

Associations between insulin-like growth factor binding protein-2 and lipoprotein kinetics in men

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Abstract Low circulating concentrations of insulin-like growth factor binding protein-2 (IGFBP-2) have been associated with dyslipidemia, notably with high triglyceride (TG) levels. However, the determinants by which IGFBP-2 influences lipoprotein metabolism, especially that of TG-rich lipoproteins (TRLs), are poorly understood. Here, we aimed to assess the relationships between IGFBP-2 levels and lipoprotein production and catabolism in human subjects. Fasting IGFBP-2 concentrations were measured in the plasma of 219 men pooled from previous lipoprotein kinetics studies. We analyzed production rate and fractional catabolic rates of TRLapoB-48, and LDL-, IDL-, and VLDLapoB-100 by multicompartamental modeling of L-[5,5,5-D³] leucine enrichment data after a 12 h primed constant infusion in individuals kept in a constant nutritional steady state. Subjects had an average BMI of 30 kg/m², plasma IGFBP-2 levels of 157 ng/ml, and TG of 2.2 mmol/l. After adjustments for age and BMI, IGFBP-2 levels were negatively associated with plasma TG ($r = -0.29$; $P < 0.0001$) and positively associated with HDL-cholesterol ($r = 0.26$; $P < 0.0001$). In addition, IGFBP-2 levels were positively associated with the fractional catabolic rate of VLDLapoB-100 ($r = 0.20$; $P < 0.01$) and IDLapoB-100 ($r = 0.19$; $P < 0.05$) and inversely with the production rate of TRLapoB-48 ($r = -0.28$; $P < 0.001$). These correlations remained statistically significant after adjustments for age, BMI, and the amount of fat given during the tracer infusion. These findings show that the association between low plasma IGFBP-2 and high TG concentrations could be due to both an impaired clearance of apoB-100-containing VLDL and IDL particles and an increased production of apoB-48-containing chylomicrons.  Additional studies are necessary to investigate whether and how IGFBP-2 directly impacts the kinetics of TRL.

Supplementary key words dyslipidemia • IGFBP-2 • lipoproteins • biomarker • tracer • clearance • production • kinetics

Insulin-like growth factor (IGF) binding proteins (IGFBPs) bind and modulate the bioavailability and half-life of IGF-I and IGF-II, which have important roles in development and energy metabolism (1). In particular, IGFBP-2 is produced mainly by the liver and is the second most abundant IGFBP in circulation. Interestingly, IGFBP-2 can also exert IGF-independent effects to promote cell migration and proliferation (2, 3) or modulate adipocyte differentiation (4, 5). In addition, several studies have now highlighted its relevance in the modulation of energy metabolism (2, 6), and multiple reports have established that IGFBP-2 is inversely and robustly associated with obesity and insulin resistance in humans (7–10). Studies in animal models further support a direct role of IGFBP-2 in the modulation of insulin sensitivity and weight gain (5, 11, 12). Finally, recent clinical longitudinal multivariate analyses have also shown that IGFBP-2 is among the best biomarkers negatively associated with incident metabolic syndrome (13).

One aspect of notable interest is the strong link between IGFBP-2 and plasma lipids. Association studies have shown that plasma IGFBP-2 levels are inversely correlated with circulating concentrations of triglycerides (TGs) in a more robust manner than with those of total or LDL-cholesterol levels (7, 9, 14, 15). IGFBP-2 levels are also positively associated with HDL-cholesterol levels (7, 8, 16). Extensive analysis of the lipoprotein-lipid profile further indicated that higher IGFBP-2 concentrations are a strong marker of low TG content in VLDL particles independently of age, waist circumference, and insulin sensitivity, but it is currently unknown if these associations are linked with changes in TG upon VLDL production or catabolism (8). In addition, increases in IGFBP-2 levels observed after weight loss have been linked to the extent of the reductions in plasma apoB and LDLapoB levels, but not those in LDL-cholesterol levels, which implies an association with LDL number rather than size (17). Taken together, these findings strongly suggest important relationships between IGFBP-2 and lipoprotein-lipid

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metabolism, which could have important clinical repercussions and impact cardiovascular risk in humans (18, 19). However, the mechanisms underlying these relationships have not been fully characterized.

In this context, we tested the hypothesis that reduced IGFBP-2 levels are linked with disturbances in lipid metabolism, especially production and clearance of TG-rich lipoprotein (TRL). To that end, we took advantage of data from previously published lipoprotein kinetic studies performed in humans that have used tracers to analyze the production rate (PR) and fractional catabolic rate (FCR) of apoB100 in VLDL, IDL, LDL and of apoB48 in TRL and investigated their associations with circulating IGFBP-2 levels.

MATERIALS AND METHODS

Study design

The present study reports IGFBP-2 data from cohorts previously described, including details about recruitment of patients and approval by the local institutional review board of Université Laval. The studies in this work abide by the Declaration of Helsinki principles. The present cross-sectional study comprised a total of 219 Caucasian men who participated in independent in vivo tracer kinetic experiments performed by our team and previously reported in detail (20–32). Herein, only data from men who either had no particular treatment (20–24), were on a control diet (25–29), or on a placebo drug regimen (24, 30–32) were used for further analyses.

None of the 219 individuals had symptomatic cardiovascular disease, monogenic hyperlipidemia, insulin-dependent diabetes, insulin therapy, acute renal or hepatic dysfunction, history of cancer, uncontrolled arterial hypertension, or recent history of drug or alcohol abuse. Subjects with clinical diagnosis of type 2 diabetes included in this study received a stable dose of metformin for at least 3 months prior to the lipid kinetics assessment. Subjects taking lipid-lowering therapy had to stop their medication for at least 6 weeks before entering the studies.

Blood biochemistry

Blood samples were collected after overnight 12 h fast on the morning before the beginning of the kinetics studies. Blood was placed in tubes containing EDTA and benzamidine 0.03%, and plasma was collected after centrifugation. Circulating lipids were quantified using enzymatic methods and ultracentrifugation as described (33). Plasma levels of glucose (colorimetry), insulin (electrochemiluminescence), and C-reactive protein (CRP; ELISA) were measured as described (34). Plasma insulin values were only available for 177 individuals. Insulin-resistant individuals were defined as having fasting insulin concentrations ≥ 90 pmol/l (26, 29), which represents the 95th percentile of the population distribution in fasting insulin levels. Plasma IGFBP-2 concentrations were quantified by ELISA using a commercial kit (Alpco Kit, Salem, NH; catalog no.: 22-BP2HU-E01) according to the manufacturer's instructions. The detection limit of this assay was 0.2 ng/ml. The interassay and intra-assay coefficients of variability were 14.4% and 4.6%, respectively.

Lipoprotein kinetics measurement

The quantification of lipoprotein kinetics is based on stable isotope-labeled leucine tracer studies in steady-state volunteers kept in a constant nutritional condition. Twenty-eight participants were studied in a fasting state. Starting at 7:00 AM, all other 177 participants received half-hourly snacks in the form of small cookies for 15 h, to maintain a continuous postprandial state. Each snack contained 1/30th of the estimated daily caloric intake. Depending on the nature and objectives of the kinetic studies, different amounts of fat were provided through the snacks: low fat, moderate fat, or high fat (22.4%, 35.1%, or 41.1% of energy as fat, respectively). However, the fatty acid profiles of the fat in each of these studies were similar. At approximately 07:00 AM, a priming dose of 10 $\mu\text{mol/kg}$ 1-[5,5,5-D₃]-leucine was given before the continuous infusion. At 10:00 AM, deuterated leucine 1-[5,5,5-D₃] at a concentration of 10 $\mu\text{mol/kg}$ was continuously infused. Blood was collected at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 11, and 12 h during the infusion. Isolation and quantification of lipoprotein fractions, apoB-100, apoB-48, and isotopic enrichment determinations were performed exactly as described previously (34–37). Kinetics analysis as a function of plasma volume was calculated exactly as described previously (34). Kinetics of apoB-48 were calculated in nonfasted participants only. Data on the kinetics of VLDLapoB-100, IDLapoB-100, and LDLapoB-100 from the 28 fasted and 191 nonfasted (postprandial) participants are shown together in the tables and supplemental figures since regression analyses and their consequent conclusions were not impacted by this factor. The total pool size (PS) of the different lipoprotein fractions was expressed as milligrams. Lipoprotein catabolism, or FCR quantified in a constant postprandial steady state, was expressed as the number of pools per day. PR of lipoproteins was expressed as $\text{mg} \cdot \text{kg body weight}^{-1} \cdot \text{day}^{-1}$.

Statistical analysis

Statistical analyses were performed using SAS (version 9.3; SAS Institute, Cary, NC). Distribution of data was checked for normality, and data were transformed when required. Correlations between IGFBP-2 and different parameters were assessed using Spearman correlation coefficient. Correlations were adjusted for age, BMI, amount of fat given during the constant feeding, tracer infusion period, or fasting TG concentration as indicated in the tables. Multivariate correlates of variations were investigated using stepwise multiple regression analyses. Statistical significance was defined as $P < 0.05$.

RESULTS

The anthropometric and fasting metabolic characteristics of the 219 men are presented in Table 1. On average, participants were 43 ± 12 years old and had a BMI of 30 ± 5 kg/m^2 . Subjects were mildly hyperlipidemic, with elevated circulating levels of TGs (2.2 ± 1.6 mmol/l), total cholesterol (5.4 ± 1.4 mmol/l), and LDL-cholesterol (3.5 ± 1.2 mmol/l). Mean HDL-cholesterol levels were 1.0 ± 0.2 mmol/l. On average, men had slightly elevated fasting glycemia (5.8 ± 1.5 mmol/l) and insulinemia (114 ± 64 pmol/l). Thirty-seven participants (17% of the cohort) had type 2 diabetes. Mean fasting concentrations of CRP and IGFBP-2 were 2.7 ± 2.3 mg/l and 157 ± 80 ng/ml, respectively

TABLE 1. Anthropometric and fasting biochemical characteristics of the subjects of the cohort

Variables	Mean ± SD (n = 219)	Range (lower—higher quartiles)
Anthropometric		
Age (years)	42.9 ± 12.1	(32.0–54.0)
BMI (kg/m ²)	30.3 ± 4.7	(27.1–32.9)
Waist circumference (cm)	104.6 ± 11.3	(97.1–111.6)
Plasma lipids		
Fasting total cholesterol (mmol/l)	5.40 ± 1.42	(4.58–5.82)
Fasting LDL-cholesterol (mmol/l)	3.47 ± 1.22	(2.81–3.92)
Fasting HDL-cholesterol (mmol/l)	1.02 ± 0.23	(0.87–1.17)
Fasting TGs (mmol/l)	2.20 ± 1.59	(1.30–2.56)
Plasma glucose-insulin homeostasis		
Fasting glucose (mmol/l)	5.82 ± 1.50	(5.00–5.90)
Fasting insulin (pmol/l)	114.3 ± 63.8	(66.0–138.0)
Type 2 diabetes		
% Yes (n)	16.89 (37)	—
% No (n)	83.11 (182)	—
CRP (mg/l)	2.69 ± 2.26	(0.94–3.81)
IGFBP-2 (ng/ml)	157.2 ± 79.6	(104.1–183.9)

n = 212 for glucose, n = 177 for insulin, n = 168 for waist circumference, n = 216 for BMI, TG, and HDL-cholesterol, n = 218 for LDL-cholesterol, and n = 214 for CRP.

(Table 1). As previously observed (8), plasma IGFBP-2 levels were negatively correlated with BMI ($r = -0.36$; $P < 0.0001$), TG ($P < 0.0001$), fasting insulin ($r = -0.26$; $P = 0.0004$), and CRP levels ($r = -0.25$; $P = 0.0002$) but positively associated with HDL-cholesterol ($r = 0.25$; $P = 0.0001$) (Table 2). When adjusted for age and BMI, associations remained highly significant, except for insulin and CRP concentrations (Table 2).

TABLE 2. Spearman correlations between plasma IGFBP-2 concentrations and cardiometabolic markers in the cohort

Variables	Correlations (n = 219)			
	Crude		Adjusted for age and BMI	
	r	P	r	P
Anthropometric				
Age (years)	0.19	0.0041	0.26 ^a	0.0001 ^a
BMI (kg/m ²)	-0.36	<0.0001	-0.39 ^b	<0.0001 ^b
Waist circumference (cm)	-0.15	0.0496	0.11	0.1695
Plasma lipids				
Fasting total cholesterol (mmol/l)	0.05	0.5036	-0.03	0.6731
Fasting LDL-cholesterol (mmol/l)	0.09	0.1824	0.05	0.4466
Fasting HDL-cholesterol (mmol/l)	0.25	0.0001	0.26	0.0002
Fasting TGs (mmol/l)	-0.29	<0.0001	-0.29	<0.0001
Plasma glucose-insulin homeostasis				
Fasting glucose (mmol/l)	0.02	0.7354	-0.02	0.7773
Fasting insulin (pmol/l)	-0.26	0.0004	-0.14	0.0602
CRP (mg/l)	-0.25	0.0002	-0.13	0.0659

n = 212 for glucose, n = 177 for insulin, n = 168 for waist circumference, n = 216 for BMI, TG, and HDL-cholesterol, n = 218 for LDL-cholesterol, and n = 214 for CRP.

^aAdjusted only for BMI.

^bAdjusted only for age.

IGFBP-2 levels are positively associated with the catabolic rate of apoB-100-containing lipoproteins and negatively associated with the PR of apoB48-containing TRL

Circulating IGFBP-2 concentrations were significantly and negatively associated with the PS of VLDLapoB-100 ($r = -0.26$; $P < 0.001$), IDLapoB-100 ($r = -0.30$; $P < 0.001$), LDLapoB-100 ($r = -0.17$; $P < 0.05$), and TRLapoB-48 ($r = -0.38$; $P < 0.001$) (Table 3). Plasma IGFBP-2 concentrations were positively correlated with the FCR of VLDLapoB-100 and IDLapoB-100 ($r = 0.20$, $P < 0.01$ and 0.19 , $P < 0.05$, respectively) (Table 3 and supplemental Fig. S1 for complete scattergrams). These correlations remained significant when adjusted for age, BMI, and the relative amount of fat consumed during the tracer studies (Table 3 and supplemental Fig. S2). In contrast, IGFBP-2 levels were negatively correlated with the PR of TRLapoB-48 ($r = -0.28$; $P < 0.001$) (Table 3) independent of age, BMI, and the fat consumed during the tracer studies (Table 3). However, IGFBP-2 concentrations were no longer statistically associated with the kinetics of these apoB48- and apoB100-containing lipoproteins after additional adjustment for fasting TG levels (Table 3).

Correlations between circulating IGFBP-2 levels and the kinetics of apoB-containing lipoproteins are influenced by insulin resistance

Because of the established and important relationship between insulin resistance and lipoprotein-lipid metabolism, the relationships between IGFBP-2 levels and lipoprotein kinetics were next evaluated separately on the basis of fasting insulinemia, using values <90 pmol/l as a cutoff to identify insulin-sensitive subjects. Similar to the observations in the whole cohort, IGFBP-2 concentrations were negatively associated with the PS of VLDLapoB-100 ($r = -0.36$; $P < 0.01$), IDLapoB-100 ($r = -0.28$; $P < 0.05$), LDLapoB-100 ($r = -0.32$; $P < 0.05$), and TRLapoB-48 ($r = -0.30$; $P < 0.05$) in the 66 men characterized with insulin levels lower than 90 pmol/l (Table 4 and supplemental Fig. S3). After adjustments for age, BMI, and relative amount of fat consumed during the tracer studies, IGFBP-2 was positively correlated with VLDL FCR ($r = 0.42$; $P < 0.001$) (Table 4) and negatively correlated with the PR of LDLapoB-100 ($r = 0.28$; $P < 0.05$) and TRLapoB-48 ($r = -0.36$; $P < 0.01$) (Table 4) among these insulin-sensitive men.

Among the III insulin-resistant men (fasting insulinemia ≥ 90 pmol/l), the relationship between fasting IGFBP-2 levels and PS remained only for IDLapoB-100 ($r = -0.34$; $P < 0.01$) and TRLapoB-48 ($r = -0.25$; $P < 0.05$) (Table 5 and supplemental Fig. S3). In these subjects, IGFBP-2 concentrations were no longer associated with the PR of apoB-100- or apoB-48-containing lipoproteins (Table 5). On the other hand, the inverse association between IGFBP-2 concentrations and the PR of

TABLE 3. Spearman correlations between the kinetics of apoB-100- and apoB-48-containing lipoproteins and plasma IGFBP-2 concentrations in the 219 men of this study

Variables	Correlations (Spearman rho) with IGFBP-2			
	Adjustments			
	None	Age, BMI	Age, BMI, and relative fat intake during the tracer study	Age, BMI, and relative fat intake during the tracer study, fasting TG
VLDLapoB-100				
PS (mg)	-0.26 ^a	-0.18 ^b	-0.21 ^b	-0.04
PR (mg • kg body weight ⁻¹ • day ⁻¹)	-0.02	0.09	0.07	0.04
FCR (pools/day)	0.20 ^b	0.29 ^c	0.29 ^c	0.12
IDLapoB-100				
PS	-0.30 ^a	-0.14	-0.14	0.04
PR	-0.008	0.08	0.08	0.14
FCR	0.19 ^d	0.24 ^b	0.24 ^b	0.15
LDLapoB-100				
PS	-0.17 ^d	-0.07	-0.07	-0.03
PR	0.02	0.04	0.05	0.05
FCR	0.06	0.09	0.09	0.03
TRLapoB-48				
PS	-0.38 ^a	-0.35 ^c	-0.39 ^c	-0.27 ^a
PR	-0.28 ^a	-0.23 ^b	-0.25 ^b	-0.14
FCR	0.09	0.17 ^d	0.17 ^d	0.07

n = 166 for IDLapoB-100 and LDLapoB-100 kinetic and *n* = 170 for TRLapoB-48 kinetic.

^a*P* < 0.001.

^b*P* < 0.01.

^c*P* < 0.0001.

^d*P* < 0.05.

apoB-48-containing lipoproteins was significant in both men with and without type 2 diabetes (supplemental Fig. S4). Finally, significant correlations were still observed between IGFBP-2 and the FCR of VLDLapoB-100 (*r* = 0.23; *P* < 0.05) and IDLapoB-100 (*r* = 0.41; *P* < 0.001) after adjustments for age, BMI, and relative amount of fat consumed during the tracer studies (Table 5).

DISCUSSION

Previous clinical reports have demonstrated that low circulating levels of IGFBP-2 are associated with a deleterious lipid profile, especially impacting levels of HDL-cholesterol and TG. The purpose of the present study was to further investigate these relationships by analyzing for the first time how IGFBP-2 levels are associated with key processes determining lipoprotein

TABLE 4. Spearman correlations between the kinetics of apoB-100- and apoB-48-containing lipoproteins and IGFBP-2 concentrations among 66 individuals characterized as insulin sensitive (fasting insulin concentrations <90 pmol/l)

Variables	Correlations (Spearman rho) with IGFBP-2			
	Adjustments			
	None	Age and BMI	Age, BMI, and relative fat intake during the tracer study	Age, BMI, and relative fat intake during the tracer study, fasting TG
VLDLapoB-100				
PS (mg)	-0.36 ^a	-0.39 ^a	-0.45 ^b	-0.35 ^a
PR (mg • kg body weight ⁻¹ • day ⁻¹)	-0.09	-0.00001	-0.03	-0.05
FCR (pools/day)	0.24	0.41 ^b	0.42 ^b	0.27 ^c
IDLapoB-100				
PS	-0.28 ^c	-0.12	-0.16	-0.03
PR	-0.04	0.005	-0.04	-0.04
FCR	-0.10	0.10	0.07	-0.03
LDLapoB-100				
PS	-0.32 ^c	-0.26 ^c	-0.38 ^a	-0.37 ^a
PR	-0.13	-0.15	-0.28 ^c	-0.28 ^c
FCR	0.09	0.12	0.10	0.08
TRLapoB-48				
PS	-0.30 ^c	-0.28 ^c	-0.48 ^b	-0.32 ^c
PR	-0.20	-0.17	-0.36 ^a	-0.21
FCR	0.04	0.15	0.10	0.08

n = 59 for IDLapoB-100 and LDLapoB-100 kinetic and *n* = 56 for TRLapoB-48 kinetic.

^a*P* < 0.01.

^b*P* < 0.001.

^c*P* < 0.05.

TABLE 5. Spearman correlations between the kinetics of apoB-100- and apoB-48-containing lipoproteins and IGFBP-2 concentrations among the III individuals in the cohort characterized as insulin resistant (fasting insulin concentrations ≥ 90 pmol/l)

Variables	Correlations (Spearman rho) with IGFBP-2			
	Adjustments			
	None	Age and BMI	Age, BMI, and relative fat intake during the tracer study	Age, BMI, and relative fat intake during the tracer study, fasting TG
VLDLapoB-100				
PS (mg)	-0.10	-0.10	-0.10	0.05
PR (mg \cdot kg body weight ⁻¹ \cdot day ⁻¹)	-0.04	0.07	0.07	0.08
FCR (pools/day)	0.10	0.23 ^a	0.23 ^a	0.10
IDLapoB-100				
PS	-0.34 ^b	-0.31 ^a	-0.35 ^b	-0.20
PR	-0.0004	0.08	0.04	0.16
FCR	0.30 ^a	0.42 ^c	0.41 ^c	0.39 ^b
LDLapoB-100				
PS	-0.09	-0.10	-0.10	-0.05
PR	0.14	0.15	0.15	0.23
FCR	0.18	0.22	0.22	0.19
TRLapoB-48				
PS	-0.25 ^a	-0.30 ^b	-0.30 ^b	-0.19
PR	-0.15	-0.13	-0.13	-0.08
FCR	0.09	0.16	0.16	0.04

$n = 67$ for IDLapoB-100 and LDLapoB-100 kinetic and $n = 102$ for TRLapoB-48 kinetic.

^a $P < 0.05$.

^b $P < 0.01$.

^c $P < 0.001$.

concentrations. Taken together, our data indicated that higher plasma IGFBP-2 levels are associated with a higher catabolism of apoB-100-containing VLDL and with a lower PR of apoB-48 TRL, the latter being modulated by the state of insulin resistance. These two potential kinetic factors are likely involved in the inverse relationship between circulating IGFBP-2 and TG levels.

Subjects were mildly hyperlipidemic men who participated in various experimental designs but included in groups that had no particular treatment, a control diet, or placebo regimen (20–32). On average, anthropometric and biochemical characteristics indicated that participants were overweight and showed established markers of a deleterious cardiometabolic profile. Specifically, analysis from these multiple studies reiterates the already established association between low IGFBP-2 levels and parameters of insulin resistance and dyslipidemia, including high TG and low HDL-cholesterol, even after adjustment for age and BMI. Thus, despite its large cross-sectional nature, this study included individuals with similar associations between IGFBP-2 and lipid metabolism, which was an important basis for further assessment of its links with lipoprotein kinetics.

The amount of the different circulating lipoprotein fractions results from the balance between their rate of production and catabolism. The quantification of specific apolipoproteins, especially apoB-100 (for VLDL, IDL, and LDL) and apoB-48 (for TRL-chylomicrons), informs about the number of lipoproteins (as there is one apoB per particle) but not about their size, which is rather dependent on the mass of their content.

Consistent with previous studies (8), plasma IGFBP-2 concentrations were robustly and negatively associated with the PS (number) of TRLapoB-48, VLDLapoB-100, IDLapoB-100, and LDLapoB-100 to a smaller yet statistically significant extent. When adjusted for age, BMI, and the relative amount of fat consumed during the tracer studies, these relationships remained significant for VLDLapoB-100 and TRLapoB-48 only, suggesting that TRLs are more susceptible to IGFBP-2 than IDL and LDL. Supporting this view, in a model further adjusted for fasting triglyceridemia, the association between IGFBP-2 levels and PS of VLDLapoB-100, which are naturally secreted in the fasted state, was no longer observed. In contrast, IGFBP-2 levels were still negatively associated with PS of TRLapoB-48 even after adjustment for fasting TG, which is consistent with the production of chylomicrons primarily occurring in the postprandial state.

The pool of VLDL, and consequently those of IDL and LDL produced after hydrolysis of their TG content by the action of lipoprotein lipase (LPL) in peripheral tissues, results from the balance between production in the liver and catabolism. Importantly, no relationship was found between IGFBP-2 and the PR of VLDLapoB-100, as analyzed in the whole cohort or specifically for insulin-sensitive or insulin-resistant individuals. This first main finding suggests that increased catabolism is the main determinant by which higher IGFBP-2 levels are associated with lower pools of VLDL particles. This finding helps to understand the previously shown associations between IGFBP-2 and VLDL levels in the circulation (8) and strengthens the possibility that IGFBP-2 influences the lipid profile through actions on

extrahepatic tissues to modulate lipoprotein metabolism. Supporting this view, plasma IGFBP-2 levels were positively linked with the catabolism of VLDLapoB-100 and IDLapoB-100, and consequently, with the production of LDLapoB-100 as a final catabolic cascade of apoB-100-containing particles. These relationships were stronger in insulin-sensitive than in insulin-resistant individuals, which for the latter was likely because of an impaired action of LPL in adipose tissue, especially in a steady postprandial state (38). However, whether and how IGFBP-2 affects LPL action remains to be fully tested. Although there is no published evidence yet for a direct molecular mechanism linking IGFBP-2 and LPL levels, its activity could be indirectly enhanced through the promotion of a higher blood flow via the demonstrated stimulating action of IGFBP-2 on the transcription of vascular endothelial growth factor (VEGF) (39). This hypothesis should also be thoroughly investigated.

The second main finding of the present study is that the negative correlations between IGFBP-2 levels and TRLapoB-48 particles may be in large part attributed to their production. In the whole cohort, this association was observed even after adjustment for age, BMI, and the relative amount of fat consumed during the tracer studies. The inverse association between plasma concentrations of IGFBP-2 and chylomicron production was also seen among participants with and those without type 2 diabetes. This suggests that IGFBP-2 may be related to chylomicron metabolism, particularly production, irrespective of the degree of insulin resistance. It would be important to confirm these concepts in different cohorts with larger degrees of insulin sensitivity and with other methods to characterize insulin resistance (for instance, the HOMA or Matsuda index). Yet, whether and how IGFBP-2 can directly impact chylomicron production in the intestinal tract is unknown. An interesting possibility is that IGFBP-2 could directly control key intestinal genes at the transcriptional level, as previously shown for VEGF in other tissues (39). In enterocytes, VEGF induces lacteal junction zippering, leading to chylomicron malabsorption in the endothelium, reducing its uptake in the bloodstream (40). In addition, direct overexpression of IGFBP-2 was recently found to reduce the expression of the transcription factor sterol regulatory element-binding protein-1c in hepatocytes (41) and could have similar effects in the gut, which would result in a reduction in chylomicron production. Again, these hypotheses need to be tested and validated experimentally using more direct cellular and murine models lacking IGFBP-2.

The present work has several weaknesses that limit the extent of the conclusions. Importantly, it is a transversal experimental design across several independent studies, allowing only associative observations. In addition, patients with type 2 diabetes included in this cohort were all under treatment with a stable dose of metformin, which may be a confounding factor in

the interpretation of our results since metformin has been shown to increase IGFBP-2 levels in mice (42). Although relationships between IGFBP-2 and VLDL catabolism and apoB48-TRL production were very similar (statistically and in magnitude) between nondiabetic individuals and diabetic patients and metformin, which strengthen our conclusions, it is thus possible that metformin treatment could have influenced the results reported for the entire cohort. Moreover, since biological sex is a major determinant of lipid metabolism and insulin sensitivity (43), it is also crucial to note that the results obtained might be specific to men, and that similar testing need to be performed in women before any generalization. In addition, by measuring the kinetics of apoB-containing lipoproteins, this work did not offer a possible explanation for the established relationships between IGFBP-2 and HDL-cholesterol. Finally, timed measurements of these lipoproteins in the circulation does neither allow any glimpse on the tissue nor allow the mechanism of action in the periphery that would contribute to TG clearance, including relationships with other apolipoproteins or enzymatic activity between lipoproteins.

In summary, we report that the inverse relationship between circulating IGFBP-2 and triglyceridemia is associated with important novel links involving the kinetics of TRLs in men. Our findings suggest that the association of IGFBP-2 with the pool of TG-rich VLDL is more closely linked with their catabolism in the periphery rather than their production. They also suggest that the negative correlation between IGFBP-2 and the amount of TG-rich chylomicrons is associated with a reduced PR in the intestine in the postprandial state. These novel observations offer a solid rationale for the replication in other cohorts. Finally, they also support the thorough investigation of the direct and indirect effects of IGFBP-2 on circulating TG levels, especially through the modulation of their clearance from VLDL and their entry into the circulation within chylomicrons.

Data availability

All data pertinent to this study are contained within the article. Data files are available upon reasonable request to F. Picard (Université Laval; frederic.picard@pha.ulaval.ca). 

Supplemental data

This article contains [supplemental data](#).

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Author contributions

B. L., A. J. T., P. C., and F. P. conceptualization; B. L., A. J. T., P. C., and F. P. methodology; C. R. formal analysis; C. R. investigation; C. R., B. L., and F. P. data curation; C. R., B. L.,

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Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations

CRP, C-reactive protein; FCR, fractional catabolic rate; IGF, insulin-like growth factor; IGFBP-2, insulin-like growth factor binding protein-2; LPL, lipoprotein lipase; PR, production rate; PS, pool size; TG, triglyceride; TRL, TG-rich lipoprotein; VEGF, vascular endothelial growth factor.

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