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Significant increase of serum B12 levels with high-dose oral vitamin B12 supplementation with or without intrinsic factor after Roux-en-Y gastric bypass: A randomized controlled trial

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ABSTRACT

Purpose: VitB12 deficiency is frequently encountered after RYGB. Low-dose oral supplements are not sufficient to avoid this deficiency, often implying treatment with intramuscular injections. As IF plays an important role in vitB12 absorption, there might be an additional effect in adding IF to oral vitB12 supplements.

Material and methods: This randomized, monocentric, double-blind, placebo-controlled trial evaluates the short-term effect of high-dose (1000 μg) oral vitB12 supplementation on serum vitB12 levels after RYGB. 225 patients were randomized into three treatment arms: vitB12, vitB12 in combination with intrinsic factor (vitB12IF) or placebo.

Results: Serum vitB12 levels 6 months postoperatively were significantly higher in patients receiving vitB12 compared to placebo ($p < 0.001$). A mean decrease of 197.6 (SD ± 37) ng/L vitB12 and an augmentation of 154 (SD ± 97.8) ng/L and 202.3 (SD ± 113) ng/L were respectively observed in the placebo, vitB12 and vitB12IF groups. Our study shows no significant benefit in adding IF compared to high dose oral vitB12 without IF ($p = 0.711$).

Conclusion: High-dose oral vitB12 supplements significantly increase serum vitB12 levels after RYGB, confirming the efficacy of such treatment, without a significant benefit of adding IF to the vitB12 supplement.

1. Introduction

VitB12 or cobalamin (Cbl) is a micronutrient essential for normal erythropoiesis, growth and overall development (Brito et al., 2018; Butler et al., 2006; Henfridsson et al., 2019). Deficiency is frequently defined as serum plasma levels below 148 pmol/L (200 pg/mL) and is associated with low holotranscobalamin (HoloTC) and elevated serum levels of homocysteine and methylmalonic acid (Allen, 2009; Brito et al., 2018; Scott, 1999). Clinical symptoms include anemia, fatigue, neurological complications and cognitive impairment (Brito et al., 2018; Butler et al., 2006; Henfridsson et al., 2019). VitB12 levels are entirely dependent on dietary intake since this necessary micronutrient cannot be produced by the human body itself.

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A review of Doets et al. showed that daily vitamin B12 losses probably range from 1.4 to 5.1 μg in apparently healthy adults. Based on the relationship between the ingested dose and the biological availability, the vitamin B12 intake needed for compensation of daily losses ranges from 3.8 to 20.7 μg (Doets et al., 2013).

VitB12 deficiency is observed in 2–18% of obese patients (Saltzman and Karl, 2013). This number rises to 6–30% when, in addition to obesity, a proton pump inhibitor (PPI) is used (Parrott et al., 2017). As early as 2 months after RYGB, the vitB12 absorption capacity is markedly decreased with a negative homeostasis 4 months later (Kornerup et al., 2019). The first year after RYGB, a significant decrease of serum vitB12 level is reported in 17–60% of patients (Alexandrou et al., 2014; Mechanick et al., 2009; Souza et al., 2020; Vargas-Ruiz et al., 2008). Furthermore, Alexandrou et al. described that 4 years after RYGB 42.1% of patients suffered from vitB12 deficiency measured by vitB12 serum levels (Alexandrou et al., 2014).

A generally accepted explanation for vitB12 deficiency after RYGB is the small gastric pouch, where HCl and IF producing cells are both bypassed. This results in an inadequate gastric processing of food, as well as a low secretion of IF, which are both critical for normal vitB12 uptake (Behrns et al., 1994; Brito et al., 2018; Butler et al., 2006; Carvalho et al., 2012; Gesquiere et al., 2017; Parrott et al., 2017). After digestion and liberation of free Cbl, this vitamin binds to IF, whereupon the IF-Cbl complex undergoes a receptor mediated endocytosis in the distal small intestine (Alpers, 2016).

Under normal circumstances the uptake of Cbl from a single dose is limited to 1–2 μg by the amount of IF-Cbl receptor in the intestine (Brito et al., 2018). Apart from the IF-Cbl receptor, a low secretion of IF is also a well-known cause of Cbl malabsorption in pernicious anemia (Green et al., 2017).

After RYGB IF production is low and therefore less IF-Cbl complexes can be formed, resulting in impaired vitB12 absorption (Marcuard et al., 1989; Smith et al., 1993). Gesquiere et al. showed that initially after RYGB the dietary vitB12 intake is significantly decreased, but gradually increases until 12 months post-RYGB, still remaining below the preoperative intake values (Gesquiere et al., 2017).

In practice, oral vitB12-supplementation after RYGB is not always sufficient. As a result, the vitamin is frequently administered by intramuscular injections (Madhok et al., 2018; Worm et al., 2015). These injections can be painful for the patient and also the organization of frequent injections burdens the health care system. A systematic review by Mahawar et al. concluded that low-dose oral preparations (<= 15 μg) are inadequate as prophylaxis after RYGB (Mahawar et al., 2018). High-dose oral vitB12 (1000 mg) can be used instead, as was shown by several studies on healthy cohorts and pernicious anemia patients. High quantities of vitB12 guarantee its unspecific permeability (1–2%), bypassing the malfunctioning specific uptake (Green et al., 2017).

To our knowledge, no clinical trial has been performed in RYGB cohorts to evaluate the effect of high oral B12 with IF added. The current work attempts to address this issue.

2. Material and methods

2.1. Study design

This study is a randomized, monocentric, double blind, placebo-controlled trial. Equal numbers of patients were assigned to one of the three study groups: placebo, oral vitB12 supplement or vitB12 with IF (vitB12IF). The intervention started 2 days after the RYGB surgery and ended 6 months post-surgery. In addition to the study intervention, all patients were instructed to start their multivitamin (without vitB12) intake 6 weeks post-surgery and until 6 months post-surgery, as advised in the standard care after RYGB. Lab values were evaluated at the day of surgery and 6 months post-surgery.

2.2. Patient selection

Patients were screened during their bariatric pre-surgery consultation at the hospital. When compliant to all eligibility criteria, they were randomized into one of the three treatment arms. The inclusion criteria were: older than 18 years, given voluntary written informed consent and scheduled for RYGB. The exclusion criteria were: other bariatric procedure, post-surgery complications impeding oral intake of supplements, pre-surgery vitB12 status below the reference value (200 pg/mL) and the intake of other vitamin supplements.

2.3. Study products

The study products consisted of chewable tablets. According to the manufacturer (BariNutrics® vitamin B12 I.F., Metagenics) the vitB12 tablets contained 1000 μg methylcobalamin (Vitamine B12 1000 μg, Metagenics), whereas the vitB12IF tablets held 1000 μg methylcobalamin and 20 mg intrinsic factor. The placebo tablets contained maltodextrine. Patients were instructed to take 1 tablet from the second day onward after the surgery.

The standard therapy after bariatric surgery implies the intake of a multivitamin supplement. All patients were provided with a multivitamin without vitB12. This multivitamin has a specific composition tailored to the needs of bariatric patients and was supplied as a chewable tablet. The composition of this multivitamin is equal to the BariNutrics® Multi (Metagenics) chewable tablets, only without methylcobalamin. Patients were instructed to take 1 tablet a day, starting 6 weeks after surgery.

2.4. Data collection and follow-up

Patients were seen during 3 visits: pre-surgery, during hospitalization for RYGB surgery and 6 months after surgery. During the pre-surgery consultation, patients were screened for eligibility and assigned to one of the intervention groups. During the other 2 visits, the following assessments were performed: measurement of weight and height, concomitant medication and food supplements listing, assessment of general health and blood sampling. The blood analysis included folic acid, vitB12 and (for a subgroup of 50 patients)

Holotranscobalamin (HoloTC), also called "active vitB12".

Serum levels were compared to baseline (determined at the time of surgery) to analyze the impact of supplementation.

2.5. Sample size and randomization

In this study, 225 patients were enrolled and equally randomized (1-1-1) into one of the three treatment arms with 75 patients per arm. The sample size was based on a one-way ANOVA corrected for 3 pairwise comparisons to detect a difference (change from baseline after 6 months) in vitB12 levels based on previous supplementation data of the hospital. To detect a difference of 100 ± 180 (mean ± SD) ng/L vitB12 between the groups with a significance of 5% and a power of 90%, a sample size of 68 subjects per group was seemingly required. Considering a possible drop-out of 10% after 6 months, a total number of 75 patients per group was assumed.

For participant allocation, a computer-generated list of random numbers was created using a blocked randomization sequence with a random block size of two or three (R version 3.2.0 blockrand, (R Core Team, 2019).

2.6. Statistical analysis

The statistical analysis was performed by an independent statistician. A per-protocol analysis was used to measure the effect of the vitB12 intervention, excluding patients who received additional vitamin supplements containing vitB12 during the trial. Non-compliance (<80% study product intake) was also seen as a drop-out criterium.

For each study arm, all data were listed by subject and visit. The data were analyzed by their mean, standard deviation, median, minimum and maximum. Further analyses were conducted using Pearson and one-way ANOVA with Tukey HSD. For all categorical measures, the frequency (n/N) and proportions (%) based on the non-missing sample size is reported. All tests were carried out at a two-tailed 5% level of significance. Estimates of treatment effects are presented with 95% confidence intervals. No imputation of missing data was performed.

2.7. Ethical committee

The Study protocol was approved by the Medical Ethics Committee of AZ Sint-Jan, Bruges. Patients were recruited by the bariatric surgeons and residents at the centrum for obesity surgery at AZ Sint-Jan Hospital, Bruges. Informed consent was obtained from all individuals included in the study. The study was conducted in accordance to ICH GCP, the declaration of Helsinki and local regulatory guidelines.

3. Results

3.1. Study population

A total of 225 patients were randomized (1:1:1) over the three treatment groups. The per-protocol analysis included 159 subjects: 59 patients in the placebo group, 52 patients in the VitB12 group and 48 patients in the VitB12IF group. A drop-out of 66 patients (29%) corresponded to 44 who missed the follow-up (67%) plus 22 due to non-compliance (33%).

Mean age was 39.7 (SD ± 12.8) years, mean BMI 41.4 (SD ± 4) kg/m^2 and 71.7% of patients were female. At baseline, 32% of patients suffered from arterial hypertension, 11.9% from diabetes and 75.8% reported sporadic alcohol use (less than 1 glass a week). Six months post-surgery, a mean reduction of 33.8 (SD ± 9.1) kg resulted in a decline by 11.9 (SD ± 3.0) kg/m^2 BMI (Table 1).

Regarding medication post-surgery, PPI intake increased by 14.4% (from 17.8% to 32.2%).

3.2. Change in Vitamin B12 levels

The mean length of study medication intake was respectively 194.2 (SD ± 22.8) days, 193.5 (SD ± 28.5) days and 196.5 (SD ± 24.9) in the placebo, vitB12 and vitB12IF groups. In all three groups, similar levels of serum vitB12 were seen at baseline (Fig. 1, Table 2) with an overall mean serum level of 509 (SD ± 179.3) ng/L.

In the placebo group, a mean decrease of - 197.6 (SD ± 37) ng/L of serum vitB12 was seen while an increase in serum levels was observed in the vitB12 supplemented groups: + 154 (SD ± 97.8) ng/L and +202.3 (SD ± 113) ng/L in the vitB12 and vitB12IF group respectively.

The within group changes of pre- and postoperative vitB12 serum levels were statistically significant in all three groups (Table 2).

Comparison of post-surgery vitB12 serum levels of the three treatment groups revealed significantly higher serum levels in the vitB12 ($p < 0.001$) and vitB12IF ($p < 0.001$) groups than in the placebo group.

When comparing the post-surgery vitB12 serum levels in the vitB12 and vitB12IF groups, the 48.2 ng/L higher outcome in the vitB12IF group was not statistically significant ($p = 0.711$) due to a high dispersion of results.

Table 1
Demographics (mean ± SD).

Age	39.7 ± 12.8 years	
Gender	71.7% female	28.3% male
	Baseline	**6 months post-op**
Weight	117,9 ± 16,8 kg	84,2 ± 13,6 kg
BMI	41.4 ± 4 kg/m^2	29.5 ± 3.5 kg/m^2
PPI use	17.8%	32.2%

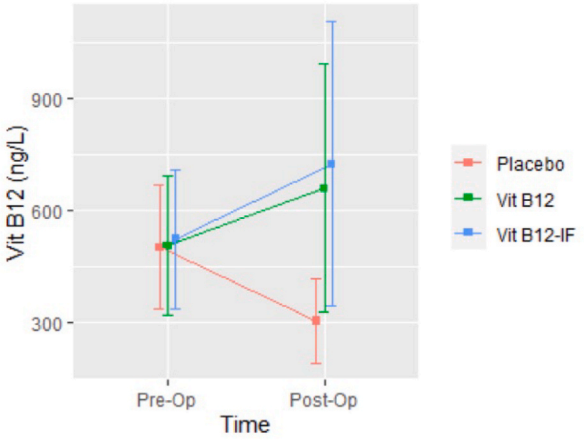

Fig. 1. Mean serum vitamin B12 levels (Mean ± SD) pre-op and post-op (6 months). The plot is based on all paired observations (N = 159).

Table 2
Serum vitamin B12 levels (mean ± SD, ng/L).

	Placebo (n = 59)	Vit B12 (n = 52)	Vit B12IF (n = 48)
Baseline	501.6 ± 166	506.1 ± 187.7	523.4 ± 188.6
6 months post-op	304.1 ± 112.5	660.2 ± 332.5	725.7 ± 382.0
Mean change	−197.6 ± 37	154.1 ± 97,8	202.3 ± 113
p-value	<0.001	0.002	<0.001

3.3. Secondary results

At the time of surgery, HoloTC or "active VitB12" was measured in a subpopulation of the trial. Of this subpopulation, most of the participants dropped out of the trial leading to too many missing values for the "active vitB12" analysis 6 months after surgery.

When comparing serum vitB12 levels with HoloTC levels, a positive correlation was found between serum vitB12 levels and HoloTC levels at baseline and 6 months post-surgery (Pearson's correlation 0.655 (df = 48) and 0.858 (df = 16), respectively).

No correlation was found between the change in serum vitB12 and the change in folic acid levels. Neither weight loss and post-RYGB serum vitB12 level nor baseline serum vitB12 level and PPI intake correlated to each other.

4. Discussion

This trial confirms the treatment efficacy of daily high dose oral vitB12 supplementation (1000 μg) without IF in the prevention of vitB12 deficiency after RYGB in patients with normal baseline vitB12 serum levels. This effect was also confirmed in a systematic review from 2006 with Cbl deficient patients (Butler et al., 2006). Schijns et al. reported similar plasma vitB12 concentrations after intramuscular injections or oral high-dose vitamin B12 (1000 μg methylcobalamin) (Schijns et al., 2018). Our results confirm research by Mahawar et al. stating that high-dose supplements are sufficient to keep vitamin B12 levels within the normal ranges (Mahawar et al., 2018).

The decline of serum B12 levels in the placebo group is mostly due to the lack of gastric acid and intrinsic factor production, which compromise vitamin B12 absorption from food (Brito et al., 2018). Yet, such individuals may absorb oral vitamin B12 if it is administered in a free form at a high level (or at any level if supplemented together with IF) (Kornerup et al., 2019).

The trend of higher serum vitB12 levels in the vitB12IF group compared to vitB12 alone is not statistically significant in this trial. In this regard we should discuss the quantities and the proportion of vitB12 and IF in the commercially available pills used in our study (B12 = 1 mg and IF = 20 mg according to the manufacturer). Such quantity of IF is sufficient to bind approximately 60% of vitB12 in the pill (≈600 μg), while the rest will remain in the free form. It is known that only 1–2 μg of B12 (bound to IF) can be absorbed per meal in the intestine (Brito et al., 2018). The rest of B12 (≈598 μg) will mostly remain bound to IF due to a high proteolytic stability of this protein (Fedosov et al., 2019). In such way, nearly 60% of the B12 dose (with IF added) will be constraint from the passive intestinal uptake. The overall balance of uptake for the aforementioned B12 and B12 + IF pills is expected to be 10–20 μg (passive diffusion) and 6–12 μg + 1–2 μg (passive + active uptake), respectively. In other words, presence of unphysiologically high IF cannot improve the uptake of vitB12, but might even hamper its intestinal adsorption. However, this reasoning cannot be confirmed by our results, suggesting a partial proteolytic processing of the excessive IF-B12 complex. The situation is noticeably different for small (physiological) quantities of B12 and IF. For example, Hvas et al. demonstrated that patients with evident vitB12 deficiency show a remarkably higher increase in HoloTC, if they are supplemented with vitB12 (3 × 9 μg) combined with slightly higher molar quantities of IF [24].

This trial confirms that passive diffusion due to a high oral vitB12 dosage compensates for the food-bound vitB12 malabsorption in

RYGB in patients with start values within the normal ranges. The equal efficacy of high-dose oral vitB12 supplements as opposed to intramuscular injections had already been demonstrated in multiple RCT's for vitB12 deficiency in the general population (Bolaman et al., 2003; Castelli et al., 2011). Results of this trial support extrapolation of these principles to the RYGB population. The significance and potential optimization of adding IF to high doses of oral vitB12 needs further research.

A prolonged intake of PPI has shown to inhibit food-bound vitamin B12 absorption by reducing gastric acid secretion (Allen, 2009; Behrns et al., 1994). This could not be confirmed in this study. Also the previously reported negative interaction between high serum folate and vitB12 biomarkers has not been found (Brito et al., 2018).

Earlier research (Coupaye et al., 2014; Gesquiere et al., 2017) showed that the significant decrease of dietary intake of vitamin B12 after RYGB relates to the lower caloric intake and intolerance to vitB12 rich food (for example red meat). Therefore, we expected a link between the amount of weight loss and vitB12 serum levels. This could not be confirmed in this study, but no detailed nutritional analysis was performed. Dietary vitB12 intake should be registered in future research to check for the relation between intake and serum vitB12 levels.

The oral intake of high dose vitB12 is considered safe on the long-term. Wolffenbuttel et al. documented a slight increase of all-cause and cardiovascular mortality with low vitB12 levels while high intake of vitB12 supplements was not associated with negative outcomes (Wolffenbuttel et al., 2020). In practice, lower dosed multivitamins could be considered following the initial start with high dose vitB12 supplements. A high dose vitB12 regimen could be proposed if lower dosed multivitamins seem to be insufficient.

Due to dropouts, no conclusion can be made regarding the effect of IF on serum HoloTC concentrations. As HoloTC is an active form of vitB12 in the body, it provides a better status of vitB12 for tissues (Nexo and Hoffmann-Lucke, 2011). Ledoux et al. reported an association between higher serum levels of Homocysteine and paresthesia, which is a typical symptom of vitB12 deficiency. This was not associated with low vitB12 levels (Ledoux et al., 2014). As Ledoux et al. did not measure HoloTC levels, this association could reflect low active vitB12 levels and thus not be related to total vitB12 levels in serum. Schijns et al. also analyzed Methyl malonic acid (MMA) and Homocysteine and concluded that no relationship could be seen between both parameters and serum vitB12 (Schijns et al., 2018). In literature, there is no clear consensus on what blood tests most adequately reflects vitB12 related symptoms.

Limitations of this trial cover limited follow-up time of 6 months and the 29% dropout. This undermines the power of the statistical analysis of the trial. An additional consideration is that if the compliance is already low in a clinical study, it is likely that this will be even worse in clinical practice. Dropouts reflect a significant problem in the bariatric surgery population, being non-compliant to therapy and post-surgical follow-up (Henfridsson et al., 2019; Ledoux et al., 2014). This observation emphasizes the importance of patient education and of a prolonged follow-up to expose the effect of long-term (non)supplementation.

5. Conclusion

This randomized controlled trial shows the efficacy of high-dose oral vitB12 supplements on increasing serum vitB12 levels and therefor avoiding vitB12 deficiency after RYGB surgery and bypassing the need for intramuscular injections.

There is no statistically significant benefit on serum vitB12 levels when adding IF to a high-dose oral vitB12 supplement, at least not for the vitB12 and vitB12IF commercial preparations used in our study.

Declining levels of serum vitB12 in the placebo group during this trial reconfirm the need for postoperative vitB12 supplementation. High-dose oral vitB12 supplementation should therefore be part of the standard postoperative care after RYGB.

CRediT authorship contribution statement

Melissa Ooms: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Greet Vanheule:** Conceptualization, Methodology, Formal analysis, Writing – original draft. **Karen Van Langenhove:** Conceptualization, Methodology. **Nick De Wever:** Writing – review & editing. **An-Katrien Vynckier:** Conceptualization, Resources. **Mieke Van Den Driessche:** Conceptualization, Resources. **Sebastiaan Van Cauwenberge:** Supervision. **Bruno Dillemans:** Supervision, Writing – review & editing.

Declaration of competing interest

Authors 2, 6 and 7 are employees of Metagenics Europe. The other authors declare that they have no conflict of interest.

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