Journal of Clinical & Translational Endocrinology 36 (2024) 100357

Contents lists available at ScienceDirect

Journal of Clinical & Translational Endocrinology

journal homepage: www.elsevier.com/locate/jcte

Effects of *Lacticaseibacillus rhamnosus HA-114* probiotic supplementation on circulating IGFBP-2 levels during a calorie-restricted diet in overweight humans

Justine Faramia [a,b], Béatrice S.-Y. Choi [a,c], Lucie Brunelle [d], André Marette [a,c,e], Vicky Drapeau [a,c,f], Angelo Tremblay [a,c,d,*], Frédéric Picard [a,b,*]

[a] Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec – Université Laval, Québec, QC, Canada
[b] Faculty of Pharmacy, Université Laval, Québec, QC, Canada
[c] Centre Nutrition, santé et société (NUTRISS) and Institute of Nutrition and Functional Foods (INAF), Université Laval, Québec, Canada
[d] Department of Kinesiology, PEPS, Université Laval, QC, Canada
[e] Department of Medicine, Faculty of Medicine, Université Laval, Québec, QC, Canada
[f] Department of Physical Education, Faculty of Education, Université Laval, QC, Canada

ARTICLE INFO

Keywords:
Insulin-like growth factor
Binding protein
Probiotics
Lacticaseibacillus rhamnosus
Eating behaviors
Humans
Weight loss
Microbiota
Circulating

ABSTRACT

Background and aim: Gut microbiota influences energy homeostasis in part through circulating hormones. Insulin-like growth factor-binding protein (IGFBP)-2 is a biomarker whose increase in systemic circulation is associated with positive effects on body weight and metabolism. In a recent clinical trial, probiotic *Lacticaseibacillus rhamnosus* HA-114 supplementation showed positive effects on eating behaviors and insulin resistance in overweight participants undergoing a weight-loss intervention. In this context, this ancillary study aimed at assessing the impact of *L. rhamnosus* HA-114 supplementation on plasma IGFBP-2 levels in these individuals, and whether this modulation correlated with changes in fat mass, energy metabolism, and eating behaviors.

Methods: Fasting plasma IGFBP-2 concentrations were quantified in 100 overweight or obese men and women enrolled in a 12-week diet-based weight reduction program (−500 kcal/day), in combination with probiotic *L. rhamnosus HA-114* or placebo supplementation. Baseline and changes in circulating IGFBP-2 concentrations were correlated with anthropometric parameter, glucose and lipid metabolism, cardiorespiratory function and eating behaviors.

Results: On average, the intervention reduced BMI by 4.6 % and increased IGFBP-2 by 13 %, regardless of supplementation group. Individuals who presented an increase in IGFBP-2 levels had significantly greater reductions in BMI. Changes in IGFBP-2 levels were correlated with loss in fat mass ($r = 0.2$, $p < 0.001$) in the probiotic-supplemented group, but not with other metabolic parameters or eating behaviors. Baseline IGFBP-2 levels were not associated with weight loss or improvements in cardiometabolic parameters.

Conclusion: Probiotic supplementation with *L. rhamnosus HA-114* did not modulate plasma IGFBP-2 levels. Changes in IGFBP-2 levels were correlated with greater reductions in BMI, but not with other metabolic parameters or eating behaviors, indicating that the benefits of HA-114 on eating behaviors are likely independent of IGFBP-2. Additional changes in microbiota might be required to modulate IGFBP-2 and observe its associations with eating behaviors and cardiometabolic improvements.

Introduction

The incidence of obesity and its comorbidities has rapidly and steadily increased in the past years, stressing the need for new therapeutic and preventive measures [1]. Quality of food and eating behaviors are factors that play an important role in the development of obesity [2]. Emerging evidence indicates that low-quality food is associated with a poorly diversified microbiota, which can impact body weight and alter metabolic functions by modulating gut and brain hormones [3,4]. Moreover, altered microbiota can induce a state of

* Corresponding authors.
E-mail addresses: Angelo.Tremblay@kin.msp.ulaval.ca (A. Tremblay), Frederic.Picard@criucpq.ulaval.ca (F. Picard).

https://doi.org/10.1016/j.jcte.2024.100357
Received 12 January 2024; Received in revised form 7 May 2024; Accepted 10 June 2024
Available online 11 June 2024

dysphoria, which changes eating behaviour in a reciprocal relation contributing to the initiation of weight gain [5,6]. Analysis of dysbiosis in disease states and fecal transplantation experiments have shown that gut microbiota can modulate energy metabolism directly through the vagus nerve or indirectly by impacting metabolite production [7–9]. On this basis, probiotic supplementation is considered a potential strategy to prevent obesity or associated disorders [1,10,11].

Insulin-like growth factor (IGF) binding protein (IGFBP)-2 is an hepatokine also produced by the gut and associated with positive effects on glucose and lipid metabolism [12]. Low plasma IGFBP-2 levels (under ~ 200 ng/mL) are observed in obese and diabetic patients as well as in individuals with high-risk cardiometabolic profiles [13,14]. Following a one-year lifestyle modification program aimed at weight loss, circulating IGFBP-2 levels were shown to increase by 43 %, along with improvements in insulin resistance and lipid metabolism [15]. Consistently, in mice, IGFBP-2 overexpression prevented against age-induced and diet-induced obesity and insulin resistance [16]. Notably, IGFBP-2 levels are robustly increased by bariatric surgery, especially after malabsorptive procedures such as Roux-en-Y gastric bypass (RYGB) and biliopancreatic diversion with duodenal switch (BPD-DS), rising a 6-fold in humans within 6 months after surgery [17,18], whereas *Igfbp2* gene deletion in mice attenuated the early metabolic improvements caused by bariatric surgery [17]. Interestingly, bacterial richness is enhanced after bariatric surgery, more specifically with a significant increase in the major *Lacticaseibacillus* (Firmicutes phylum*)* and *Bifidobacterium* (Actinobacteria phylum) genera [19]. Changes in the relative amount of these two genera have been associated with surgery-induced improvements in energy balance, body composition, and glucose and lipid metabolism [20]. In particular, *Lacticaseibacillus rhamnosus* has been identified as an anxiolytic species in animals [21], and thus may influence food intake and eating behaviors in the context of weight loss. Altogether, these data suggest that increasing circulating IGFBP-2 levels may be beneficial in obesity prevention or management and its comorbidities, especially when gut microbiota is modified after energy restriction caused by nutritional or surgical approaches.

Because IGFBP-2 is also produced in the intestine, the parallel changes in both gut microbiota composition and the increase in IGFBP-2 levels reported after bariatric surgery suggest that IGFBP-2 levels could be modulated by gut microbiota, and/or that changes in IGFBP-2 could also potentially contribute in parts to the beneficial effects of a healthy gut microbiota. However, the mutual relationships between gut microbiota and circulating IGFBP-2 levels remain unknown. We thus hypothesized that the supplementation with a specific probiotic strain could positively impact circulating IGFBP-2 levels, which may in turn support improvements in cardiometabolic health.

To assess this hypothesis, we analysed IGFBP-2 levels in plasma samples from a previously described cohort of healthy participants with overweight and obesity enrolled in a 12-week calorie restricted weight loss program (− 500 kcal/d) and randomized to receive either *Lacticaseibacillus rhamnosus* HA-114 or placebo [22]. In this cohort, *L. rhamnosus* HA-114 supplementation resulted in a significant decrease in the circulating levels of insulin, LDL-cholesterol and triglycerides in the probiotic-supplemented group only, along with a reduction in Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index [22]. In addition, significant beneficial effects of the HA-114 supplementation were observed of eating behaviors and mood-related factors, including decrease in binge eating tendencies and disinhibition and food-cravings [22]. In the present ancillary analysis, baseline and changes in circulating IGFBP-2 levels were correlated with modifications in anthropometric and cardiometabolic parameters, as well as food-related behaviors.

Materials and methods

Cohort

The present post-hoc analysis was conducted using plasma samples obtained from a cohort whose details about volunteer recruitment and approval by the institutional ethics committee of Université Laval were previously described [22].

Briefly, the original study was designed as a randomized, double-blind, placebo-controlled 12-week clinical trial conducted in 152 overweight or obese (BMI between 27.0 and 39.9 kg/m^2) men and women, aged between 18 and 55 years old [22]. Volunteers followed a personalized program to lower their calorie intake by 500 kcal/day and were concomitantly treated daily with a placebo or a similar capsule containing 10.10^9 CFU of probiotic *L. rhamnosus* HA-114 (provided by Lallemand Health Solutions, Montreal, QC, Canada), for 12 weeks. Importantly, baseline characteristics of participants were similar between the placebo and the probiotic groups (Supplementary Table 1). Capsules were kept refrigerated all along the intervention, and participants were asked to ingest them during a meal, not exceeding one capsule per day. The absolute presence of *L. rhamnosus* HA-114 in fecal samples could only be detected in the probiotic group at the end of the intervention [22], confirming the compliance of volunteer and the proper conduct of the intervention. Volunteers were included in the initial study following several specific criteria: sedentary or moderately active individuals (<90 min physical activity per week) with a stable body weight (<4.5 kg change within 3 months before the study) and had to stop consuming any laxatives, fermented foods and prebiotics during the study. Exclusion criteria were antibiotics intake or any treatments (medication or nutritional program) affecting body weight, food intake and/or energy expenditure, smoking or a history of drug and/or alcohol abuse (>9 drinks weekly). Women could not be in menopause, or known to be pregnant, breastfeeding, or planning on becoming pregnant in the 18 months after enrollment. Any of the following health conditions also resulted in exclusion: celiac disease, inflammatory bowel syndrome, short bowel syndrome or any other malabsorptive syndrome, uncontrolled angina within the past six months, type 1 diabetes, serious and/or unstable medical conditions (e.g. cardiovascular, renal, pulmonary, psychiatric diseases, bleeding disorders), cancer treatment within past six months, allergy to yeast, soy or milk, abnormal thyroid hormone levels, immune-compromised conditions, or chronic nausea, fever, vomiting, bloody diarrhoea or severe abdominal pain. The study was conducted in accord with the Declaration of Helsinki principles. Informed written consent was obtained from all participants prior to their inclusion in the study.

Data reported herein were obtained at baseline and at the end of the study immediately after the intervention. For 52 individuals, matched data for plasma IGFBP-2 levels at both baseline and end of the intervention were lacking, resulting in an analysis on 100 men and women.

Measurements

Before and at the end of the intervention (12 weeks ± 1 week), volunteers were subjected to a visit after an overnight fast and blood samples were collected and anthropometrics variables, blood pressure, heart rate, resting metabolic rate (indirect calorimetry) were measured exactly as described [22]. During visits, eating behaviors such as binge eating, disinhibition, susceptibility for hunger were determined using established Binge Eating questionnaire, the Three Factor Eating (TFEQ) and the Food Cravings (FCQ) Questionnaires before and after a standardised breakfast test meal (733 kcal for men and 599 for women, consisting of bread, butter, peanut butter, cheddar cheese, and orange juice) exactly as described [22].

Plasma IGFBP-2 concentrations were quantified by ELISA (Alpco kit #22-BP2HU-E01, Salem, NH, USA) according to the manufacturer's instructions. The detection limit was 0.2 ng/mL; the inter-assay

coefficient of variability was below 10 %. Plasma biochemistry and levels of other circulating factors described in this study were quantified as previously reported [22]. Using a portable chemistry analyzer (Piccolo Xpress, Abaxis inc.) glucose, total cholesterol, HDL-cholesterol, LDL- cholesterol, ALT and AST levels were measured according to the manufacturer's instructions. Ghrelin and leptin were quantified by ELISA (Human Leptin Instant ELISA Kit and Human Ghrelin ELISA Kit, Invitrogen, ThermoFisher Scientific). C-reactive protein and insulin were assessed by the biochemical analysis platform of the Quebec Heart and Lung Institute.

Statistical analysis

Data are expressed as the mean ± SD in text and tables and mean ± SEM in figures. Statistical analyses were performed with GraphPad Prism version 8.4.2 (GraphPad Software, La Jolla, CA, USA), using one or two-way ANOVA followed by Bonferroni post-hoc test for multiple comparisons as appropriate. Correlations were analyzed by bilateral partial correlation test adjusted or not with BMI, age and sex using JMP software (Cary, NC, USA). Differences were considered statistically significant at $p \leq 0.05$.

Results

Mild caloric restriction increases circulating IGFBP-2 levels independently of probiotic supplementation

Participants were 34 ± 9 years old and had a BMI of 32 ± 3 kg/m^2 at baseline (Suppl. Table 1). The dietary restriction regimen stimulated a similar extent of weight loss in both groups, with an average BMI reduction by 4.6 %. Baseline IGFBP-2 levels were under 200 ng/mL in both men and women (180 ± 82 ng/mL and 187 ± 100 ng/mL, respectively, p = 0.8). Baseline IGFBP-2 levels were not different between placebo and probiotics groups (Suppl. Table 1). The dietary regimen increased IGFBP-2 levels by 13 %, an average augmentation of 26 ± 70 ng/mL (p < 0.001), without any sex interaction (Fig. 1A). When analysed according to probiotic or placebo groups, plasma IGFBP-2 concentrations increased similarly over the 12-week study (Fig. 1B).

The change in circulating IGFBP-2 levels from baseline to week 12 (Δ IGFBP-2) was not dependent upon the treatment (probiotic vs placebo supplementation, p = 0.8) or baseline IGFBP-2 levels (low vs high levels, based on the median of 160 ng/mL, p = 0.6) (Fig. 1C). In this cohort, there were 37 IGFBP-2 responders, defined as participants showing an increase in plasma IGFBP-2 levels from baseline to week 12 (Δ IGFBP-2 > 0) and 63 non-responders who showed a reduction in plasma IGFBP-2 levels (Δ IGFBP-2 < 0). There was no difference in the IGFBP-2 responder status between treatment groups (p = 0.4), sex (p = 0.7) or their interaction (p = 0.1, Fig. 1D). These results clearly indicate that IGFBP-2 was modulated by the reduced caloric intake (−500 kcal/day) of the nutritional intervention but not amplified by *L. rhamnosus* HA-114 supplementation.

IGFBP-2 "responders" exhibit larger weight loss but similar improvements in cardiometabolic parameters

To evaluate whether IGFBP-2 was implicated in the beneficial effects of dietary restriction and probiotics, the response in IGFBP-2 was first correlated with anthropometric parameters. Interestingly, the decrease in BMI associated with the nutritional intervention was 30 % higher in IGFBP-2 responders (p = 0.05, Fig. 2A), but when divided into treatment groups (placebo or probiotic supplementation), this observation was no longer significant (Fig. 2B). Nonetheless, IGFBP-2 response (Δ IGFBP-2) was still positively associated with BMI reductions in the probiotic group (r^2 = 0.2, p < 0.001, Fig. 2C). Similarly, in the probiotic group, the IGFBP-2 responder status was slightly positively associated with loss of fat mass (r^2 = 0.1, p < 0.01, Fig. 2D, E) but not lean mass (Fig. 2F).

The IGFBP-2 responder status was next correlated with cardiometabolic parameters (Fig. 3). Overall, IGFBP-2 responders did not show greater metabolic improvements (data not shown). When considering probiotic or placebo supplementation, changes in IGFBP-2 levels were weakly but significantly associated with reductions in insulinemia in the placebo group (r^2 = 0.1, p < 0.01, Fig. 3A) and glycaemia in the probiotic group (r^2 = 0.08, p = 0.03, Fig. 3B). However, no other association was observed between the changes in IGFBP-2 and those in HOMA index, lipid metabolism or cardiorespiratory variables (Fig. 3C-I). Taken together, these findings indicate that, within the present cohort, changes in IGFBP-2 are associated with diet-induced loss of weight but are not correlated with important changes in glucose, lipid or cardiorespiratory variables.

Eating behaviors are improved with probiotic supplementation independently of IGFBP-2

Next, IGFBP-2 levels were correlated with modifications in eating behaviors. Regression analyses between IGFBP-2 response and changes in eating behaviors were tested in each treatment group (Fig. 4). Although probiotic supplementation by itself had a positive effect on binge eating (BINGE), hunger (TFEQ_HUN), intention and planning to

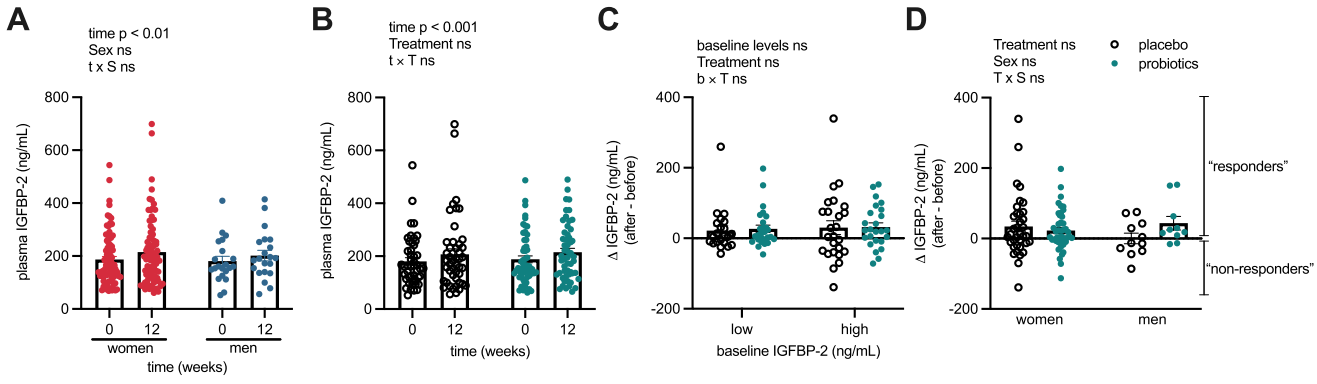

Fig. 1. Dietary restriction but not probiotic supplementation increases plasma IGFBP-2 levels independently of sex. Plasma IGFBP-2 levels before and 12-weeks after a mild energy restriction intervention in men (blue dots) and women (red dots) independently of the supplementation group (A). IGFBP-2 levels in the cohort were then analyzed before and after 12-weeks of diet dependently of the treatment, either in the placebo (black cercle) or probiotic group (turquoise dots) (B). IGFBP-2 modulation in each study arm was also analyzed based on median baseline IGFBP-2 levels (160 ng/mL) (C). Men and women were divided into "responders" when IGFBP-2 levels were increased after 12-weeks protocol (Δ IGFBP-2 > 0) and "non responders" when IGFBP-2 levels were decreased after 12-weeks protocol (Δ IGFBP-2 < 0). Bars are mean ± SEM. p < 0.05, ** p < 0.01. Baseline clinical characteristics are detailed in Supplementary Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

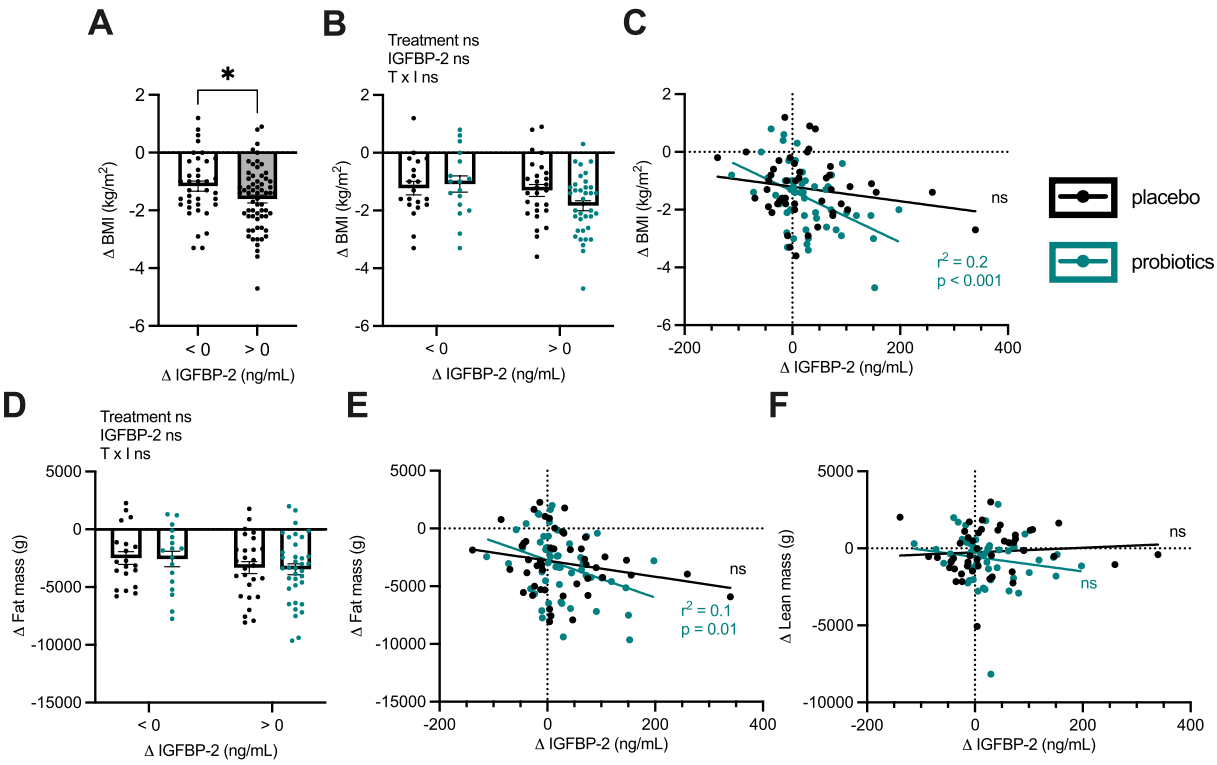

Fig. 2. Correlations between changes in IGFBP-2 levels and anthropometric parameters. Differences in BMI (A-C) and fat mass (D-F) before and 12-weeks after diet were analyzed according to IGFBP-2 response status ("responders" or "non responders") in the whole cohort (A) or for each treatment (placebo in black and probiotic supplementation in turquoise) (B, D, F, H). Correlations between IGFBP-2 response (Δ IGFBP-2) and BMI (C), fat mass (E) or lean mass (F) was then assessed in each treatment groups. * $p < 0.05$. Bars are mean ± SEM.

consume (FCQ-T_INT), lack of control (FCQ-T_CTR) and thoughts or preoccupation (FCQ-T_PEP) (Fig. 4A and Suppl. Fig. 1A, B, C, E, F), neither IGFBP-2 response (Δ IGFBP-2) or higher baseline IGFBP-2 levels were associated with a greater improvement of these parameters (Fig. 4). However, in the placebo group, baseline IGFBP-2 levels were negatively associated with binge eating ($r = -0.29$, $p = 0.05$), but this correlation was no longer significant when adjusted with age, sex and BMI (Table 1). After the 12-weeks program, IGFBP-2 levels were significantly and negatively associated with craving variables such as intention to consume (FCQ-T_INT), anticipation of relief from negative states (FCQ-T_ANM), lack of control (FCQ-T_CTR), thoughts or preoccupation (FCQ-T_PEP) or emotion-related cravings (FCQ-T_EAN) (Table.1). Again, these correlations were no longer statistically significant when adjusted for age, sex and BMI (Table 1). No significant correlation between IGFBP-2 levels and improvement of eating behaviors was observed in the probiotic group (Fig. 4B, D and Table.1), suggesting that the beneficial effects of HA-114 on eating behaviors are independent of IGFBP-2.

Baseline IGFBP-2 levels do not predict cardiometabolic improvements after weight loss

We then tested whether baseline IGFBP-2 levels (below or over the cohort median of 160 ng/mL) were associated with the magnitude in cardiometabolic improvements induced by dietary restriction and weight loss. Overall, baseline IGFBP-2 did not predict improvements in either anthropometric parameters such as BMI (Fig. 5A,B), loss of fat mass (Fig. 5C,D), or cardiometabolic parameters such as insulinemia (Fig. 5E,F) or triglyceridemia (Fig. 5G,H). Consistent with the lack of association between baseline IGFBP-2 levels and changes in eating behaviors (Suppl. Fig. 1), these additional data suggest that baseline IGFBP-2 concentrations are not predictive of the efficacy of the diet intervention, regardless of the study arm.

Discussion

How microbiota influences hormone production is still not well understood. Previous studies have shown that bariatric surgery, which greatly influences gut microbiota [20,23], also stimulates an increase in IGFBP-2 production, which in turn contributes to metabolic improvements [17]. In this study, direct supplementation with *L. rhamnosus* HA-114 probiotic strain was tested as a modulator of the circulating biomarker IGFBP-2, and changes in IGFBP-2 levels were analyzed for correlations with cardiometabolic improvements and eating behaviors.

In accordance with previous reports [13–15,17], we observed that overweight and obese participants display low IGFBP-2 levels (under 200 ng/mL), and that mild calorie restriction can increase IGFBP-2 levels by approximately 13 % after 3 months of nutritional intervention, in both men and women. Also consistent with previous findings [15], individuals with positive changes in circulating IGFBP-2 concentrations had a larger reduction in BMI. Larger increases in IGFBP-2 levels (43 %) have been achieved in a similar population of obese men (BMI ~ 31 kg/m^2) experiencing a 500 kcal/day deficit, but after a one-year intervention [15]. Thus, when compared with the 6-fold increase in plasma IGFBP-2 concentrations induced by bariatric surgery [17], nutritional dietary changes are significant but weaker modulators of circulating IGFBP-2 levels. The mechanisms for such differences are not yet understood. However, it suggests that the short duration of the present intervention (12 weeks) was not sufficient to induce major changes in IGFBP-2 levels. Combined with the latter premise, the relatively healthy cardiometabolic condition of the participants, which possibly reduced the potential magnitude of positive changes in most cardiometabolic parameters (including glucose or insulin levels, lipid metabolism, and blood pressure) [22], may explain, at least in part, the poor associations of these variables with circulating IGFBP-2 levels. Longitudinal studies with longer intervention periods might reveal stronger correlations between changes in IGFBP-2 and those in energy

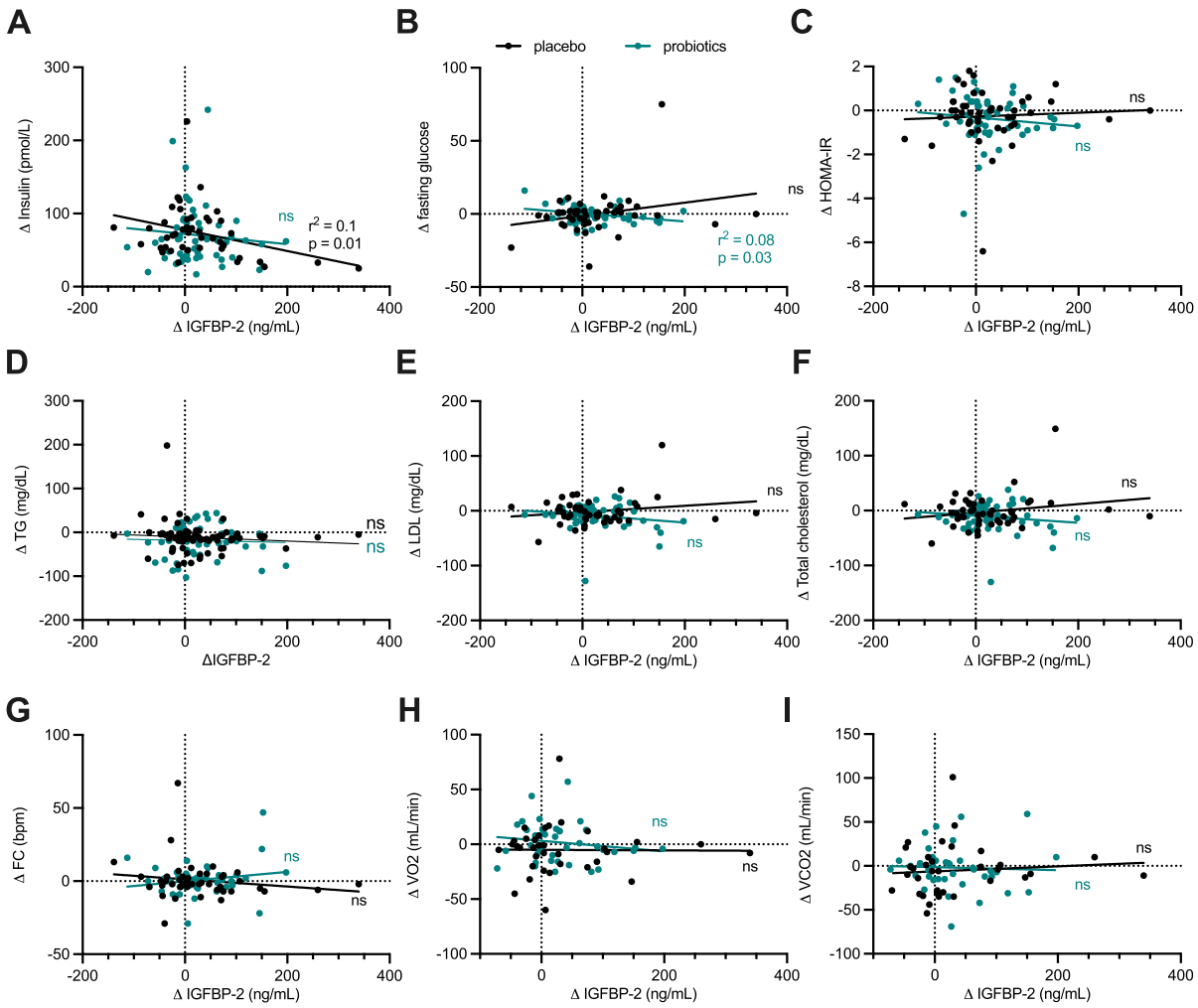

Fig. 3. Changes in IGFBP-2 levels do not correlate with cardiometabolic improvements. Correlation between IGFBP-2 response (Δ IGFBP-2) and parameters of glucose metabolism: insulinemia (A), glycaemia (B) and insulin resistance HOMA index (C); lipid metabolism: triglycerides (D), LDL cholesterol (E) and total cholesterol (F); and cardiorespiratory function: heart rate (G), O_2 and CO_2 max volumes (H,I).

metabolism.

One aim of this analysis was to assess the effect of *L. rhamnosus* HA-114 on circulating IGFBP-2 levels in humans with overweight and obesity in the context of a weight loss intervention. Indeed, available preclinical data suggest that probiotics could be useful to potentiate weight loss (or mitigate weight gain), and that this effect could be mediated by IGFBP-2. First, in high fat-fed mice, the weight loss and insulin sensitization effects of bariatric surgery were associated with increased circulating IGFBP-2 levels, and these benefits of bariatric surgery were attenuated in obese *igfbp2*-deficient mice[17]. In humans, circulating levels of IGFBP-2 were also increased after bariatric surgery, and also correlated with insulin sensitization [17]. In vitro and in vivo studies in male mice suggests that IGFBP-2 can modulate loss of weight by inhibiting preadipocyte differentiation and visceral but not subcutaneous fat development [24,25]. Another study showed that *Bifidobacterium pseudocatenulatum* supplementation for 7 weeks in a mouse model of diet-induced obesity mitigated weight gain, which was associated with an 63 % increase in hepatic IGFBP-2 levels [26]. In the current analysis, an increase in circulating IGFBP-2 was associated with a superior weight loss (reduced BMI and fat mass) when considering the cohort as a whole, and this effect was similar in both groups. The mechanisms by which higher IGFBP-2 is associated with weight reduction is currently not established, as previous reports in mice have observed that IGFBP-2 can changes food intake [17] or energy expenditure depending [16] on the context and sex. In addition, as *L. rhamnosus HA-114* probiotic supplementation did not potentiate the loss of weight in the subjects of the study [22], it suggests that the modulation of IGFBP-2 levels occurring during the course of the regimen is more likely related to the calorie-restricted diet. However, an important limitation of our study is that it cannot be excluded that the current probiotic intervention (12 weeks) could have been too short to capture beneficial effects on IGFBP-2 levels, that other probiotic strains may have been more potent, or that potential HA-114-induced modulation of IGFBP-2 production in the gut, brain or liver may have been impeded by proportional changes in degradation. Another limitation is that it is possible that the energy restriction intervention *per se* could target and saturate similar effectors as *L. rhamnosus HA-114* supplementation, which could mask additional outcomes on circulating IGFBP-2 levels. A complete analysis of the changes in gut microbiota induced by *L. rhamnosus HA-114* supplementation used in the present study would likely shed light on this issue. Further studies are needed to verify if manipulation of gut microbiota can modulate IGFBP-2 production in humans independently of weight loss.

To the best of our knowledge, the potential associations between IGFBP-2 and eating behaviors have not yet been studied in humans. Considering that the absence of IGFBP-2 in mice was shown to stimulate intake of low-energy density (chow) food over that of high-density diets [17] and that IGFBP-2 is also produced in the brain [27,28], this paradigm is even more interesting because positive changes in eating behaviors were among the largest effects of *L. rhamnosus HA-114*

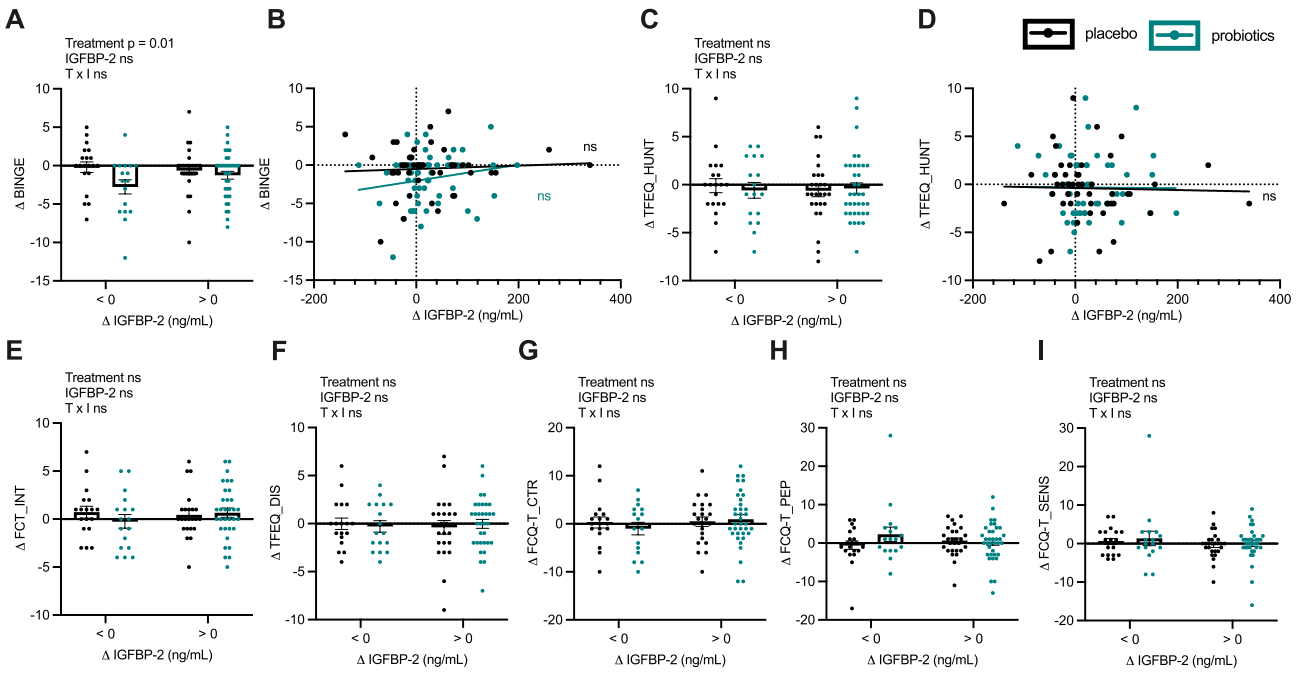

Fig. 4. Changes in IGFBP-2 levels do not correlate with improvements in eating behaviors. Differences in eating behaviors, namely binge scale (BINGE, A-B), hunger (TFEQ_HUN, C-D), intention and planning to consume (FCT_INT, E), disinhibition (TFEQ_DIS, F), lack of control (FCT_CTR, G), thoughts or preoccupation (FCT_PEP, H) and food cues from biological or environmental signals (FCT_SENS, I) before and after 12-weeks diet were analyzed according to the IGFBP-2 responder status and intervention (placebo in black and probiotic supplementation in turquoise). Bars are mean ± SEM.

Table 1
Regression analysis between plasma IGFBP-2 levels and eating behaviors before and after intervention with or without probiotics supplementation.

	Placebo						Probiotic					
	Before			After			Before			After		
	Non-adjusted		Adjusted*	Non-adjusted		Adjusted*	Non-adjusted		Adjusted*	Non-adjusted		Adjusted*
IGFBP-2 vs	**r**	**p**	**p**	**r**	**p**	**p**	**r**	**p**	**p**	**r**	**p**	**p**
BINGE	−0.29	0.05	NS	−0.27	0.07	NS	−0.03	0.84	NS	0.11	0.44	NS
FCQ-T_INT	−0.09	0.53	NS	−0.35	0.02	NS	−0.07	0.60	NS	−0.18	0.20	NS
FCQ-T_ANM	−0.18	0.24	NS	−0.29	0.05	NS	0.23	0.10	NS	0.03	0.82	NS
FCQ-T_CTR	−0.15	0.31	NS	−0.34	0.02	NS	−0.03	0.81	NS	−0.01	0.95	NS
FCQ-T_PEP	−0.19	0.20	NS	−0.29	0.05	NS	0.00	0.99	NS	−0.08	0.59	NS
FCQ-T_EAN	−0.21	0.15	NS	−0.36	0.01	NS	0.27	0.05	NS	−0.01	0.92	NS
FCQ-T_SEN	−0.15	0.33	NS	−0.17	0.25	NS	−0.02	0.88	NS	−0.13	0.36	NS
TFEQ_RES	−0.01	0.96	NS	0.06	0.71	NS	0.10	0.49	NS	0.04	0.75	NS
TFEQ_DIS	−0.17	0.24	NS	−0.27	0.07	NS	0.15	0.29	NS	0.06	0.65	NS
TFEQ_HUN	−0.24	0.10	NS	−0.35	0.02	NS	−0.03	0.81	NS	−0.05	0.72	NS

* Adjusted for age, sex and BMI.
FCQ: Food Craving Questionnaire – Trait; **TFEQ**: Three Factor Eating Questionnaire. **BINGE**: binge eating; **FCQ-T_INT**: intention and planning to consume; **FCQ-T_ANM**: anticipation of relief from negative states; **FCQ-T_CTR**: lack of control; **FCQ-T_PEP**: thoughts or preoccupation; **FCQ-T_EAN**: emotion-related cravings; **FCQ-T_SEN**: food cues from biological or environmental signals; **TFEQ_RES**: restraint; **TFEQ_DIS**: disinhibition; **TFEQ_HUN**: hunger.

supplementation in the cohort studied herein, notably a decrease in binge eating tendencies, disinhibition and food-cravings [22]. However, regression analyses indicated that IGFBP-2 levels were not associated with eating behaviors after adjustments for age, sex and BMI. Moreover, when divided on the basis of baseline or changes in IGFBP-2 levels, subjects were not characterized with better behaviors in either placebo or probiotic groups. Thus, although further experiments might reveal potential effects of IGFBP-2 on eating behaviors in different settings, the present findings suggest that IGFBP-2 levels did not contribute to the changes induced by energy restriction or *L. rhamnosus HA-114* supplementation.

In summary, the present study confirmed previous reports that IGFBP-2 is modulated by energy restriction and its associated weight loss. The small extent of these changes was not sufficient after a 12-week program to be associated with cardiometabolic improvements, even with probiotic supplementation. Increases in IGFBP-2 levels after intervention was associated with a greater loss of weight, but not of cardiometabolic function or eating behaviors, suggesting that IGFPB-2 did not mediate the beneficial effects of diet on these parameters within these experimental settings. However, the lack of data on other gut microbiota changes and the short duration of the study could have hindered a potential link with IGFBP-2 levels. Additional studies are needed to investigate relation between a long-term or different species change in gut microbiota and IGFBP-2.

ClinicalTrials.gov Identifier

NCT02962583

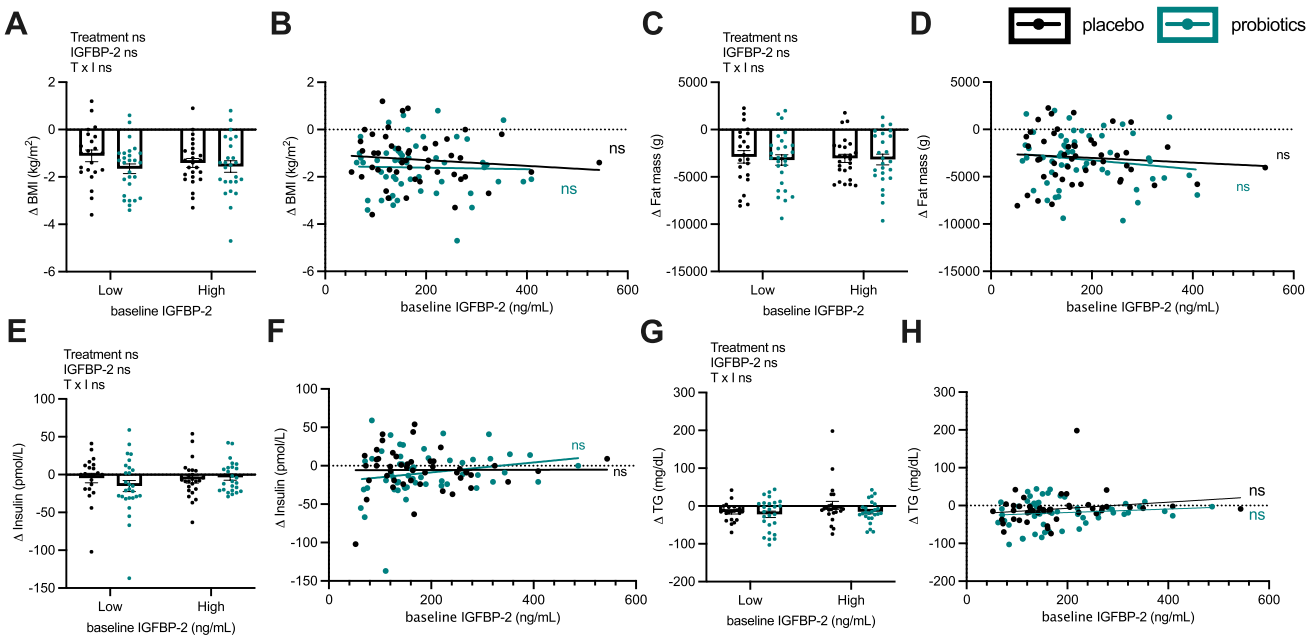

Fig. 5. Baseline IGFBP-2 levels do not predict cardiometabolic improvements. Cardiometabolic changes in BMI (A), fat mass (C), insulin (E) and TG levels (G) were analyzed according to baseline IGFBP-2 levels (based on median of 160 ng/mL) and intervention (placebo in black and probiotic supplementation in turquoise). Data were also probed for correlations between baseline IGFBP-2 levels and variations in BMI (B), fat mass (D), insulin (F) and TG levels (H) according to intervention group (placebo in black and probiotic supplementation in turquoise). Bars are mean ± SEM.

Disclosure statement

The authors have nothing to disclose. This analysis uses data and blood samples from a clinical trial in which Lallemand Health Solutions Inc. (LHS) was involved. LHS provided the probiotics and was involved in the study design and reviewing of the present manuscript.

Author statement

All authors have contributed to at least one part of the study, including planning, execution, analysis of the data, and redaction. All have read and approved the present version of the paper.

CRediT authorship contribution statement

Justine Faramia: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Béatrice S.-Y. Choi:** Writing – review & editing, Methodology, Investigation. **Lucie Brunelle:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **André Marette:** Writing – review & editing, Conceptualization. **Vicky Drapeau:** Writing – review & editing, Resources, Project administration, Formal analysis, Conceptualization. **Angelo Tremblay:** Writing – review & editing, Project administration, Formal analysis, Conceptualization. **Frédéric Picard:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Frederic Picard reports financial support was provided by Canadian Institutes of Health Research. This analysis uses data and blood samples from a clinical trial in which Lallemand Health Solutions Inc. (LHS) was involved. LHS provided the probiotics and was involved in the study design and reviewing of the present manuscript. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are thankful to Brunella Gonzalez Cautela (LHS) and Thomas Tompkins (LHS) for their contribution to the design and conduct/analysis of the original study [22], and to Annie Tremblay (LHS) for reviewing this manuscript. The present work was supported by operating grants to FP from the Canadian Institutes of Health Research (PJT-148550). JF was supported by a studentship from Fond de Recherche du Québec en Santé (FRQS). AT is the recipient of a Canada Research Chair in Environment and Energy Balance. AM is the recipient of a CIHR/Pfizer research Chair in the pathogenesis of insulin resistance and cardiovascular diseases. BSYC is funded by doctoral scholarships from the Sentinel North program at Laval University (Canada First Research Excellence Fund) and from the Natural Sciences and Engineering Research Council of Canada (NSERC).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcte.2024.100357.

References

[1] Van Hul M, Cani PD. The gut microbiota in obesity and weight management: microbes as friends or foe? Nat Rev Endocrinol 2023. https://doi.org/10.1038/s41574-022-00794-0.
[2] Jacob R, Provencher V, Panahi S, et al. Eating behaviour traits mediate the association between satiety responsiveness and energy intake among individuals with overweight and obesity. Appetite 2023;180:106373. https://doi.org/10.1016/j.appet.2022.106373.
[3] Tagliabue A, Elli M. The role of gut microbiota in human obesity: recent findings and future perspectives. Nutr Metab Cardiovasc Dis 2013;23:160–8. https://doi.org/10.1016/j.numecd.2012.09.002.
[4] Sanz Y, Rastmanesh R, Agostoni C. Understanding the role of gut microbes and probiotics in obesity: how far are we? Pharmacol Res 2013;69:144–55. https://doi.org/10.1016/j.phrs.2012.10.021.

[5] Alcock J, Maley CC, Aktipis CA. Is eating behavior manipulated by the gastrointestinal microbiota? evolutionary pressures and potential mechanisms. Bioessays 2014;36:940–9. https://doi.org/10.1002/bies.201400071.
[6] Takeuchi T, Kameyama K, Miyauchi E, et al. Fatty acid overproduction by gut commensal microbiota exacerbates obesity. Cell Metab 2023;35(361–375):e369.
[7] Bui TI, Britt EA, Muthukrishnan G, et al. Probiotic induced synthesis of microbiota polyamine as a nutraceutical for metabolic syndrome and obesity-related type 2 diabetes. Front Endocrinol 2022;13:1094258. https://doi.org/10.3389/fendo.2022.1094258.
[8] O'Donnell MP, Fox BW, Chao PH, et al. A neurotransmitter produced by gut bacteria modulates host sensory behaviour. Nature 2020;583:415–20. https://doi.org/10.1038/s41586-020-2395-5.
[9] Wahlstrom A, Sayin SI, Marschall HU, et al. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. Cell Metab 2016;24:41–50. https://doi.org/10.1016/j.cmet.2016.05.005.
[10] Liu H, Hu C, Zhang X, et al. Role of gut microbiota, bile acids and their cross-talk in the effects of bariatric surgery on obesity and type 2 diabetes. J Diabetes Investig 2018;9:13–20. https://doi.org/10.1111/jdi.12687.
[11] Delzenne NM, Neyrinck AM, Backhed F, et al. Targeting gut microbiota in obesity: effects of prebiotics and probiotics. Nat Rev Endocrinol 2011;7:639–46. https://doi.org/10.1038/nrendo.2011.126.
[12] Haywood NJ, Slater TA, Matthews CJ, et al. The insulin like growth factor and binding protein family: novel therapeutic targets in obesity & diabetes. Mol Metab 2019;19:86–96. https://doi.org/10.1016/j.molmet.2018.10.008.
[13] Heald AH, Kaushal K, Siddals KW, et al. Insulin-like growth factor binding protein-2 (IGFBP-2) is a marker for the metabolic syndrome. Exp Clin Endocrinol Diabetes 2006;114:371–6. https://doi.org/10.1055/s-2006-924320.
[14] Carter S, Li Z, Lemieux I, et al. Circulating IGFBP-2 levels are incrementally linked to correlates of the metabolic syndrome and independently associated with VLDL triglycerides. Atherosclerosis 2014;237:645–51. https://doi.org/10.1016/j.atherosclerosis.2014.09.022.
[15] Carter S, Lemieux I, Li Z, et al. Changes in IGFBP-2 levels following a one-year lifestyle modification program are independently related to improvements in plasma apo B and LDL apo B levels. Atherosclerosis 2019;281:89–97. https://doi.org/10.1016/j.atherosclerosis.2018.12.016.
[16] Wheatcroft SB, Kearney MT, Shah AM, et al. IGF-binding protein-2 protects against the development of obesity and insulin resistance. Diabetes 2007;56:285–94. https://doi.org/10.2337/db06-0436.
[17] Faramia J, Hao Z, Mumphrey MB, et al. IGFBP-2 partly mediates the early metabolic improvements caused by bariatric surgery. Cell Rep Med 2021;2:100248. https://doi.org/10.1016/j.xcrm.2021.100248.
[18] Li Z, Martin J, Poirier P, et al. Upregulation of plasma insulin-like growth factor binding protein 2 levels after biliopancreatic diversion in humans. Obesity 2012;20:1469–73. https://doi.org/10.1038/oby.2012.90.
[19] Guo Y, Huang ZP, Liu CQ, et al. Modulation of the gut microbiome: a systematic review of the effect of bariatric surgery. Eur J Endocrinol 2018;178:43–56. https://doi.org/10.1530/eje-17-0403.
[20] Kong LC, Tap J, Aron-Wisnewsky J, et al. Gut microbiota after gastric bypass in human obesity: increased richness and associations of bacterial genera with adipose tissue genes. Am J Clin Nutr 2013;98:16–24. https://doi.org/10.3945/ajcn.113.058743.
[21] Reis DJ, Ilardi SS, Punt SEW. The anxiolytic effect of probiotics: a systematic review and meta-analysis of the clinical and preclinical literature. PLoS One 2018;13:e0199041.
[22] Choi BS, Brunelle L, Pilon G, et al. Lacticaseibacillus rhamnosus HA-114 improves eating behaviors and mood-related factors in adults with overweight during weight loss: a randomized controlled trial. Nutr Neurosci 2022:1–13. https://doi.org/10.1080/1028415x.2022.2081288.
[23] Mukorako P, Lopez C, Baraboi ED, et al. Alterations of Gut microbiota after biliopancreatic diversion with duodenal switch in wistar rats. Obes Surg 2019;29:2831–42. https://doi.org/10.1007/s11695-019-03911-7.
[24] Xi G, Solum MA, Wai C, et al. The heparin-binding domains of IGFBP-2 mediate its inhibitory effect on preadipocyte differentiation and fat development in male mice. Endocrinology 2013;154:4146–57. https://doi.org/10.1210/en.2013-1236.
[25] Li Z, Miard S, Laplante M, et al. Insulin stimulates IGFBP-2 expression in 3T3-L1 adipocytes through the PI3K/mTOR pathway. Mol Cell Endocrinol 2012;358:63–8. https://doi.org/10.1016/j.mce.2012.02.022.
[26] Moya-Pérez A, Romo-Vaquero M, Tomás-Barberán F, et al. Hepatic molecular responses to Bifidobacterium pseudocatenulatum CECT 7765 in a mouse model of diet-induced obesity. Nutr Metab Cardiovasc Dis 2014;24:57–64. https://doi.org/10.1016/j.numecd.2013.04.011.
[27] Bonham LW, Geier EG, Steele NZR, et al. Insulin-like growth factor binding protein 2 is associated with biomarkers of alzheimer's disease pathology and shows differential expression in transgenic mice. Front Neurosci 2018;12:476. https://doi.org/10.3389/fnins.2018.00476.
[28] Elmlinger MW, Deininger MH, Schuett BS, et al. In vivo expression of insulin-like growth factor-binding protein-2 in human gliomas increases with the tumor grade. Endocrinology 2001;142:1652–8. https://doi.org/10.1210/endo.142.4.8084.