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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

Sophie Carter[a,b,2], Isabelle Lemieux[a,2], Zhuo Li[a,1], Natalie Alméras[a,c], Angelo Tremblay[a,c], Jean Bergeron[d], Paul Poirier[a,b], Jean-Pierre Després[a,c], 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] Department of Kinesiology, Faculty of Medicine, Université Laval, Québec, QC, Canada
[d] Endocrinology and Nephrology Unit, CHU de Québec-Université Laval Research Center, Québec, QC, Canada

HIGHLIGHTS

- A 1-year lifestyle modification program increased circulating IGFBP-2 levels by 43% in men.
- Changes in IGFBP-2 levels contributed to improvements in plasma apo B.
- This association was independent from those between IGFBP-2, insulin resistance, and fat mass.

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ABSTRACT

Background and aims: Recent transversal studies have associated insulin-like growth factor binding protein (IGFBP)-2 levels with glucose tolerance and parameters of the lipoprotein-lipid profile. Here, we aimed at determining the longitudinal effects of a one-year lifestyle modification program on IGFBP-2 levels and to identify specific metabolic improvements impacted by the changes in IGFBP-2.

Methods: 99 middle-aged Caucasian men were involved in a lifestyle modification program consisting in personalized healthy eating and physical activity counseling, combined to elicit a daily 500 kcal deficit. Anthropometric and metabolic parameters as well as circulating IGFBP-2 levels were measured before and after one year of the lifestyle modification program.

Results: The intervention triggered positive changes in many metabolic parameters and a 43% ($p < 0.0001$) increase of IGFBP-2 levels. Subjects with the most substantial increases in IGFBP-2 also experienced the most important metabolic improvements. Changes in IGFBP-2 levels (both absolute and relative) were correlated with markers of body fat distribution and lipoprotein-lipid profile, and independently associated with changes in LDL apolipoprotein (apo) B but not VLDL apo B concentrations. Further analyses showed that for similar changes in BMI, waist circumference and visceral adipose tissue volume, large changes in IGFBP-2 levels were required to observe improvements in LDL apo B levels.

Conclusions: The 1-year lifestyle modification program was associated with increased IGFBP-2 concentrations. Increases in IGFBP-2 levels were closely associated with reduced LDL apo B concentrations and independently of the modifications in fat mass and insulin sensitivity. Further mechanistic studies are required to assess the effects of IGFBP-2 levels on LDL metabolism.

Abbreviations: Apo, apolipoprotein; AT, adipose tissue; AUC, area under the curve; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IGFBP, insulin-like growth factor binding protein; LDL, low-density lipoprotein; TG, triglycerides; VLDL, very-low-density lipoprotein

* Corresponding author. Institut universitaire de cardiologie et de pneumologie de Québec, Université Laval, 4255, 2725, chemin Sainte-Foy, Québec, QC, G1V 4G5, Canada.
E-mail address: Frederic.Picard@criucpq.ulaval.ca (F. Picard).
[1] Current address: Endocrinology and Metabolism Department, The First Hospital of Jilin University, Jilin, Changchun, China 130021.
[2] These authors contributed equally to this work.

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1. Introduction

The increase in the prevalence of obesity has been associated with a concomitant rise in the occurrence of metabolic diseases such as dyslipidemia, type 2 diabetes and hypertension. These metabolic dysregulations increase cardiovascular risk via the overproduction of apolipoprotein (apo) B-containing triglyceride (TG)-rich very-low-density lipoproteins (VLDL), decreased peripheral vascular catabolism of VLDL, and increased quantities of small, atherogenic LDL particles [1]. Studies conducted in humans have shown that visceral and hepatic fat accumulation are associated with decreased insulin sensitivity and increased VLDL secretion, suggesting that lipid accretion in these compartments contributes to the development of dyslipidemia [2,3]. In parallel, visceral obesity is characterized by a decreased peripheral catabolism leading to a higher LDL particle number (as assessed by apo B levels) and to the formation of small, dense LDL [4]. These alterations in the lipoprotein-lipid profile are also associated with a decreased cardiorespiratory fitness [5]. Recently, an increased cardiorespiratory fitness was shown to delay the onset of age-related dyslipidemia [6], suggesting its protective role in the development of metabolic alterations.

Lower circulating levels of insulin-like growth factor (IGF) binding protein-2 (IGFBP-2) have been associated with increased fat mass, insulin resistance and abnormalities in the lipoprotein-lipid profile in children [7] and adults [8–11]. We recently showed that human subjects with low IGFBP-2 levels have an increased risk of showing metabolic alterations linked to the metabolic syndrome [12]. Additional transversal associations between IGFBP-2 levels and components of the lipoprotein-lipid profile, mostly TG and HDL cholesterol, were also described by others [11,13]. Further analyses suggested that subjects with low IGFBP-2 levels display an increased number of TG-rich particles as assessed by elevated apo B concentrations [12]. In turn, both overexpression of IGFBP-2 in mice and IGFBP-2 treatment to 3T3-L1 adipocyte cultures enhance uptake and metabolism of energy substrates, through yet unclear, partially IGF-1-independent mechanisms, including a possible attenuation of α5β1 integrins and intracellular PTEN signaling [14,15]. Taken together, these findings identify IGFBP-2 as a potentially relevant biomarker for the development of features of the metabolic syndrome, but also suggest that IGFBP-2 could be an important endocrine factor for the modulation of glucose and lipid metabolism.

Little is known about lifestyle interventions that change IGFBP-2 levels in humans. In patients with type 1 diabetes, insulin infusion results in upregulation of IGFBP-2, which peaks after 3–4 h and decreases thereafter [16]. In addition, leptin stimulates hepatic IGFBP-2 production in mice and humans through yet unestablished pathways [17,18]. A robust and sustained upregulation of plasma IGFBP-2 concentrations (up to 700 ng/mL) was described after biliopancreatic diversion with duodenal switch in severely obese men and women [8]. However, it remains unclear whether the observed effects on IGFBP-2 levels were attributable to weight loss or due to specific effects of biliopancreatic diversion with duodenal switch *per se*. Interestingly, weight loss due to a reduction in caloric intake has been shown to generate a 30–40% increase in IGFBP-2 levels [19,20]. These changes were concomitant to improvements in insulin sensitivity and fasting glucose levels [19]. However, the independent contribution of IGFBP-2 to improving insulin action could not be established.

In order to assess the extent of the effects of IGFBP-2 on metabolic improvements in humans, the present study aimed at determining the longitudinal impact of a 1-year lifestyle modification program (diet and exercise) on IGFBP-2 circulating levels, and to assess whether lifestyle-related changes in IGFBP-2 concentrations were independently related to improvements in the cardiometabolic risk profile, notably insulin sensitivity and apo B levels as prime indices of enhanced glucose and lipid metabolism.

2. Patients and methods

2.1. Study participants

One hundred and forty-four men, between the ages of 30–65 years, presenting abdominal obesity (waist circumference ≥ 90 cm), TG levels ≥ 1.69 mmol/L and/or HDL-cholesterol < 1.03 mmol/L, were recruited by solicitation in the media for a 1-year lifestyle modification program. Participants had to be sedentary, which was defined as less than 30 min of moderate to vigorous physical activity per week performed over the past two months. Subjects with type 2 diabetes, body mass index (BMI) values below 25 or over 40 kg/m^2, or taking medication targeting glucose or lipid metabolism or blood pressure were excluded. Informed written consent was obtained from all participants prior to their inclusion in the study. This protocol has been approved by the ethics committees of Université Laval and of the Institut universitaire de cardiologie et de pneumologie de Québec – Université Laval.

2.2. Intervention

The intervention consisted of a lifestyle modification program in which subjects obtained personalized healthy eating and physical activity counseling by a nutritionist and a kinesiologist as previously described [21]. Briefly, for the first four months of the program, counseling was scheduled every two weeks followed by monthly visits for the following eight months. The nutritional counseling was designed to induce an overall daily energy deficit of about 500 kcal. The physical activity program was individualized for each participant based on their physical activity history and preferences. The objective was to reach 160 min per week of aerobic activity of moderate intensity, along with an increase in occupational physical activity.

2.3. Anthropometric, body composition and fat distribution measurements

Waist and hip circumferences were measured using standardized procedures previously described [22]. Body composition was assessed by dual energy X-ray absorptiometry (DEXA, Lunar Prodigy, GE, Madison, WI, USA) in viscerally obese men. Cross-sectional areas of visceral and subcutaneous abdominal adipose tissue (AT) at L2-L3 and L4-L5 were assessed by computed tomography, using previously described procedures [23] and partial volumes of visceral and subcutaneous AT (between L2-L3 and L4-L5) were calculated. Briefly, participants were examined while being in the supine position with both arms stretched above the head. Using a specially designed image-analysis software (sliceOmatic, Tomovision, Montréal, Canada) AT areas were calculated using an attenuation range of −190 to −30 Hounsfield units.

2.4. Cardiorespiratory fitness

Cardiorespiratory fitness was assessed using a submaximal standardized exercise test on a TMX 425 treadmill (Trackmaster, Newton, KS) linked to a QuarkB2 monitor (Cosmed, Rome, Italy). Two variables were retained as fitness endpoints to evaluate cardiorespiratory fitness: 1) the subject's heart rate (mean of the last 3 min) at a standardized treadmill stage (3.5 mph, 2% slope); and 2) the estimated metabolic equivalent of task (MET) reached by the subject at a heart rate of 150 beats/min.

2.5. Lipoprotein-lipid profile and apolipoproteins

Blood samples were collected from the antecubital vein after a 12-h overnight fast for the measurement of plasma lipid and lipoprotein levels. TG and cholesterol levels were determined in plasma and lipoprotein fractions using Technicon RA-500 (Bayer Corporation, Tarrytown, NY, USA); enzymatic reagents were obtained from Randox (Crumlin, UK). TG-rich lipoproteins (VLDL) were first removed by ultracentrifugation [24]. The HDL fraction was obtained after precipitation of the remaining apo B-containing lipoproteins, primarily LDL, in the infranatant (density > 1.006 g/mL) with heparin and $MnCl_2$ [25].

Table 1
Characteristics of the sample of 99 men at baseline and after the 1-year lifestyle modification program.

Variables	Baseline	1-year follow-up	1-year changes	*p* value
Anthropometric and fat distribution data				
Weight (kg)	95.0 ± 12.0	88.9 ± 12.4	−7.0 ± 4.6	< 0.0001
Body mass index (kg/m^2)	31.1 ± 3.1	28.8 ± 3.3	−2.3 ± 1.5	< 0.0001
Waist circumference (cm)	108.3 ± 9.0	99.6 ± 10.2	−8.8 ± 5.3	< 0.0001
Fat mass (kg)	29.5 ± 7.1	23.3 ± 7.9	−6.2 ± 3.8	< 0.0001
L4-L5 visceral AT (cm^2)	253.6 ± 74.1	175.9 ± 73.7	−77.7 ± 49.9	< 0.0001
L4-L5 subcutaneous AT (cm^2)	313.3 ± 98.4	253.2 ± 96.4	−60.6 ± 43.5	< 0.0001
Visceral AT volume (cm^3)	1926.7 ± 487.0	1418.8 ± 540.3	−520.9 ± 343.2	< 0.0001
Subcutaneous AT volume (cm^3)	1809.2 ± 644.5	1460.6 ± 588.8	−346.5 ± 262.5	< 0.0001
Physical activity and cardiorespiratory fitness				
Heart rate – 3.5 mph; 2% (beats/min)	117.4 ± 13.8	103.4 ± 13.7	−13.8 ± 11.4	< 0.0001
Exercise output at 150 beats/min (METs)	7.65 ± 1.21	8.70 ± 1.62	+1.18 ± 1.26	< 0.0001
Daily step count (no. steps/day)	7619 ± 2847	9704 ± 3090	+1968 ± 2793	< 0.0001
Plasma glucose-insulin homeostasis				
Fasting glucose (mmol/L)	5.93 ± 0.41	5.82 ± 0.38	−0.12 ± 0.36	0.0012
AUC glucose (mmol/L × 180 min)	1441 ± 226	1330 ± 218	−130 ± 220	< 0.0001
Fasting insulin (pmol/L)*	164.1 ± 80.3	110.9 ± 46.3	−54.2 ± 72.25	< 0.0001
AUC insulin (pmol/L × 180 min)	174669 ± 77878	103863 ± 51973	−71152 ± 64484	< 0.0001
HOMA-IR*	6.06 ± 3.12	4.00 ± 1.74	−2.10 ± 2.86	< 0.0001
Plasma lipids				
Triglycerides (mmol/L)*	2.45 ± 0.95	1.87 ± 0.69	−0.59 ± 0.86	< 0.0001
Cholesterol (mmol/L)	5.11 ± 0.79	5.06 ± 0.81	−0.05 ± 0.65	NS
Cholesterol/HDL cholesterol	5.47 ± 0.94	4.80 ± 0.91	−0.69 ± 0.82	< 0.0001
Apolipoprotein B (g/L)	1.09 ± 0.18	1.05 ± 0.20	−0.04 ± 0.15	0.0066
Apolipoprotein A1 (g/L)	1.13 ± 0.15	1.30 ± 0.17	+0.17 ± 0.12	< 0.0001
VLDL				
VLDL apo B (g/L)	0.18 ± 0.05	0.16 ± 0.05	−0.02 ± 0.06	0.0083
VLDL triglycerides (mmol/L)*	1.92 ± 0.85	1.44 ± 0.65	−0.48 ± 0.80	< 0.0001
VLDL cholesterol (mmol/L)*	1.02 ± 0.47	0.72 ± 0.33	−0.30 ± 0.40	< 0.0001
LDL				
LDL apo B (g/L)	0.91 ± 0.17	0.89 ± 0.19	−0.03 ± 0.13	0.058
LDL triglycerides (mmol/L)*	0.31 ± 0.14	0.24 ± 0.09	−0.07 ± 0.09	< 0.0001
LDL cholesterol (mmol/L)	3.14 ± 0.68	3.26 ± 0.73	+0.13 ± 0.58	0.0342
HDL				
HDL triglycerides (mmol/L)*	0.23 ± 0.06	0.20 ± 0.05	−0.04 ± 0.05	< 0.0001
HDL cholesterol (mmol/L)	0.95 ± 0.17	1.08 ± 0.19	+0.13 ± 0.14	< 0.0001

Apo: Apolipoprotein, AT: Adipose tissue, AUC: Area under the curve, HDL: High-density lipoprotein, HOMA-IR: Homeostatic model assessment of insulin resistance, IGFBP: Insulin-like growth factor binding protein, LDL: Low-density lipoprotein, VLDL: Very-low density lipoprotein.
Values are mean ± SD.
*Log-transformed variables.

Cholesterol and TG concentrations in the infranatant were measured before and after the precipitation step, allowing the calculation of LDL cholesterol levels [26]. Plasma apo B and apo A1 concentrations were measured according to standardized procedures [27,28].

2.6. Oral glucose tolerance test

After a 12-h overnight fast, participants were subjected to a 75 g oral glucose load. Blood samples were taken at −15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min for the measurement of plasma glucose and insulin. Plasma glucose was measured enzymatically (Olympus, America Inc., PA) [29], whereas plasma insulin levels were determined by radioimmunoassay [30]. The total glucose and insulin areas under the curve (AUC) during the OGTT were determined by the trapezoid method between 0 and 180 min. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin concentrations [31].

2.7. IGFBP-2 quantification

Plasma IGFBP-2 levels were measured by ELISA (Mediagnost, Reutlingen, Germany) according to the manufacturer's instructions. The detection limit was 0.2 ng/mL; the inter-assay coefficient of variability was 8.7%. Samples and reagents handling as well as signal quantification steps were all automated (Biomek 2000, Beckman Coulter, Brea, CA, USA).

2.8. Statistical analyses

Data are reported as means ± SD in Tables and as means ± SE in Fig.s. Variables that were not normally distributed were log transformed as indicated in Tables and Fig.s. Subjects were divided according to quartiles of changes in IGFBP-2 levels (Q1: 169.5–11.7 ng/mL; Q2: 12.8–44.8 ng/mL; Q3: 45.4–73.6 ng/mL; Q4: 73.7–389.6 ng/mL). Differences between quartiles were evaluated using ANOVA and post hoc Tukey's test. Relationships between variables have been reported using Pearson correlations. Pearson partial correlation coefficients adjusting for changes in fasting insulin or HOMA-IR were also computed between changes in IGFBP-2 and changes in apo B subfractions. Stepwise regression analyses were performed to quantify the independent contributions of changes in waist circumference, cardiorespiratory fitness and IGFBP-2 to the variance in fasting insulin, HOMA-IR, AUC glucose as well as plasma apo B subfractions. To further determine the impact of changes in IGFBP-2, subjects were also divided according to their improvements (above or below their respective medians) in IGFBP-2 levels (+45.4 ng/mL), BMI (−2.1 kg/m^2), waist circumference (+8.75 cm) and visceral AT volume (+529.5 cm^3). Differences between these groups were assessed by two-way ANOVA. A p value < 0.05 was considered significant. All statistical analyses were performed using the SAS statistical system (version 9.4; SAS Institute, Cary, NC).

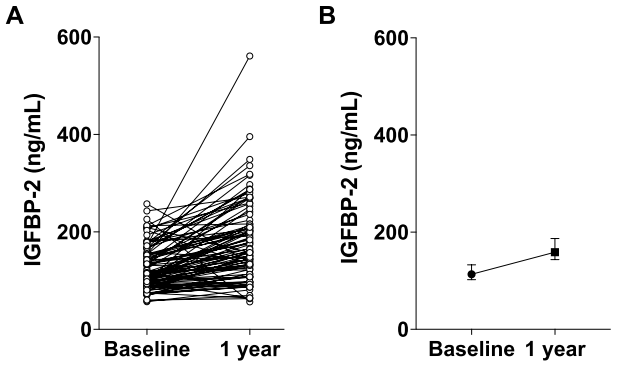

Fig. 1. IGFBP-2 circulating levels.
(A) Individual baseline and 1-year IGFBP-2 circulating levels in response to a lifestyle modification program in every 99 subjects of the cohort. (B) Median baseline and 1-year IGFBP-2 circulating levels ± 95% confidence intervals.

3. Results

3.1. Clinical data

Our study included non-diabetic middle-aged men with abdominal obesity characterized by high TG and/or low HDL cholesterol levels. From the 144 subjects who initially started the lifestyle intervention program, we kept only participants for whom we had available both IGFBP-2 and visceral AT at baseline and 1 year. We also excluded subjects who meet diagnostic criteria for type 2 diabetes (in addition to having excluded diabetic patients at screening) at baseline and 1 year (fasting glucose ≥7.0 mmol/L and 2-h glucose ≥11.1 mmol/L). Thus, 99 men were included in the present analyses. These 99 men were not different at baseline from the 45 excluded, except for slightly lower triglycerides (2.45 ± 0.95 *vs.* 2.67 ± 0.71 mmol/L, $p = 0.0303$, respectively), VLDL TG (1.92 ± 0.85 *vs.* 2.13 ± 0.66 mmol/L, $p = 0.0210$, respectively) and VLDL apo B (0.18 ± 0.05 *vs.* 0.20 ± 0.05, $p = 0.0069$ mmol/L, respectively).

A 1-year lifestyle modification program led to improvements in numerous cardiometabolic risk parameters such as reductions in fat mass, waist circumference, visceral AT volume, fasting glucose, fasting insulin, HOMA-IR, plasma TG, plasma apo B and an increase in HDL cholesterol (Table 1). The lifestyle modification program also produced a significant 43% increase in IGFBP-2 levels, ($p < 0.0001$, from 125.6 ± 44.4 to 177.0 ± 83.0 ng/mL, an average rise of 51.4 ± 68.9 ng/mL). Only 10 out of 99 subjects showed a reduction in IGFBP-2 levels during the program (Fig. 1A), notwithstanding their loss of waist circumference or visceral AT volume. The median values of IGFBP-2 at baseline and 1 year are shown in Fig. 1B (median rise of 45.4 ± 68.9 ng/mL). In addition, the changes in IGFBP-2 levels in the 99 patients (either up or down) were not statistically correlated with baseline levels (data not shown).

We next investigated the impact of changes in IGFBP-2 brought about by the lifestyle regimen on cardiometabolic risk variables on the basis of quartiles of changes in IGFBP-2 (n = 24, 25, 25, 25 for quartiles 1, 2, 3 and 4, respectively, Table 2). Following the 1-year lifestyle modification program, subjects who experienced the largest increase in IGFBP-2 levels were those who showed the greatest decreases in body weight ($p < 0.0001$) fat mass ($p < 0.0001$), BMI ($p < 0.0001$), waist circumference ($p < 0.0002$), and subcutaneous ($p = 0.0002$) and visceral ($p = 0.0008$) AT volumes (Table 2). Moreover, superior improvements in exercise output at 150 beats/min ($p = 0.0132$) and in heart rate at a standardized submaximal workload ($p = 0.0328$) were observed in men who had the largest increases in IGFBP-2 (Table 2). Similar observations were observed for several variables of the lipoprotein-lipid profile where the most important reductions in plasma TG (−1.01 ± 0.76 mmol/L, $p = 0.0004$), cholesterol (−0.30 ± 0.82 mmol/L, $p = 0.0104$) and apo B (−0.12 ± 0.17 g/L, $p = 0.0006$) levels and the highest increases in HDL cholesterol (+0.16 ± 0.16, $p = 0.0359$) concentrations were found in men with the greater increases in IGFBP-2 (Table 2). More specifically, the lifestyle modification-induced decrease in apo B levels was mostly observed in the LDL fraction (−0.09 ± 0.15 g/L, $p = 0.0064$ for LDL apo B *vs.* −0.03 ± 0.06 g/L, $p = 0.0404$ for VLDL apo B) whereas the reduction in TG levels was the largest in the VLDL fraction (−0.78 ± 0.51 mmol/L, $p = 0.0004$, Table 2).

3.2. Univariate analyses

Among the several significant associations observed between lifestyle intervention-induced changes in IGFBP-2 and those in variables of the cardiometabolic risk profile (Table S1), we especially noted significant univariate Pearson correlations between changes in IGFBP-2 levels and changes in waist, visceral AT volume, HOMA-IR, fasting insulin, apo B and LDL apo B ($-0.34 \leq r \leq -0.47$, $p < 0.05$) (Suppl. Fig. 1). None of these variables were significantly correlated with baseline IGFBP-2 levels (data not shown). Moreover, after adjusting for fasting insulin or HOMA-IR as covariate did not alter the significance of the associations between changes in IGFBP-2 levels and changes in total and LDL apo B levels (not shown).

3.3. Multivariable regression analyses

To further explore these associations, one-year changes in IGFBP-2, submaximal heart rate (for cardiovascular fitness) and visceral AT volume (for abdominal adiposity) were first entered in a stepwise multivariable regression model as independent variables to quantify their respective contributions to the variance in three indices of glucose homeostasis, namely fasting insulin, HOMA-IR and AUC glucose (Table 3A). Only one-year changes in IGFBP-2 were independently associated with 1-year changes in the three glucose-insulin homeostasis parameters investigated (Table 3A). However, when visceral AT volume was replaced by waist circumference, the latter significantly contributed to the variance in fasting insulin, HOMA-IR and AUC glucose (Table S2A). Moreover, when relative changes in IGFBP-2 were used instead of absolute changes in IGFBP-2 levels, results remained similar (data not shown).

Next, the same independent variables were entered in a second stepwise multivariable regression model to study their influence on apo B levels as indices of lipoprotein metabolism (Table 3B). One year after the beginning of the lifestyle intervention program, modifications in IGFBP-2 levels were independently associated with 1-year changes in total plasma apo B and LDL apo B, but not VLDL apo B levels (Table 3B). Changes in IGFBP-2 independently explained 17.4% of the variation in total apo B levels (Table 3B), or 14.0% when replacing it by non-HDL cholesterol levels (not shown). Only changes in submaximal heart rate was independently associated with 1-year changes in VLDL apo B (explaining 9.9% of the variation, Table 3B). No independent association between abdominal adiposity and apo B levels was observed when visceral AT volume was replaced by waist circumference in the same model, whereas changes in IGFBP-2 remained significantly associated (Table S2B). Again, similar data were observed when relative changes in IGFBP-2 were used instead of absolute changes in IGFBP-2 levels (data not shown). In addition, as reported [32], changes in fasting insulin, HOMA-IR and AUC glucose were not significantly associated with changes in plasma, LDL or VLDL apo B levels (data not shown). Taken together, these findings suggest that the increase in IGFBP-2 levels following the one-year intervention program overwhelmed the improvements in insulin sensitivity and potentially visceral adiposity as the main modulators of LDL apo B concentrations.

Table 2
1-year changes in characteristics of the sample of 99 men according to quartiles of 1-year changes in IGFBP-2 levels.

Variables	ΔQ1 (−169.5–11.7 ng/mL)	ΔQ2 (12.8–44.8 ng/mL)	ΔQ3 (45.4–73.6 ng/mL)	ΔQ4 (73.7–389.6 ng/mL)	*p* value
Anthropometric data					
Weight (kg)	−4.05 ± 3.20[a]	−5.90 ± 5.37[ab]	−7.82 ± 2.86[bc]	−10.1 ± 4.49[c]	< 0.0001
Body mass index (kg/m^2)	−1.32 ± 1.04[a]	−1.93 ± 1.70[ab]	−2.49 ± 0.96[bc]	−3.29 ± 1.51[c]	< 0.0001
Waist circumference (cm)	−5.64 ± 4.02[a]	−7.66 ± 5.75[a]	−9.12 ± 3.68[ab]	−12.1 ± 5.59[b]	0.0002
Fat mass (kg)	−3.70 ± 2.75[a]	−5.09 ± 3.75[ab]	−7.05 ± 2.69[bc]	−8.90 ± 3.93[c]	< 0.0001
L4-L5 visceral AT (cm^2)	−58.9 ± 40.9	−72.8 ± 54.6	−82.3 ± 35.5	−96.2 ± 59.8	0.0610
L4-L5 subcutaneous AT (cm^2)	−35.7 ± 25.0[a]	−50.6 ± 48.2[ab]	−72.1 ± 33.9[bc]	−82.7 ± 48.1[c]	0.0003
Visceral AT volume (cm^3)	−346.5 ± 294.1[a]	−466.8 ± 48.2[a]	−535.9 ± 178.6[ab]	−726.0 ± 369.9[b]	0.0008
Subcutaneous AT volume (cm^3)	−189.0 ± 132.6[a]	−296.9 ± 386.8[ab]	−402.1 ± 189.8[bc]	−493.8 ± 292.1[c]	0.0002
Physical activity and cardiorespiratory fitness					
Heart rate − 3.5 mph; 2% (beats/min)	−9.5 ± 10.2[a]	−11.4 ± 12.3[ab]	−14.6 ± 9.0[ab]	−18.8 ± 12.1[b]	0.0328
Exercise output at 150 beats/min (METs)	+0.59 ± 0.93[a] (14)	+0.70 ± 1.39[a] (16)	+1.67 ± 1.01[a] (15)	+1.67 ± 1.28[a] (17)	0.0132
Daily step count (no. steps/day)	+1903 ± 2217 (16)	+1646 ± 2576 (19)	+2749 ± 3529 (21)	+1483 ± 2535 (19)	NS
Plasma glucose-insulin homeostasis					
Fasting glucose (mmol/L)	+0.02 ± 0.24	−0.17 ± 0.35	−0.21 ± 0.31	−0.11 ± 0.47	NS
AUC glucose (mmol/L × 180 min)	+21 ± 239[a] (20)	−112 ± 173[ab] (19)	−2145 ± 158[b] (23)	−196 ± 232[b] (21)	0.0012
Fasting insulin (pmol/L)[†]	−12.3 ± 67.1[a]	−75.2 ± 80.3[b]	−62.5 ± 61.7[b]	−65.4 ± 65.5[b]	0.0019
AUC insulin (pmol/L × 180 min)	−36727 ± 65940[ab] (20)	−94964 ± 54576[b] (19)	−79559 ± 61827[ab] (23)	−73187 ± 64984[ab] (21)	0.0304
HOMA-IR[†]	−0.38 ± 2.40[a]	−2.94 ± 3.22[b]	−2.55 ± 2.57[b]	−2.49 ± 2.59[b]	0.0019
Plasma lipids					
Triglycerides (mmol/L)[†]	−0.12 ± 0.55[a]	−0.65 ± 1.01[a]	−0.68 ± 1.05[ab]	−1.01 ± 0.76[b]	0.0004
Cholesterol (mmol/L)	+0.30 ± 0.60[a]	−0.13 ± 0.43[ab]	−0.06 ± 0.59[ab]	−0.30 ± 0.82[b]	0.0104
Cholesterol/HDL cholesterol	−0.28 ± 0.69[a]	−0.54 ± 0.83[ab]	−0.89 ± 0.86[b]	−1.01 ± 0.76[b]	0.0064
Apolipoprotein B (g/L)	+0.05 ± 0.14[a]	−0.04 ± 0.09[ab]	−0.06 ± 0.14[b]	−0.12 ± 0.17[b]	0.0006
Apolipoprotein A1 (g/L)	+0.17 ± 0.12	+0.14 ± 0.08	+0.20 ± 0.11	+0.17 ± 0.14	NS
VLDL					
VLDL apo B (g/L)	+0.01 ± 0.06[a]	−0.03 ± 0.05[a]	−0.02 ± 0.07[a]	−0.03 ± 0.06[a]	0.0404
VLDL triglycerides (mmol/L)[†]	−0.06 ± 0.52[a]	−0.54 ± 0.95[a]	−0.53 ± 0.96[a]	−0.78 ± 0.51[b]	0.0004
VLDL cholesterol (mmol/L)[†]	−0.10 ± 0.25[a]	−0.30 ± 0.41[a]	−0.37 ± 0.53[a]	−0.44 ± 0.25[b]	0.0002
LDL					
LDL apo B (g/L)	+0.04 ± 0.15[a]	−0.01 ± 0.08[ab]	−0.04 ± 0.12[ab]	−0.09 ± 0.15[b]	0.0064
LDL triglycerides (mmol/L)[†]	−0.05 ± 0.07	−0.08 ± 0.11	−0.09 ± 0.09	−0.06 ± 0.06	NS
LDL cholesterol (mmol/L)	+0.29 ± 0.61	+0.10 ± 0.44	+0.14 ± 0.61	−0.02 ± 0.65	NS
HDL					
HDL triglycerides (mmol/L)[†]	−0.02 ± 0.04	−0.03 ± 0.05	−0.05 ± 0.07	−0.05 ± 0.05	0.0814
HDL cholesterol (mmol/L)	+0.11 ± 0.11[ab]	+0.07 ± 0.12[a]	+0.17 ± 0.14[b]	+0.16 ± 0.16[ab]	0.0359

Apo: Apolipoprotein, AT: Adipose tissue, AUC: Area under the curve, HDL: High-density lipoprotein, HOMA-IR: Homeostatic model assessment of insulin resistance, IGFBP: Insulin-like growth factor binding protein, LDL: Low-density lipoprotein, VLDL: Very-low density lipoprotein.
Values are mean ± SD. [a,b,c]Quartiles with the same letters are not statistically different. Depending on the variable, the number of subjects per quartile is between 21 and 25 except when indicated in parentheses.
[†] Log-transformed variables.

3.4. Subgroup analyses

To further examine this intriguing, unique relationship between changes in IGFBP-2 and modifications in apo B subfractions, subjects were classified into four groups (using the median values) according to their changes in IGFBP-2 and adiposity (studied either as BMI, waist circumference or visceral AT volume). Fig. 2 shows the combined effect of concomitant variations in IGFBP-2 and those of the three markers of adiposity on the changes in apo B levels in the total plasma and in the VLDL and LDL fractions. One-year changes in IGFBP-2 levels modulated, to a statistically significant extent, 1-year changes in plasma apo B (Fig. 2, left panels) and LDL apo B (Fig. 2, right panels) associated with variations in BMI, waist circumference and visceral AT volume. Not surprisingly considering the highly significant relationship observed between VLDL apo B levels and weight loss ($r = 0.37$, $p = 0.0002$), changes in adiposity (BMI and visceral AT volume) was the main driver of changes in VLDL apo B (Fig. 2, middle panels).

We finally compared post-intervention plasma apo B levels, adjusted for baseline IGFBP-2 and apo B levels, on the basis of 1-year changes in IGFBP-2 quartiles (Suppl. Fig. 2). As a group, the only participants who reached a plasma apo B level of 1.0 g/L or lower were the one who experienced the largest improvement in IGFBP-2 levels on average (Q4: apo B: 0.96 g/L, $p < 0.0001$), although some subjects who achieved apo B levels below 1.0 g/L were also present in the other quartiles (Q1:6 subjects, Q2: 9 subjects, Q3: 12 subjects). A similar analysis performed according to quartiles of post-intervention IGFBP-2 levels yielded no significant difference in apo B concentrations (data not shown).

4. Discussion

The goal of the present study was to evaluate the longitudinal associations between IGFBP-2 levels and the beneficial changes in cardiometabolism induced by a lifestyle intervention regimen. Here, we show that a one-year lifestyle modification program targeting healthy eating and physical activity leads to robust metabolic improvements and to an increase in circulating IGFBP-2 levels. Notably, men with the largest increases in IGFBP-2 displayed the most substantial improvements in lipoprotein-lipid levels. In our cohort, upregulation of IGFBP-2 was associated with improvements in LDL apo B levels but not VLDL apo B. Most of the subjects who reached a plasma apo B level of 1.0 g/L following the 1-year intervention were those with the most important increases in IGFBP-2 concentrations. The changes in IGFBP-2 concentrations were associated with those in total and LDL apo B levels independently of changes in visceral adiposity and insulin sensitivity, suggesting a direct role of IGFBP-2 in the metabolism of apo B-containing lipoproteins beyond other markers of cardiometabolic health.

As expected, adhesion to a lifestyle modification program resulted in metabolic improvements and in increased IGFBP-2 levels. In the present study, total circulating IGFBP-2 levels were within the range reported in men with mild dyslipidemia and increased adiposity

Table 3
Multivariable regression analyses showing the contribution of 1-year changes in plasma IGFBP-2, heart rate (3.5 mph; 2% slope) and VAT volume to 1-year changes in (A) fasting insulin, HOMA-IR and AUC glucose and (B) apolipoprotein B, VLDL apolipoprotein B and LDL apolipoprotein B in response to the lifestyle intervention.

A

1-year changes	Fasting insulin[a]					HOMA-IR[a]					AUC glucose				
	Univariate		Multivariable regression			Univariate		Multivariable regression			Univariate		Multivariable regression		
	r	*p* value	Partial R^2%	Total R^2%	*p* value	r	*p* value	Partial R^2%	Total R^2%	*p* value	r	*p* value	Partial R^2%	Total R^2%	*p* value
IGFBP-2[a]	−0.47	< 0.0001	26.4	26.4	< 0.0001	−0.47	< 0.0001	26.3	26.3	< 0.0001	−0.32	0.0036	5.5	5.5	0.0288
Heart rate (3.5 mph; 2%)	0.23	0.0327	–	–	NS	0.23	0.0313	–	–	NS	0.39	0.0005	–	–	NS
VAT volume	0.35	0.0005	–	–	NS	0.35	0.0005	–	–	NS	0.36	0.001	–	–	NS

B

1-year changes	Apolipoprotein B					VLDL apolipoprotein B					LDL apolipoprotein B				
	Univariate		Multivariable regression			Univariate		Multivariable regression			Univariate		Multivariable regression		
	R	*p* value	Partial R^2%	Total R^2%	*p* value	r	*p* value	Partial R^2%	Total R^2%	*p* value	r	*p* value	Partial R^2%	Total R^2%	*p* value
IGFBP-2[a]	−0.46	< 0.0001	17.4	17.4	< 0.0001	−0.20	0.0553	–	–	NS	−0.43	< 0.0001	16.7	16.7	< 0.0001
Heart rate (3.5 mph; 2%)	0.31	0.0032	–	–	NS	0.30	0.0050	9.9	9.9	0.0031	0.21	0.0457	–	–	NS
VAT volume	0.36	0.0003	6.6	24.0	0.0086	0.22	0.0317	–	–	NS	0.31	0.0023	4.0	20.7	0.0430

IGFBP-2: Insulin-like growth factor binding protein-2; NS: Not significant; VAT: visceral adipose tissue;AUC: Area under the curve; HOMA-IR: Homeostasis model assessment of insulin resistance; IGFBP-2: Insulin-like growth factor binding protein-2; NS: Not significant; VAT: visceral adipose tissue.

[a] Log-transformed variables.

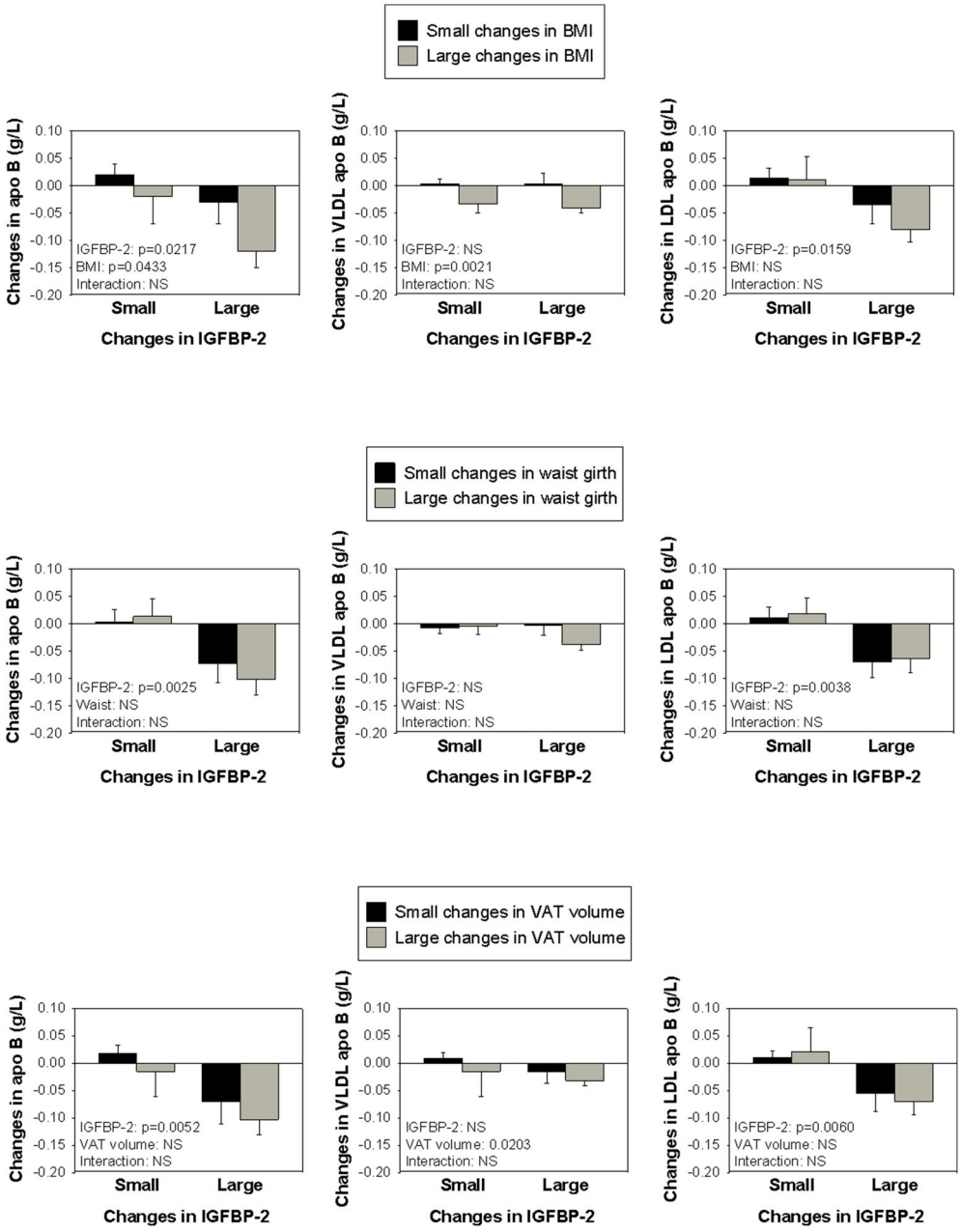

Fig. 2. Impact of combined 1-year changes in IGFBP-2 and body mass index (BMI) or waist circumference or visceral adipose tissue (VAT) volume on 1-year changes in plasma apolipoprotein (apo) B, VLDL apo B and LDL apo B in response to a lifestyle modification program.
Men were divided on the basis of their median values (below or above) of 1-year changes in IGFBP-2 (+45.4 ng/mL), BMI (−2.1 kg/m^2), waist circumference (+8.75 cm) or VAT volume (+529.5 cm^3). NS: Not significant.

[8,12,33,34]. Following the 1-year intervention, average IGFBP-2 levels were increased by 43%, which is consistent with the upregulation observed after weight loss achieved by dieting alone [19]. In addition, for a similar baseline IGFBP-2 levels, men experienced various responses in IGFBP-2 levels. Genetic factors could have played a role in the IGFBP-2 response to diet and exercise. In addition, we did not assess the levels of IGFBP-2 fragments [35,36] or those of other IGFBPs possibly modified by the lifestyle regimen, which could have both influenced the changes in IGFBP-2 as a compensatory feedback. Nonetheless, these data complement the numerous transversal studies showing that total IGFBP-2 levels are closely linked to insulin sensitivity, fat mass and components of the lipoprotein-lipid profile [8,9,11,34,37–39] and suggest that these associations are within a time continuum as well. The molecular mechanism responsible for the stimulation of IGFBP-2 production upon lifestyle modifications remain to be tested in humans. However, our observations reinforce the work previously done by Espelund et al. [19], which showed that changes in BMI were associated with changes in IGFBP-2 levels. These observations diverge, however, from the one emerging from biliopancreatic diversion, since in this model IGFBP-2 levels experienced a large and rapid increase (more than 120% within 24 h), which was not yet associated with significant fat loss [8]. This suggests that bariatric surgery could specifically modulate the increase in IGFBP-2; potentially via the anatomical changes inherent to this type of surgery. Interestingly, changes in IGFBP-2 did not correlate with baseline IGFBP-2. This implies that subjects with the most important changes in IGFBP-2 were not the one with the lowest baseline concentrations. From a metabolic standpoint, this could suggest that for a given individual, the increase in IGFBP-2 levels seems to bear more importance than final levels reached, or that at least a certain threshold in IGFBP-2 needs to be attained.

Remarkably, changes in IGFBP-2 were strongly and negatively associated with changes in both plasma apo B and LDL apo B, supporting our observation of a univariate relationship between IGFBP-2 and apo B in a previous cross-sectional analysis [12]. Stepwise analyses showed that these associations were independent of changes in cardiorespiratory fitness. Moreover, although changes in IGFBP-2 concentrations and those in visceral adiposity appear to have additional effects on plasma apo B and LDL apo B levels, the stratification of subjects according to their changes in different anthropometric parameters and those in IGFBP-2 clearly strengthens and extends our previous observations. Indeed, our analyses distinctly indicate that changes in VLDL apo B were mostly driven by weight loss (changes in BMI and visceral AT volume) whereas those in plasma apo B and LDL apo B were mostly affected by changes in IGFBP-2 levels.

The associations found between IGFBP-2 levels and markers of insulin sensitivity have previously been described [12,34]. The data presented herein further demonstrate that longitudinal changes in IGFBP-2 levels are significantly and independently linked with the modulation of apo B levels, besides the established effects of insulin on the production of apo B and secretion of apo B-containing VLDL [40]. Specifically, the strong independent correlation between changes in IGFBP-2 and those in LDL apo B, together with the lack of association between changes in IGFBP-2 and those in VLDL apo B, suggests that the up-regulation of IGFBP-2 could be associated with increased LDL catabolism rather than changes in hepatic VLDL production. It is also possible that the circulating levels of IGFBP-2, a protein mostly produced by the liver, are the reflect of a state in which hepatocytes stimulate LDL reuptake. Whereas only speculative at this point, it is indeed possible that higher intracellular IGFBP-2 concentrations enhance the level and activity of LDL reuptake mechanisms within hepatocytes, and that circulating levels mirror a subsequent consequence of this process. To test this hypothesis, a detailed mechanistic study on the influence of IGFBP-2 on apo B100 kinetics would be required.

Previous studies by our group performed in two reference samples of 38 and 60 nonobese, normolipidemic, healthy men reported circulating apo B concentrations of 0.96 and 0.99 g/L, which corresponded to their 50th percentile and mean value, respectively [21,41]. These data are consistent with the reported 50th percentile and mean value of 1.00 and 1.03 g/L in plasma apo B in a random reference Canadian population of 1755 men [42]. In the present study, lipoprotein-lipid profile analyses revealed that many subjects reached this plasma apo B threshold of 1.0 g/L following their lifestyle intervention. Remarkably, notwithstanding their post-intervention IGFBP-2 levels, there was a greater proportion of subjects with plasma apo B levels below 1.0 g/L in the quartile who experienced the largest changes in IGFBP-2 compared to other quartiles. However, some subjects who achieved final apo B levels below 1 g/L were also present in the other quartiles. This observation further strengthens the hypothesis that the variation in IGFBP-2 levels seems to bear more importance than the final levels reached. Notably, addition of growth hormone modulates IGFBP-2 levels and apo B levels in both hepatocytes and growth hormone-deficient patients [43,44]. The effects of growth hormone on apo B levels appear to be mediated by IGF-1, at least in type 1 diabetic patients [45]. This is interesting because exercise increases growth hormone levels. Additional studies will be needed to determine whether the relationship between IGFBP-2 and apo B levels reflects a direct or indirect phenomenon. Moreover, considering the atherogenic potential of apo B LDL particles, it would be highly interesting to test whether IGFBP-2 impacts on the progression of atherosclerotic plaques.

4.1. Study limitations

This study is subjected to the inherent limitations of a descriptive lifestyle intervention study. In particular, this design does not allow to establish a causal relationship between IGFBP-2 and LDL apo B levels. All participants were Caucasian males between 20 and 65 years old. Therefore, our results need to be validated in other cohorts.

4.2. Clinical implication

The present study shows the notion that a lifestyle modification program can produce increases in IGFBP-2 levels, which are associated with improvements in metabolic parameters. Our findings are consistent with and extend reports of the use of IGFBP-2 as an early marker of the metabolic syndrome [12,34]. Here, we suggest that changes in IGFBP-2 levels could be used as a marker of global improvements in cardiometabolic risk markers in response to lifestyle modifications. Considering that more than a quarter of the world population is overweight, large-scale, low-cost strategies are needed to limit cardiovascular consequences of our “toxic” lifestyle. In this regard, quantification of IGFBP-2 levels could allow physicians to keep track of their patient's overall metabolic improvements and to adjust intervention accordingly. As many subjects who experienced the largest increase in IGFBP-2 achieved the strongest lowering of plasma apo B levels, interventions aimed at increasing IGFBP-2 levels should be targeted. The strong and independent associations between changes in IGFBP-2 and those in total plasma and LDL apo B direct towards the possibility that increases in IGFBP-2 are associated with a decreased cardiovascular risk. Further studies are needed to delineate the possible links between the risk of cardiovascular events and IGFBP-2 levels.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

N.A., A.T., J.B., P.P., J.-P. D., F.P. conceived and designed research project. Z.L. performed IGFBP-2 quantification. S.C., I.L., Z.L. analyzed data. S.C., I.L., J.B., P.P. J.-P. D., F.P. interpreted the experimental results. I.L. and F.P. prepared Fig.s. S.C., I.L, and F.P. drafted manuscript. S.C., I.L., N.A., J.B., P.P. J.-P. D., F.P. edited and revised the manuscript. All approved final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2018.12.016.

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