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## **Behaviour of plasma citrulline after bariatric surgery in the BARIASPERM cohort**

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## Abstract

**Background:** Plasma citrulline is currently used in clinical practice as a marker of small bowel functional mass. Behaviour of plasma citrulline after bariatric surgery and its link with post-operative outcome are still poorly understood.

**Objective:** Primary objective was to compare plasma citrulline 12 months after two types of bariatric surgery with pre-operative concentrations. Secondary objectives were to search for correlation between plasma citrulline variation and body weight and fat mass loss.

**Design:** This is an ancillary study of the BARIASPERM study. Forty-six adult men (mean age  $38.9 \pm 7.9$  years) who underwent gastric bypass (GB, n=20) or sleeve gastrectomy (SG, n=26) were included in this prospective study. Plasma citrulline was measured at baseline, 6 months and 12 months after surgery, as well as total body weight and fat mass measured by Dual x-ray absorptiometry (DEXA).

**Results:** Plasma citrulline increased significantly 12 months after surgery, both after gastric bypass and sleeve gastrectomy (respectively 30.2% [18.3-42.2] and 17.8% [5.8-29.7]). The increase was significantly higher after GB than after SG ( $p=0.02$ ) while total body weight and fat mass loss were not significantly different between GB and SG. The increase in plasma citrulline levels tended to be positively correlated with both weight and fat mass loss however the association did not reach statistical significance ( $p=0.07$  and  $p=0.06$  respectively).

**Conclusion:** These results confirm the increase in plasma citrulline after GB published in two previous small studies. Citrulline also significantly increased after SG, and in spite of similar weight loss obtained with both surgery types, citrulline increase was higher after GB than SG. This suggests different modifications of intestinal functional mass after these two anatomically different techniques.

**Keywords:** Bariatric surgery, gastric bypass, sleeve gastrectomy, citrulline, functional enterocyte mass.

28

## 29 **Introduction**

30 Plasma citrulline is now currently used in clinical practice as a non-invasive marker of  
31 functional enterocyte mass in severe small bowel diseases such as short bowel syndrome (1),  
32 villous atrophy (2,3), radiation enteritis (4,5) and other alterations of small bowel absorptive  
33 function(see ref. (6) for a recent review on this topic). Citrulline is a non protein amino acid  
34 involved as an intermediary product in the urea cycle (7, 8). Plasma citrulline is mainly  
35 produced in small bowel enterocytes through the glutamate pathway and is considered not to  
36 depend on regimen (9). Fasting venous concentration of plasma citrulline varies between 20  
37 and 60  $\mu\text{mol/L}$  in healthy subjects (10). In patients with intestinal failure, it has been shown to  
38 correlate with the remnant functional enterocyte mass, independently of the nutritional and  
39 inflammatory status (11). Citrulline concentration increases in patients with renal failure as a  
40 consequence of its renal metabolism (8,11). As citrulline can increase from a glomerular  
41 filtration rate below 50 ml/min, it is commonly slightly elevated in patients over 70 years of  
42 age (10). In the field of obesity, it has been shown in a cohort of 70 severely obese patients  
43 that citrulline concentration is increased in patients with type 2 diabetes versus non-diabetic  
44 patients (12). Bariatric surgery can take the form of a restrictive (SG) or malabsorptive  
45 technique (which combines reduced stomach volume and intestinal bypass) (GB). Behaviour  
46 of plasma citrulline after bariatric surgery is still unclear. A preliminary study in 20 patients  
47 first showed that citrulline decreased by 20% but stayed in the normal range three months  
48 after a Roux-en-Y gastric bypass (13). This was confirmed in a transversal study in 13  
49 patients who had malabsorptive bariatric surgery in the past with citrulline levels comparable  
50 to controls after a mean follow-up of 43 months (14). In a more recent series of 30 patients  
51 undergoing RYGB, citrulline concentrations significantly increased over time after surgery,  
52 and citrulline was higher in patients who had successfully lost weight compared to patients in  
53 whom RYGB had failed (15). Studying the behaviour of citrulline after different types of  
54 bariatric surgeries could therefore allow a better understanding of the links between intestinal

functional modifications and generated weight loss. We investigated plasma citrulline changes at 6 and 12 months in 46 patients from the BARIASPERM cohort undergoing either Roux-en-Y GB or SG. Our main outcome was the variation of plasma citrulline at 12 months after surgery according to the two surgery types. The secondary goals were to study the relation between citrullinemia behaviour and the surgical outcome (weight loss and fat mass loss).

## **Subjects and methods**

The present work is an ancillary study of the BARIASPERM cohort (16) which included 52 male patients from 5 different hospital centers undergoing either GB or SG. The 46 patients who have completed the one year follow-up were included in the present study.

Twenty patients underwent a GP (19 Roux-en-Y and 1 Omega bypass) and 26 underwent a SG, according to a joint decision between the surgeon, the reference physician and the patient. In patients operated by Roux-en-Y GB, length of alimentary limb ranged from 1.2 to 1.5 m (mean length 1.4 m, standard deviation 0.15 m).

Plasma citrulline was measured in these subjects, before surgery, and during follow-up at 6 months and 12 months after surgery.

Anthropometric variables were measured according to a standardized protocol. Fat mass was measured by bioelectrical impedance analysis (SECA, Belgium). Blood samples were drawn before surgery, 6 months, and 12 months after surgery. Plasma citrulline was measured in all patients in post-absorptive state (after 8 hours of fasting) by ion exchange chromatography with post-column ninhydrine derivatization using an Aminotac Jeol apparatus (Jeol, Tokyo, Japan). Plasma glucose was measured routinely.

## **Ethics**

Written informed consent was signed by all participants prior to enrollment. The study was approved by the local ethic committee (CPP2012-08-01), and was performed in accordance

with the guidelines proposed in the Declaration of Helsinki (2000) of the World Medical Association.

## Outcome

The primary outcome was the variation of plasma citrulline concentration 12 months after surgery. We also assessed the impact of surgery type, total body weight loss, fat mass loss and of the presence of diabetes prior to surgery on citrulline variation.

## Statistical analysis

Association between baseline citrulline and clinical covariates was tested with linear regression analysis for continuous covariates and Student t-test for categorical covariates. The evolution of citrulline during follow up was modeled by a linear mixed effects model using the R library nlme, the latter model was used to test the possible impact of surgery type and other covariates on citrulline evolution after surgery compared to baseline. The association between post-operative citrulline changes and weight/fat mass loss was studied by linear regression analysis. All tests were conducted with a level of significance of 0.05.

## Results

Participants mean age and BMI were 38.9 ( $\pm 7.9$ ) years old and 44.1 ( $\pm 5.5$ ) kg/m<sup>2</sup> respectively as shown in **Table 1**. 58% of patients had known diabetes mellitus before surgery. Mean plasma citrulline before surgery was 27.7  $\mu\text{mol/L}$  ( $\pm 7.9$ ). Four patients had a plasma citrulline below 20  $\mu\text{mol/L}$  before surgery (**Figure 2A**). Baseline citrullinemia did not differ according to age at inclusion and baseline BMI (**Figure 2B and 2C**). Diabetic patients did not have a significantly different plasma citrulline at baseline than non-diabetic patients ( $p=0.058$ ). Mean plasma citrulline was not different at baseline between the two surgery type groups (**Figure 3A**).

Plasma citrulline levels significantly increased both in patients operated by SG ( $p<0.01$ ) and by GB ( $p<0.001$ ) (Figure 2A). Indeed when tested in the linear mixed effect model, the increase in citrulline at 12 months was significantly higher after GB than after SG ( $p=0.02$ ) (Figure 3B).

Both bariatric surgery types resulted in a massive decrease in weight and fat mass at 6 and 12 months, with no significant difference (Table 2). The increase in plasma citrulline levels with time tended to be correlated with both weight loss and fat mass loss (Figure 4A and 4B), however the association did not reach statistical significance ( $p=0.07$  and  $p=0.06$  respectively for correlation with weight loss and fat mass).

Patients with diabetes did not have a significantly different increase in citrulline levels 12 months after surgery (Supplementary figure 2). Similarly no difference was found according to age, weight or fat mass at baseline (data not shown).

## Discussion

This is the largest study to date that measures variations of plasma citrulline 6 and 12 months after bariatric surgery. In addition, to our knowledge, it is the first study to compare the evolution of citrulline in the two main types of bariatric surgery i.e. gastric bypass and sleeve gastrectomy. Plasma concentrations of citrulline at baseline were comparable with the general population. However four subjects had a plasma citrulline lower than  $20\text{ }\mu\text{mol/L}$  which might suggest unknown intestinal failure but could be simply explained by un-compliance to the fasting period before blood sampling, as plasma citrulline concentration has been shown to decrease after food intake (10). Indeed clinical data of these four patients did not suggest any reason to any decrease functional enterocyte mass. By contrary to one previous report, we did not find a significant increase in baseline concentrations of citrulline in patients with diabetes compared to non-diabetic ones (12). Interestingly we confirmed the significant increase in citrulline levels with time 12 months after gastric bypass and for the very first time, we shown that the increase is observed in both GB and SG. The mechanism underlying this increase in

135 plasma citrulline is still unclear and suggests modifications of the small bowel function after  
136 bariatric surgery. In these two surgery types, the totality of the jejunum is left in place but the  
137 montages are very different. In particular, it is worth noting that an upstream intervention  
138 such as sleeve gastrectomy could affect downstream physiology. This has been previously  
139 described in gastric surgery and could be explained by a downstream effect on amino  
140 absorption in the gut (17). The significantly higher increase of citrullinemia after GB  
141 compared with SG, despite comparable weight and fat mass loss, could suggest that  
142 functional intestinal modifications induced by each of these two very different types of  
143 surgery are different. Malabsorption induced by GB may result in greater compensatory  
144 growth of the enterocyte mass. Indeed Cavin et al. (18) have demonstrated intestinal  
145 adaptation after two types of gastric bypass with intestinal hyperplasia, increased jejunum  
146 diameter, higher villi, and deeper crypts than in sham non operated rats. We could also  
147 hypothesize that malabsorption could play an additional role in compensatory action  
148 compared to restriction alone. Furthermore, it was healthy small bowel and therefore with an  
149 ability to compensate for the intact enterocyte mass. During the first year after bariatric  
150 surgery, the catabolism and weight loss are at maximal rate but we can suppose that the signal  
151 for this compensatory growth of enterocyte mass is also at maximal rate. The impact of this  
152 intestinal adaptation after surgery on long term weight regain remains to be investigated.  
153 Although the effect did not reach statistical significance due probably to the relatively small  
154 sample size, we showed a positive correlation between the post-operative increase in citrulline  
155 and both weight loss and fat mass loss. We also looked at whether the change in the level of  
156 inflammation or insulin after surgery bariatric could influence our results and it was not the  
157 case. Even if causality cannot be formally proven from these data, it is possible that the  
158 functional modifications underlying the increase in citrulline are associated with the  
159 effectiveness of bariatric surgery. It is thus tempting to suggest that citrullinemia could be  
160 used as a potential biomarker of the surgical outcome but its interpretation in clinical practice  
161 remains to clarify. In addition, large fat mass is associated with low grade inflammation (19,



20) which in turn leads to a decrease in lean mass (21). This organ cross-talk is mediated by adipokines but also by pro-inflammatory cytokines synthesized and released by immune cells. Actually, in immune cells citrulline is both the product and the precursor of nitric oxide (22–24) a major player of non-specific immunity. Thus, bariatric surgery, by reducing systemic inflammation, decreases the use of citrulline by immune cells thus causing an increase in its plasma levels. The study of citrulline metabolism in gut-associated lymphoid tissue (GALT) before and after bariatric surgery should be therefore of major interest to get mechanistic data. There are several limitations to our study. First, the sample size is limited and caused likely a lack of statistical power. The BARIASPERM cohort included only male patients, the generalization of these results to women is therefore unsure especially because the intestinal synthesis of citrulline is affected by the gender (25). Follow-up was limited to 12 months after surgery and we did not record markers of renal function, which is known to impact the plasma concentration of citrulline (26).

In conclusion this study strengthens the idea of an increase in plasma citrulline 12 months after gastric bypass and sleeve gastrectomy. We found that gastric bypass was followed by a significantly higher citrulline increase than sleeve gastrectomy, despite comparable weight loss, suggesting distinct modifications of intestinal function after these two anatomically different techniques.

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182 The authors declare no relevant conflict of interest.

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184 Authors contribution

185 F. Mifsud done the statistcal analysis and wrote de manuscript

186 N. Neveux was responsible for citrulline analysis and corrected the manuscript.

187 L. Cynober had a significant contribution correcting the manuscript.

188 C. Carette, S. Czernichow, C. Rives-Lange had correcting the manuscript

189

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## Tables

**Table 1. Characteristics of the 46 study participants at baseline**

Characteristics at inclusion	Surgery group		Both groups (n=46)
	Gastric By-pass (n=20)	Sleeve Gastrectomy (n=26)	
Anthropometrics measures, mean (SD)			
Age, years	41.7 (6.9)	36.8 (8.0)	38.9 (7.9)
Weight, kg [Range]	138.4 (18.2) [106-178]	141.8 (23.5) [106-192]	140.3 (21.2) [106-192]
BMI, kg/m²	44.1 (4.9)	44.1 (6.0)	44.1 (5.5)
Waist circumference, cm [Range]	140.1 (12.1) [117-158]	137.5 (15.0) [113-171]	138.6 (13.8) [113-171]
Fat mass percentage (%)	41.4 (7.0)	40.6 (7.3)	41.0 (7.1)
Fasting Laboratory values, mean (SD)			
Fasting glucose, mmol/L	6.0 (2.1)	6.1 (1.7)	6.1 (1.8)
Known diabetes mellitus (n)	Yes=5, No=9 NA=6	Yes :10, No : 7, NA=9	Yes :15, No :16, NA=15
Plasma citrulline, umol/L	29.5 (8.7)	26.1 (6.9)	27.7 (7.9)

**Table 2: Weight loss, fat mass loss and plasma citrulline variation according to surgery type (means and 95% confidence intervals)**

		6 months		12 months	
		Mean	95% CI	Mean	95% CI
Sleeve	Percent weight change	25.0	21.7 - 28.2	28.9	25.4 - 32.5
	Percent fat mass change	50.0	44.3 - 55.7	56.4	49.1 - 63.7
	Percent citrulline change	4.4	-4.8 - 13.7	17.8	5.8 - 29.7
Bypass	Percent weight change	24.9	21.3 - 28.4	29.1	25.5 - 32.6
	Percent fat mass change	47.3	39.4 - 55.3	54.3	45.7 - 62.9
	Percent citrulline change	11.8	-0.8 - 24.3	30.2	18.3 - 42.2