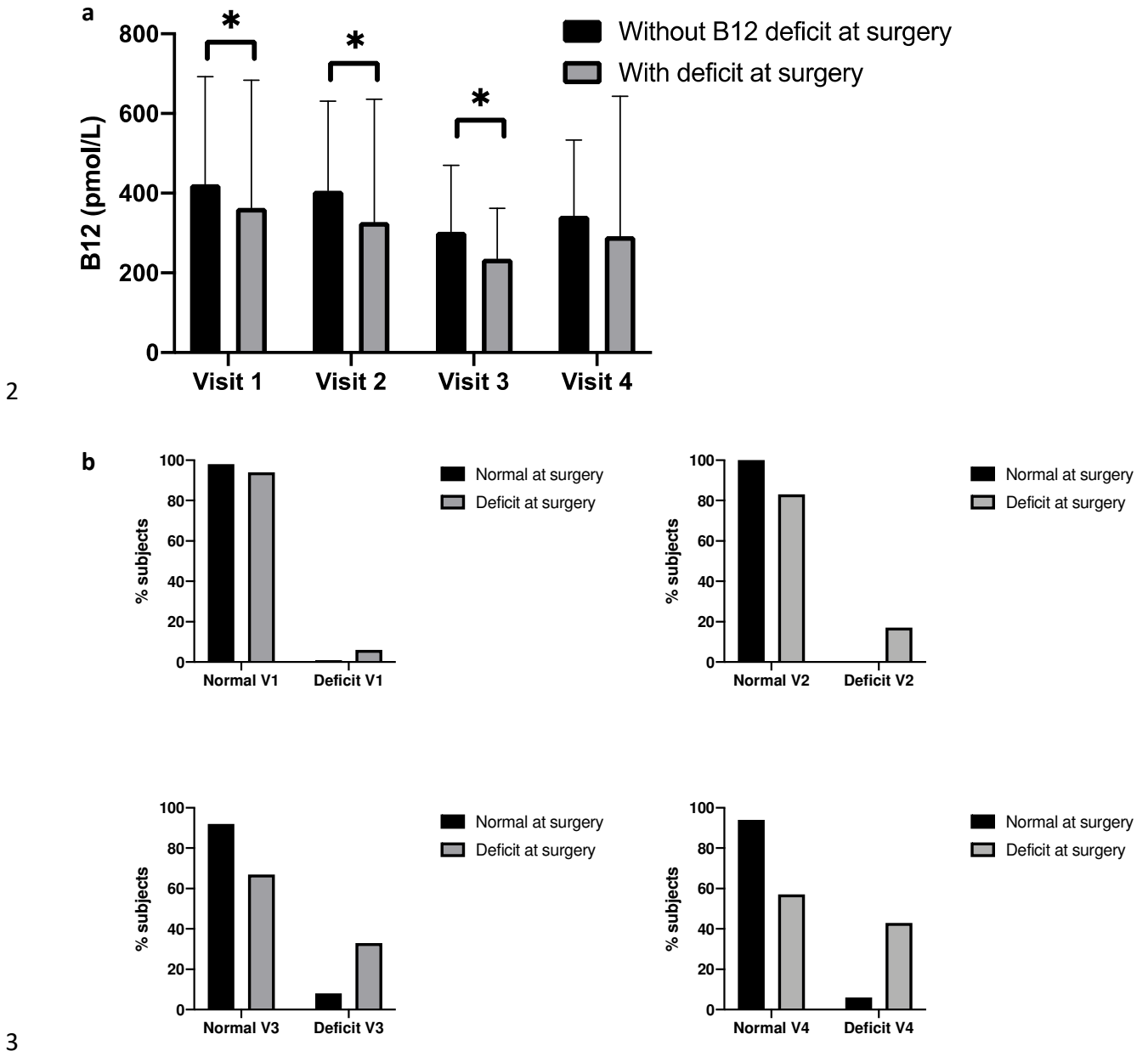
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1 **Figure 1**

4 **Figure 1: a.** Evolution of the concentration of vitamin B12, deficient patients at surgery are still
5 conserving low level of B12 at follow-up even when their status seemed to be improved. **b.**
6 Percentage of B12 deficits at visits 1-4, according to the presence or absence of B12 deficit
7 at time of surgery. Patients with B12 deficit at surgery maintain a lower concentration of
8 vitamin B12 throughout the follow-up visits and a higher risk of recurrence of B12 deficit
9 after visit 1.