



Original article

Early changes in vitamin B12 uptake and biomarker status following Roux-en-Y gastric bypass and sleeve gastrectomy

L.S. Kornerup^{a, b, *}, C.L. Hvas^c, C.B. Abild^d, B. Richelsen^d, E. Nexø^a

^a Department of Clinical Biochemistry, Aarhus University Hospital, Denmark

^b Department of Medicine, Regional Hospital Unit West Jutland, Denmark

^c Department of Hepatology and Gastroenterology, Aarhus University Hospital, Denmark

^d Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Denmark



ARTICLE INFO

Article history:

Received 16 November 2017

Accepted 3 February 2018

Keywords:

Bariatric surgery

Transcobalamins

Homocysteine

Methylmalonic acid

Vitamin B12

SUMMARY

Background & aims: Bariatric surgery increases the risk of micronutrient deficiencies, including vitamin B12 (B12) deficiency. We analysed early changes in biomarkers of B12 status following bariatric surgery. **Methods:** We prospectively included adult patients (n = 27) referred for either Roux-en-Y Gastric Bypass (RYGB) (n = 19) or Sleeve Gastrectomy (SG) (n = 8). Blood samples were drawn before surgery and 2 and 6 months following surgery for measurement of B12, holotranscobalamin (holoTC), and methylmalonic acid (MMA). The B12 absorption capacity was estimated from the increase in plasma holoTC two days after a standardised oral B12 challenge.

Results: B12 status decreased following both RYGB and SG. While a decrease in plasma B12 was not evident until 6 months postoperatively, we observed a statistically significant decrease in plasma holoTC and increase in MMA already 2 months postoperatively. These changes were more pronounced at 6 months post surgery. Correspondingly, the B12 absorption capacity was decreased following surgery.

Conclusions: HoloTC and MMA were superior to B12 to detect early changes in B12 status following bariatric surgery. Our data challenge the current concept that liver B12 stores secure long-term maintenance of B12 status. They indicate that B12 treatment in pharmacological doses may be warranted immediately after surgery.

© 2018 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Morbidly obese patients who undergo bariatric surgery are at high risk of developing micronutrient deficiencies due to the extensive changes of the anatomy and physiology of the gastrointestinal (GI) tract. The risk may be higher following Roux-en-Y gastric bypass (RYGB) than following sleeve gastrectomy (SG) [1–6]. Notably, the RYGB GI rearrangement compromises vitamin B12 (cobalamin, B12) absorption, because almost no gastric acid remains in the gastric pouch [7]. Consequently, liberation of food-bound B12 is substantially decreased [8]. In addition, the

production of intrinsic factor (IF), a parietal cell-derived protein necessary for intestinal B12 absorption, is decreased or absent in the bypassed stomach [9]. B12 malabsorption is potentiated by the delayed introduction of pancreatic enzymes in the distal jejunum. Thus, little or no IF is available to bind the limited amount of B12 liberated from food or recycled with bile, and the receptor-mediated absorption in the distal ileum is thereby jeopardized. SG may also compromise B12 absorption, but some acid secretion and IF production remain following surgery. A few studies examined food-bound B12 absorption and consistently found a significantly decreased absorption capacity following gastric surgery [5,10,11]. Most studies conclude that a negative B12 status is evident 10–36 months following bariatric surgery as mirrored by decreased plasma B12 [2–6].

We aimed to explore changes in B12 status and B12 absorption following bariatric surgery and to describe biomarker changes during the postoperative period.

Abbreviations: B12, vitamin B12, cobalamin; GI, gastrointestinal; holoTC, holotranscobalamin; Hcy, homocysteine; IF, intrinsic factor; MCV, mean cell volume; MeB12, methyl-B12; MMA, methylmalonic acid; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy.

* Corresponding author. Dept. of Clinical Biochemistry, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark.

E-mail address: lindajen@rm.dk (L.S. Kornerup).

<https://doi.org/10.1016/j.clnu.2018.02.007>

0261-5614/© 2018 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

2. Materials and methods

2.1. Study design

This was a single-centre prospective observational study. The study design is summarised in Fig. 1. The study population included patients undergoing RYGB or SG. Eligibility criteria were: 1) Age ≥ 25 years and 2) Referral to RYGB or SG.

Exclusion criteria were: 1) B12-treatment (other than a dose corresponding to the B12 content of regular multivitamin pills (2.5 μg)), 2) Changed metformin treatment between 1st and 2nd examination of B12 uptake (metformin may influence markers of B12 status [12]), and 3) Unable to give an informed consent.

Participation implied a total of five venous blood samples. Baseline blood samples were drawn at the day of the inclusion interview. Two days of B12 administration of with $3 \times 9 \mu\text{g}$ cyano-B12

(Naturdrogeriet, Hørning, Denmark) followed by blood sampling the next day was planned as soon as possible after baseline blood sampling. A blood sample was drawn at the first postoperative outpatient control (approximately two month after surgery). Two days of B12 administration and blood sampling as described above was performed soon thereafter. The final blood sample was drawn at the day of the patients' six-month outpatient control.

All study participants were included after having provided written consent in accordance with the Helsinki Declaration principles. The Scientific Ethics Committee of Central Denmark Region approved this study (project number M-2014-184-14).

2.2. Patients

All patients were consecutively included from September 2014, and the last blood samples were collected in January 2017. Study

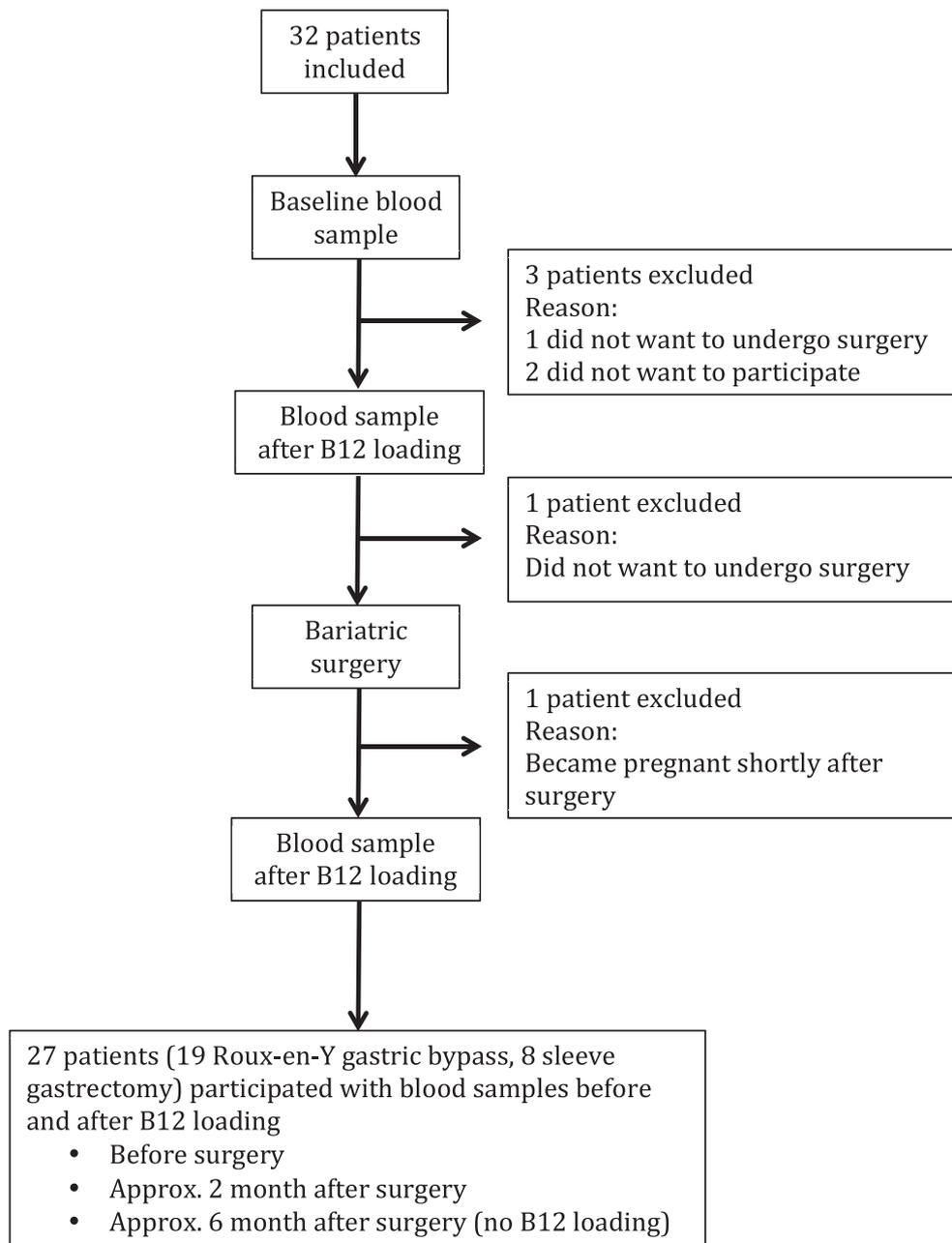


Fig. 1. Study design.

participants were recruited among patients referred to Department of Endocrinology and Internal medicine, Aarhus University Hospital, Denmark for bariatric surgery. If they met the inclusion criteria and no exclusion criteria, an inclusion interview was planned at their next visit to the outpatient clinic. The bariatric surgery was performed at Department of Surgery, Aarhus University Hospital and Department of Surgery, Viborg Regional Hospital.

All patients were instructed to take two daily regular multivitamin tablets (containing a total of 5 µg B12 and 400 µg folic acid) starting the day after surgery. Supplementation with iron, vitamin D, and calcium was started at the first postoperative outpatient control approximately two months following surgery. After completion of the present study, the patients were supplemented with a daily dose of 1 mg cyano-B12.

2.3. Sample collection and analysis

Blood samples (2 x heparin tubes 4 mL, 2 x EDTA tubes 4 mL, 2 x serum tubes with clot activator 4 mL) were drawn at Aarhus University Hospital or at a regional hospital in Central Denmark Region. Haemoglobin, mean cell volume (MCV) (Sysmex XN-9000, Sysmex Denmark), creatinine, ferritin (Cobas 6000, Roche Diagnostics), methylmalonic acid (MMA) (6500 QTRAP mass spectrometer, AB Sciex, with a Shimadzu HPLC-system), homocysteine (Hcy) and folate (ADVIA Centaur XPT immunoassay System, Siemens Healthcare Diagnostics) were analysed at our ISO certified (DS/EN ISO/IEC 15189, DS/EN ISO 22870) routine lab immediately after sample removal employing the indicated equipment.

Remaining project samples were transported to our research lab and centrifuged (1850 G, 9 min). Plasma/serum, was collected and stored at -80 °C until further analysis of B12 (ADVIA Centaur CP immunoassay System, Siemens Healthcare Diagnostics) and holotranscobalamin (holoTC) (in-house method; CV 7–8%, ref. [13,14]). All B12 and holoTC analyses on samples from the same patient were performed together after the patients had concluded the study. Based on the results for B12, holoTC, Hcy, MMA, and folate, we calculated the recently introduced combined indicator of vitamin B12 status, cB12 ($cB12 = \log_{10} [(holoTC \cdot B12)/(MMA \cdot Hcy)] - (3.79/1 + (age/230)^{2.6})$) corrected for folate. For details, see [15].

2.4. Study size calculation and statistical analyses

We based the study size calculation on the assumptions of the CobaSorb test [16]. Baseline holoTC is assumed to be normal (>40 pmol/L) in all study participants. A normal B12 absorption capacity corresponds to a holoTC increase of >22% and 10 pmol/L after two days of 3 × 9 µg cyano-B12. The intraindividual variance is reported to be 14%. For SG, the smallest detectable effect was set at 22%, since the B12 absorption capacity was expected to be nearly normal. Following RYGB, the absorption capacity was expected to decrease or lack totally. Thus, the smallest detectable effect was set at 16%. With a two-sided significance of 0.05 and a power of 0.9, a total of 18 RYGB patients and 10 SG patients were required.

For data analysis, Stata/IC 13.1 for Mac and Prism 6 for Mac OS X, version 6 h were used. Student's t-test and the chi [2]-test were applied to describe patient demography. Paired t-test was applied to compare plasma analyses at baseline, 2 months and 6 months following surgery. Repeated measures ANOVA was used to analyse correlated plasma samples from blood samples baseline, 2 month and 6 month postoperatively. All tests were considered statistically significant, when two-tailed p-values were <0.05.

For mean/median values given for a specific time point (e.g. baseline, 2 months and 6 months following surgery), all available data is included. In case of patients starting post-operative B12 supplementation within the study period, the plasma analyses

following this time point were excluded from statistical analysis. When comparing observations for two time points, the paired t-test was employed, and in case of missing data at one time point, the patient was censored from the paired t-test in question.

3. Results

3.1. Patient demography

We studied B12 absorption and changes in B12 biomarkers in patients undergoing bariatric surgery (Fig. 1). Table 1 displays the summary of patient demography. The study population consisted of 27 patients, 18 women (67%) and 9 men. Nineteen patients (70%) underwent RYGB and 8 underwent SG. The distributions of men and women in both the RYGB and the SG groups were comparable (RYGB 37%; SG 25%, chi [2], $p = 0.6$). Median age at the time of surgery was 47.2 years (y) in the RYGB group and 39.4 y in the SG group ($p = 0.6$) with no difference in age among men or women (men 46.9 y; women 42.2 y, t-test, $p = 0.3$). Median BMI at enrolment was higher in the RYGB group compared with the SG group (RYGB: 44.1; SG: 38.1, t-test, $p = 0.06$). Five patients were treated with metformin (19%) with no difference between RYGB and SG (4 (21%) vs. 1 (13%), $p = 0.6$) and no change in dose during the study.

3.2. Plasma analyses

Table 2 displays a summary of plasma analyses. For all examined analyses, no differences between RYGB patients and SG patients were found. Therefore, the results are presented together for the two surgery types in Fig. 2. Data for each surgery type are depicted in Supplementary Table 1 and Supplementary Fig. 1. Two patients (7%) had low haemoglobin values at baseline, further two at the first follow up (9%), and one patient at the second follow up (6%). Bariatric surgery had a statistically significant effect on decline in total B12 ($F(2,36) = 13.03$, $p < 0.001$), and holoTC ($F(2,36) = 18.95$, $p < 0.0001$) and increase in MMA ($F(2,37) = 8.70$, $p < 0.001$) (Table 2, one-way repeated measures ANOVA), though none of the biomarkers reached values severely outside the reference interval. The folate increase ($F(2,37) = 6.66$, $p = 0.003$) following surgery may be likely explained by the supplementation of multivitamin pills (400 µg folic/day). Additionally, the test showed a trend towards effect on haemoglobin levels only following RYGB ($F(2,28) = 2.66$, $p = 0.09$), but not following SG ($F(2,6) = 0.03$, $p = 0.97$). There was no statistically significant effect on Hcy or MCV. Early signs of B12 deficiency were evaluated from mean plasma levels of B12 biomarkers approximately 2 month after surgery compared with mean baseline levels (paired data, Table 2). Two months after surgery, there was no change in total B12, (paired t-test $p = 0.8$) while MMA was increased (paired t-test $p = 0.06$). When analysing cB12 (the combined indicator of vitamin B12 status), the mean preoperative value was 0.17, corresponding to 'B12 adequacy'. Two months postoperatively, the mean value was 0.020

Table 1
Patient baseline demography.

	RYGB (n = 19)	SG (n = 8)	p
Age at surgery, years (median (range))	47.2 (26–64)	39.4 (29–65)	0.63
Male (n (%))	7 (37)	2 (25)	0.55
BMI, kg/(m ²) (median, (range))	44.1 (35.7–58.5)	38.1 (34.2–49.0)	0.06
Metformin (n (%))	4 (21)	1 (13)	0.6

RYGB: Roux-en-Y gastric bypass; SG: sleeve gastrectomy; BMI: body mass index.

Table 2

Laboratory data at baseline and approximately 2 and 6 months after bariatric surgery in 27 patients (19 undergoing RYGB and 8 SG). Reference intervals for each biomarker are indicated in brackets.

	Baseline n = 27 Median (range)	2 months post-OP n = 22 Median (range)	P, baseline versus 2 months	6 months post-OP n = 17 Median (range)	P, baseline versus 6 months [2 months versus 6 months]
Time relative to surgery, (days (range))	-121 (-559-(-5))	63 (27-231)		179 (140-286)	
B12 (pmol/L) [200-600]	294 (129-554)	276 (189-450)	0.8	211 (129-380)	0.003 [0.0001]
holoTC (pmol/L) [>40]	56 (27-111)	54 (20-90)	0.005	30 (15-69)	0.0004 [0.001]
MMA (μmol/L) [0.08-0.28]	0.18 (0.12-0.41)	0.19 (0.11-0.49)	0.06	0.22 (0.12-0.52)	0.007 [0.02]
Homocystein (μmol/L) [< 12]	11.5 (6.5-18.3)	12.9 (7.2-21.3)	0.1	13.4 (7-20.6)	0.2 [0.2]
Folate (nmol/L) [> 9]	17 (7-45)	20 (12-45)	0.008	29 (11-45)	0.009 [0.5]
MCV (fL) [82-98]	87 (76-92)	88 (79-93)	0.9	88 (81-94)	0.3 [0.2]

B12: vitamin B12, Cobalamin; holoTC: holo-transcobalamin; MMA: methylmalonic acid; MCV: mean cell volume.

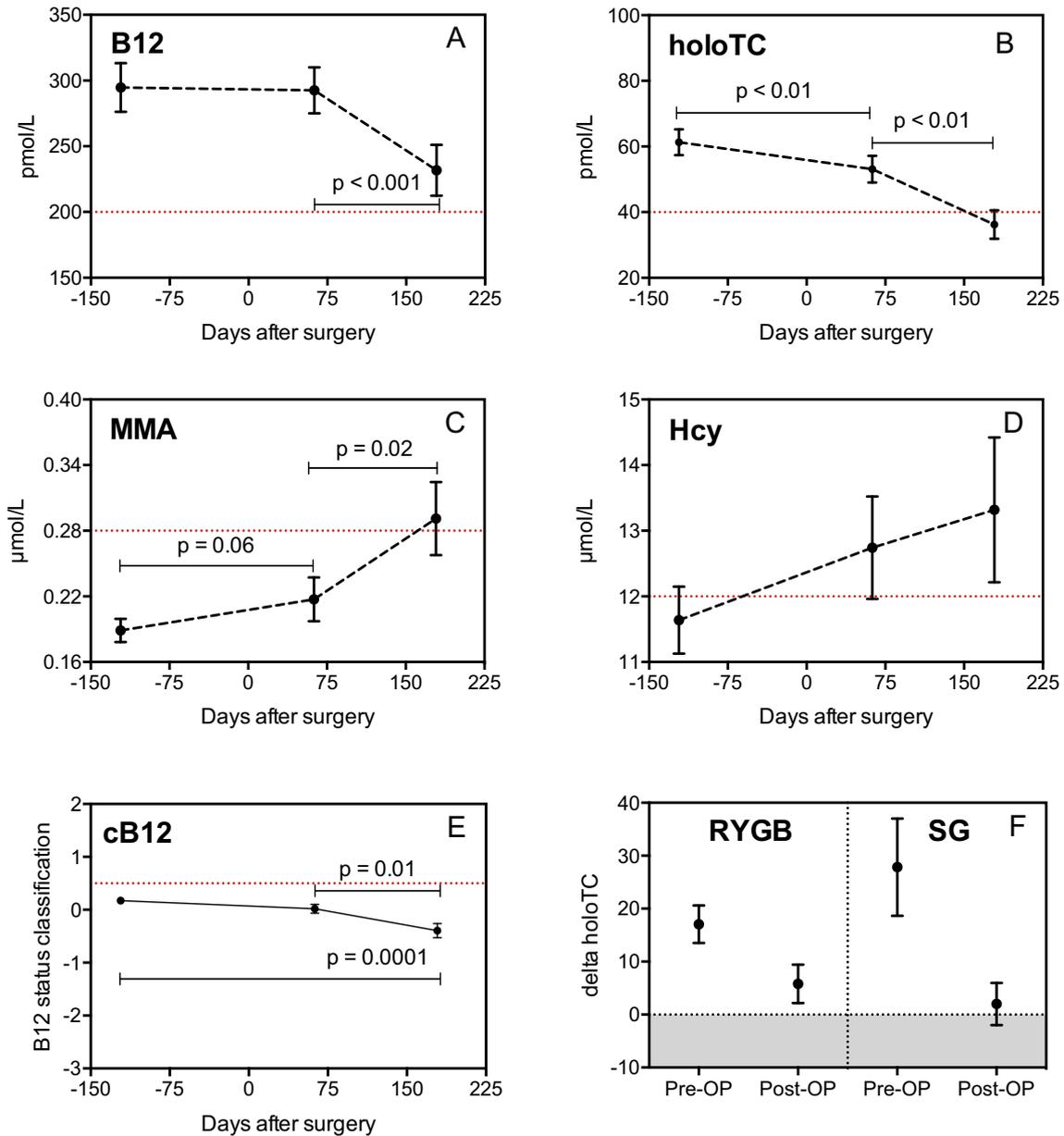


Fig. 2. Changes in biomarkers at baseline, 2 months after surgery and 6 months after surgery. Mean and SEM. Red dotted lines represents the lower limit of the 95% reference interval of the biomarker. A: plasma B12; B: plasma holoTC; C: plasma MMA; D: plasma Hcy; E: cB12; B12 status by a combination of B12, MMA, Hcy, folate and HoloTC; The cB12 values represents the B12 status and the suggested treatment (if relevant): 2: elevated B12; 1: adequate B12 status; -1: decreased B12 (start B12 supplements); -2: possibly B12 deficiency (start oral B12); -3: probably B12 deficiency (start B12 injections); F: B12 absorption capacity judged by changes in plasma holoTC before and after RYGB and SG. B12: vitamin B12, cobalamin; MMA: methylmalonic acid; Hcy: homocysteine; holoTC: holo-transcobalamin; RYGB: Roux-en-Y gastric bypass, SG: sleeve gastrectomy.

and six months postoperatively -0.39 , both also corresponding to 'B12 adequacy'.

The B12 absorption capacity was judged by the holoTC increase following two days of $3 \times 9 \mu\text{g}$ cyano-B12 administration. As evident from Fig. 2F, the holoTC increase was markedly lower two months postoperatively compared with the preoperative holoTC increase in both RYGB and SG patients. Mean Δ holoTC after preoperative B12 administration was 20 pmol/L (range -4 – 64) and 5 pmol/L (-21 – 38) after postoperative B12 administration (paired *t*-test, $p < 0.001$) for all patients together.

4. Discussion

This study demonstrated changes in biomarkers of B12 status following two types of bariatric surgery, RYGB and SG. Notably, we found that as early as two months following surgery, the B12 absorption capacity evaluated by MMA as well as holoTC, but not total B12, is markedly decreased. This indicates a negative B12 homeostasis immediately following surgery and applies to both types of bariatric surgery. Six months postoperatively, all three markers indicated a negative B12 homeostasis.

Based on the current knowledge, bariatric surgery causes poor absorption of food-bound B12. Dawson et al. studied B12 absorption and found that no patients with partial gastrectomy absorbed protein-bound B12 [11]. Likewise, Yale et al. found that patients with RYGB had significantly decreased food-B12 absorption compared with controls [5]. In both studies, a modified Schillings test was applied, but this is no longer available for clinical use because it includes radioactivity administration.

Individuals with food-B12 malabsorption may absorb oral B12 if it is administered in its free form such as in vitamin pills [10,17]. Our results question this statement with regard to patients who have undergone bariatric surgery. We demonstrated a significantly smaller increase in holoTC after bariatric surgery compared with before, which indicates a disrupted postoperative B12 absorption both in patients who had RYGB and those who had SG. Importantly, the observed changes in B12-related biomarkers occurred in patients who had a daily supplement of $5 \mu\text{g}$ free B12. This underscores the impaired B12 absorption and the subsequent negative B12 homeostasis that is demonstrated as early as 2 months following surgery. Despite preoperative and postoperative cB12 values all correlate to 'B12 adequacy', the 6 months postoperative cB12 is significantly lower than both previous cB12 values. The limit between 'B12 adequacy' and 'low B12' is -0.5 [15].

Most authors and clinicians agree to the importance of monitoring B12 status in patients after bariatric surgery in order to prevent and treat B12 deficiency in due time. It has been questioned whether patients who had SG are in the same risk of developing B12 deficiency as patients who had RYGB. Despite a small number of patients with SG in our study, the results for SG alone show a trend towards the significant changes observed in patients with RYGB (Suppl. Fig. 1). No consensus has been reached on when to start B12 substitution therapy. Brolin and colleagues claimed in 1998 that the high frequency of low B12 and folate levels found in their study population were clinically unimportant, because no patients had symptoms of deficiency [4]. Thus, prophylactic supplementations with B12 or folate were not recommended. The American Association of Endocrinologists, The Obesity Society, and the American Society for Metabolic and Bariatric Surgery 'Medical Guidelines for the Clinical Practice for the Perioperative Nutritional, Metabolic, and Nonsurgical Support of the Bariatric Surgery Patient' from 2008 states that some debate exists about B12 supplementation, but that most bariatric surgery groups recommend starting within the first six postoperative

months [18]. They suggest to supplement from around the fifth postoperative week for RYGB patients and to monitor B12 biomarkers (plasma B12; MMA and Hcy are optional) every 3–6 months for the first year and annually thereafter. These recommendations have been underscored by measures of total B12 in plasma. Our data challenge the value of judging an early B12 status by total B12 measurements in these patients and also challenge whether B12 status remains normal up to 6 months after surgery. Total B12 roughly represents 20% B12 bound to TC (holoTC) and 80% bound to haptocorrin [14,19]. Only B12 bound to TC is available for the tissues, and is thus called 'active B12'. Measurement of total B12 may not be sensitive enough to detect an early decrease in holoTC [15,20]. MMA is considered a sensitive marker of B12 status. B12 deficiency causes decreased methylmalonyl-CoA mutase activity and thereby accumulation of MMA, the enzyme substrate [21]. Hcy is another biomarker of B12 status, and decreased activity in methionine synthase (MS) causes accumulation of Hcy. However, since the bioactive form of folate (5-methyl-tetrahydrofolate) is substrate for MS, folate status will also influence the level of Hcy. Thus, Hcy is a less sensitive B12 biomarker and should not stand alone in evaluating B12 status [22].

The current belief is that the liver contains a sufficient amount of B12 in order to maintain a normal B12 status for at least 6–12 months. Several studies reported B12 deficiency to develop between 12 and 24 months after surgery [1,4,23–28]. Skroubis et al. suggested that patients would develop B12 deficiency after 10.7 months judged by extrapolating their data from total B12 analyses [6]. Several authors claim that B12 deficiency has a late onset owing to the liver's large storage of B12 [1,4,29,30]. Our results challenge this view, because we found increased MMA and decreased holoTC already 2 months after surgery.

Our study has important limitations. Compared with other studies in this field, our study population is smaller, especially the SG population. Additionally, the study was performed in a routine setting with patients following their controls at the outpatient clinic. Thus, we were unable to follow the patients closely and at strictly controlled intervals. On the other hand, this also means that the study validly reflects clinical practice. Therefore, we believe that important information can be extracted from our results.

In conclusion, our data demonstrate that bariatric surgery patients develop signs of negative B12 homeostasis already within a few months after surgery despite B12 supplementation with a physiological dose of the vitamin. This seems explained by the observed decrease in B12 absorption capacity. Additionally, our results question the concept that the liver stores serve for maintaining a normal B12 status for a prolonged time after changes in the B12 absorption has occurred. Finally, our study supports previous observations, which indicate that holoTC and MMA may be better markers of early changes in B12 status than total B12. Collectively, our data suggest that patients who undergo bariatric surgery should start high dose vitamin B12 supplementation immediately after surgery.

Author contributions

Study concept and design: Kornerup LS, Nexo E, Hvas CL, Richelsen B.

Acquisition of data: Kornerup LS, Abild CB.

Analysis and interpretation of data: Kornerup LS, Nexo E.

Drafting of the manuscript: Kornerup LS.

Critical revision of the manuscript for important intellectual content: Kornerup LS, Nexo E, Hvas CL, Richelsen B.

Statistical analysis: Kornerup LS.

Study supervision: Nexo E.

Administrative support: Abild CB

Conflicts of interest

The authors declare no conflicts of interest.

Financial support

This study is part of the TRIM-Project (www.trim-project.dk), financed by Innovation Fund Denmark; grant no. 12–132437. Innovation Fund Denmark has no role in the design, analysis or writing of this manuscript. No authors received personal funding for this study.

Acknowledgements

We warmly appreciate the competent technical assistance provided by Jette Fisker Pedersen and Inger Marie Jensen. Dorthe Møller has been of great help in organizing patient contact.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2018.02.007>.

References

- [1] Gehrler S, Kern B, Peters T, Christoffel-Courtin C, Peterli R. Fewer nutrient deficiencies after laparoscopic sleeve gastrectomy (LSG) than after Laparoscopic Roux-Y-gastric bypass (LRYGB)-a prospective study. *Obes Surg* 2010;20:447–53.
- [2] Kwon Y, Kim HJ, Lo Menzo E, Park S, Szomstein S, Rosenthal RJ. Anemia, iron and vitamin B12 deficiencies after sleeve gastrectomy compared to Roux-en-Y gastric bypass: a meta-analysis. *Surg Obes Relat Dis* December 2013;1–9.
- [3] Aarts EO, van Wageningen B, Janssen IMC, Berends FJ. Prevalence of anemia and related deficiencies in the first year following laparoscopic gastric bypass for morbid obesity. *J Obes* 2012;2012:193705.
- [4] Brodin RE, Gorman JH, Gorman RC, Petschenik AJ, Bradley LJ, Kenler HA, et al. Are vitamin B12 and folate deficiency clinically important after roux-en-Y gastric bypass? *J Gastrointest Surg* 1998;2:436–42.
- [5] Yale CE, Gohdes PN, Schilling RF. Cobalamin absorption and hematologic status after two types of gastric surgery for obesity. *Am J Hematol* 1993;42:63–6.
- [6] Skroubis G, Sakellaropoulos G, Pougouras K, Mead N, Nikiforidis G, Kalfarentzos F. Comparison of nutritional deficiencies after Roux-en-Y gastric bypass and after biliopancreatic diversion with Roux-en-Y gastric bypass. *Obes Surg* 2002;12:551–8.
- [7] Smith CD, Herkes SB, Behrens KE, Fairbanks VF, Kelly KA, Sarr MG. Gastric acid secretion and vitamin B12 absorption after vertical Roux-en-Y gastric bypass for morbid obesity. *Ann Surg* 1993;218:91–6.
- [8] King CE, Leibach J, Toskes PP. Clinically significant vitamin B12 deficiency secondary to malabsorption of protein-bound vitamin B12. *Dig Dis Sci* 1979;24:397–402.
- [9] Marcuard SP, Sinar DR, Swanson MS, Silverman JF, Levine JS. Absence of luminal intrinsic factor after gastric bypass surgery for morbid obesity. *Dig Dis Sci* 1989;34:1238–42.
- [10] Doscherholmen A, Swaim WR. Impaired assimilation of egg Co57 Vitamin B12 in patients with hypochlorhydria and achlorhydria and after gastric resection. *Gastroenterology* 1973;64:913–9.
- [11] Dawson DW, Sawers AH, Sharma RK. Malabsorption of protein bound vitamin B12. *Br Med J (Clin Res Ed)* 1984;288:675–8.
- [12] Jager J De, Kooy A, Lehert P, Wulffélé MG, van der Kolk J, Bets D, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. *Br Med J* 2010; c2181.
- [13] Nexo E, Christensen A-L, Hvas A-M, Petersen TE, Fedosov SN. Quantification of holo-transcobalamin, a marker of vitamin B₁₂ deficiency. *Clin Chem* 2002;48: 561 LP–2 LP.
- [14] Nexo E, Christensen AL, Petersen TE, Fedosov SN. Measurement of transcobalamin by ELISA. *Clin Chem* 2000;46:1643–9.
- [15] Fedosov SN, Brito A, Miller JW, Green R, Allen LH. Combined indicator of vitamin B12 status: modification for missing biomarkers and folate status and recommendations for revised cut-points. *Clin Chem Lab Med* 2015;0: 1215–25.
- [16] Hvas A-M, Morkbak AL, Nexo E. Plasma holotranscobalamin compared with plasma cobalamins for assessment of vitamin B12 absorption; optimisation of a non-radioactive vitamin B12 absorption test (CobaSorb). *Clin Chim Acta* 2007;376:150–4.
- [17] Carmel R. In: *Current concepts in cobalamin deficiency*, vol. 51; 2000. p. 357–75. New York.
- [18] Mechanick JL, Kushner RF, Sugerman HJ, Gonzalez-Campoy JM, Collazo-Clavell ML, Spitz AF, et al. American association of clinical Endocrinologists, the obesity society, and american society for metabolic & bariatric surgery medical guidelines for clinical practice for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient. *Obesity* 2009;17:S3–72.
- [19] Markle HV, Greenway DC. Cobalamin. *Crit Rev Clin Lab Sci* 1996;33:247–356.
- [20] Nexo E, Hoffmann-Lücke E. Holotranscobalamin, a marker of vitamin B-12 status: analytical aspects and clinical utility. *Am J Clin Nutr* 2011;94: 359S–65S.
- [21] Clarke R, Refsum H, Birks J, Evans JG, Johnston C, Sherliker P, et al. Screening for vitamin B-12 and folate deficiency in older persons 1 – 3. 2003.
- [22] Huemer M, Kozich V, Rinaldo P, Baumgartner MR, Merinero B, Pasquini E, et al. Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines. *J Inherit Metab Dis* 2015;38: 1007–19.
- [23] Vargas-Ruiz AG, Hernández-Rivera G, Herrera MF. Prevalence of iron, folate, and vitamin B12 deficiency anemia after laparoscopic Roux-en-Y gastric bypass. *Obes Surg* 2008;18:288–93.
- [24] Halverson JD, Zuckerman GR, Koehler RE, Gentry K, Michael HE, DeSchryver-Keckskemeti K. Gastric bypass for morbid obesity: a medical–surgical assessment. *Ann Surg* 1981;194:152–60.
- [25] Amaral JF, Thompson WR, Caldwell MD, Martin HF, Randall HT. Prospective hematologic evaluation of gastric exclusion surgery for morbid obesity. *Ann Surg* 1985;201:186–93.
- [26] Moizé V, Andreu A, Flores L, Torres F, Ibarzabal A, Delgado S, et al. Long-term dietary intake and nutritional deficiencies following sleeve gastrectomy or roux-en-y gastric bypass in a mediterranean population. *J Acad Nutr Diet* 2013;113:400–10.
- [27] van Rutte PWJ, Aarts EO, Smulders JF, Nienhuijs SW. Nutrient deficiencies before and after sleeve gastrectomy. *Obes Surg* 2014 Oct;24(10):1639–46. <https://doi.org/10.1007/s11695-014-1225-y>.
- [28] Clements RH, Katasani VG, Rajendra P, Leeth RR, Leath TD, Roy BP, et al. Incidence of Vitamin Deficiency after Laparoscopic Roux-en-Y Gastric Bypass in a University Hospital Setting. *Am Surg* 2006;72:1196–205.
- [29] Aills L, Blankenship J, Buffington C, Furtado M, Parrott J. ASMBS allied health nutritional guidelines for the surgical weight loss patient. *Surg Obes Relat Dis* 2008;4.
- [30] Gasteyger C, Suter M, Calmes J. Changes in body composition, metabolic profile and nutritional status 24 months after gastric banding. *Obes Surg* 2006;16:243–50.