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Serum FABP4 concentrations decrease after Roux-en-Y gastric bypass but not after intensive medical management ☆

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ABSTRACT

Background: Serum concentrations of fatty acid binding protein 4, an adipose tissue fatty acid chaperone, have been correlated with insulin resistance and cardiovascular risk factors. The objective of this study were to assess relationships among Roux-en-Y gastric bypass, intensive lifestyle modification and medical management protocol, fatty acid binding protein 4, and metabolic parameters in obese patients with severe type 2 diabetes mellitus; and to evaluate the relative contribution of abdominal subcutaneous adipose and visceral adipose to the secretion of fatty acid binding protein 4.

Methods: Participants were randomly assigned to intensive lifestyle modification and medical management protocol ($n=29$) or to intensive lifestyle modification and medical management protocol augmented with Roux-en-Y gastric bypass ($n=34$). Relationships among fatty acid binding protein 4 and demographic characteristics, metabolic parameters, and 12-month changes in these values were examined. Visceral and subcutaneous adipose tissue explants from obese nondiabetic patients ($n=5$) were obtained and treated with forskolin to evaluate relative secretion of fatty acid binding protein 4 in the different adipose tissue depots.

Results: The intensive lifestyle modification and medical management protocol and Roux-en-Y gastric bypass cohorts had similar fasting serum fatty acid binding protein 4 concentrations at baseline. At 1 year, mean serum fatty acid binding protein 4 decreased by 42% in Roux-en-Y gastric bypass participants ($P=.002$) but did not change significantly in the intensive lifestyle modification and medical management protocol cohort. Percentage of weight change was not a significant predictor of 12-month fatty acid binding protein 4 within treatment arm or in multivariate models adjusted for treatment arm. In adipose tissue explants, fatty acid binding protein 4 was secreted similarly between visceral and subcutaneous adipose tissue.

Conclusion: After Roux-en-Y gastric bypass, fatty acid binding protein 4 is reduced 12 months after surgery but not after intensive lifestyle modification and medical management protocol in patients with type 2 diabetes mellitus. Fatty acid binding protein 4 was secreted similarly between subcutaneous and visceral adipose tissue explants.

Introduction

Obesity and type 2 diabetes mellitus (T2DM) are significant sources of morbidity and mortality in the United States and are

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increasing at epidemic rates.[1] For decades, bariatric surgery has been the most effective treatment for obesity and more recently has been recognized for its value in the treatment of T2DM. Previously published results from the Diabetes Surgery Study (DSS) have found that augmenting lifestyle modification and intensive medical management (LS/IMM) with Roux-en-Y gastric bypass (RYGB) increases weight loss and improvements in hemoglobin A1c (HbA1c) in patients with severe T2DM and body mass index (BMI) 30.0 to 39.9.[2] Other studies have corroborated these observations.[3]

Currently less than 1% of the population with obesity and T2DM who meet Centers for Medicare and Medicaid Services criteria for surgical intervention undergo surgery, given its cost, invasiveness, and patient suitability requirements.[4,5] Identifying mechanisms responsible for the efficacy of surgery may allow us to improve nonsurgical care for patients with T2DM. Proposed mechanistic explanations underlying the effectiveness of bariatric surgery include changes in bile acids, increases in glucagon-like peptide-1 (GLP-1) and fibroblast growth factor 19 secretion, and shifts toward more metabolically favorable intestinal microbiota, among others.[6-9] However, adipose tissue dysfunction, a central feature of insulin resistance, remains inadequately characterized.

Fatty acid binding protein 4 (FABP4, also known as aP2) is a 15 kDa lipid carrier that is abundantly expressed in adipose tissue and plays a major role as a fatty acid chaperone facilitating lipolysis and additionally is secreted into circulation.[10] In mice fed a high saturated fat diet, deletion of FABP4 reduces endoplasmic reticulum stress and inflammation and improves insulin sensitivity without altering weight, thus uncoupling insulin resistance from obesity.[11-13] FABP4 has primarily been investigated as a cytosolic protein, and we have previously reported a rapid reduction in the messenger RNA and protein levels of FABP4 in subcutaneous adipose tissue after bariatric surgery.[14]

Although primarily investigated as a cytosolic protein, several studies have found associations between higher serum FABP4 and a number of diseases.[15-18] The Framingham Heart Study found that higher circulating FABP4 is positively correlated with insulin resistance and multiple cardiovascular risk factors, including total cholesterol, lower high-density lipoprotein cholesterol, and hypertension.[18] Circulating FABP4 is known to be elevated in patients with obesity and has been identified as a predictor of the development of metabolic syndrome independent from adiposity and insulin resistance.[19] Furthermore, a polymorphism in the promoter region of FABP4 that results in decreased expression is associated with a reduced risk of heart disease and development of T2DM in participants with obesity.[20] Even cancer outcomes, including morbidity and mortality in breast, ovarian, and prostate cancers, have been associated with higher serum FABP4 concentrations.[21-23] However, the secretion of FABP4 remains poorly understood. It remains unclear which adipose depot (ie, visceral versus subcutaneous) contributes to its secretion. Although both depots have been found to be independent contributors to cardiovascular disease and metabolic syndrome and play active endocrine roles, there are important physiologic differences, including adipokine secretion and rates of lipolysis and triglyceride synthesis.[24-26] In fact, many diseases that affect fat (ie, glucocorticoid excess and congenital lipodystrophy) do so in a depot-specific manner.[27] Although FABP4 appears to be linked to a number of diseases, studies identifying a direct relationship between FABP4 and weight change are limited, but it appears that it may also serve as a prognostic marker for weight loss.[28] Given its known biology, it would be expected that FABP4 concentrations increase with rapid weight loss because of an increase in lipolysis and fatty acid transport, but long-term reductions in weight would result in its reduction.[29]

Although causality has not been established, the association between more favorable metabolic parameters and lower FABP4 concentrations suggests that achieving reductions in FABP4 may be of value in treating metabolic syndrome. One intervention to accomplish this may be bariatric surgery. Although serum FABP4 concentrations have been reported to decrease after RYGB, it is unclear how this decrease is related to weight loss and glucose homeostasis. It is also unclear how current medical management of obesity and T2DM affects serum FABP4 concentrations.[30,31] In this study we measured serum FABP4 in a pilot cohort of participants with T2DM, HbA1c > 8.0%, and BMI 30.0 to 39.9 at baseline. Participants were randomly assigned to LS/IMM with or without RYGB. FABP4, BMI, and metabolic parameters were measured at baseline and at 1 year after intervention; in this ancillary study we explored potential relationships between FABP4 and the other values, both within and between treatment arms. To identify the major adipose depot contributing to serum concentrations of FABP4, we then used visceral and subcutaneous adipose tissue explants from obese patients at the time of bariatric surgery and assessed the relative contribution of each depot to circulating FABP4 level.

Methods

Participants

The Diabetes Surgery Study was a randomized trial comparing the effectiveness of RYGB and LS/IMM to achieve established therapeutic targets for the treatment of T2DM. Details of participant recruitment and allocation have been previously reported.[2,8,32] All institutions involved had Institutional Review Board approval. Between 2008 and 2011, 60 of the 120 obese participants participating in an intensive lifestyle and medically managed weight control program were randomly assigned to undergo RYGB while continuing a lifestyle/medical management protocol. Inclusion criteria included age > 30, BMI of 30.0 to 39.9 kg/m^2, undergoing treatment for T2DM for at least 6 months, HbA1c of 8.0% or higher, and C peptide > 1.0 ng/mL 90 minutes after a liquid mixed meal. Participants were excluded from the study if they had serious conditions precluding surgery. The lifestyle intervention was modeled after the Diabetes Prevention Program and the Look AHEAD protocol.[33,34] Participants met regularly with a trained bariatric registered dietician. Lifestyle interventions were augmented with intensive medical therapy to obtain treatment goals established by American Diabetes Association: HbA1c < 7.0%; serum LDL cholesterol < 100 mg/dL; and systolic blood pressure < 130 mm Hg. Techniques for the RYGB have been previously published.[2]

Candidates for the present analysis were selected from among the 88 DSS participants randomly allocated at 3 clinics in the United States (University of Minnesota, Columbia University in New York, and Mayo Clinic in Rochester, MN) (Fig 1). Participants were excluded if they lacked follow-up data or available sera at 12 months, if they had type 1 diabetes, if they used thiazolidinediones (TZDs) at baseline or during the first year of the clinical trial, or if they crossed over after the study was underway. Participants on TZDs were excluded from the present analysis because TZDs are known to increase the activity of the nuclear receptor responsible for FABP4 expression.[16]

Participants recruited for the evaluation of visceral and subcutaneous adipose tissue provided informed consent approved by the University of Minnesota Institutional Review Board. Tissue biopsy specimens were obtained from these participants at the time of bariatric surgery.

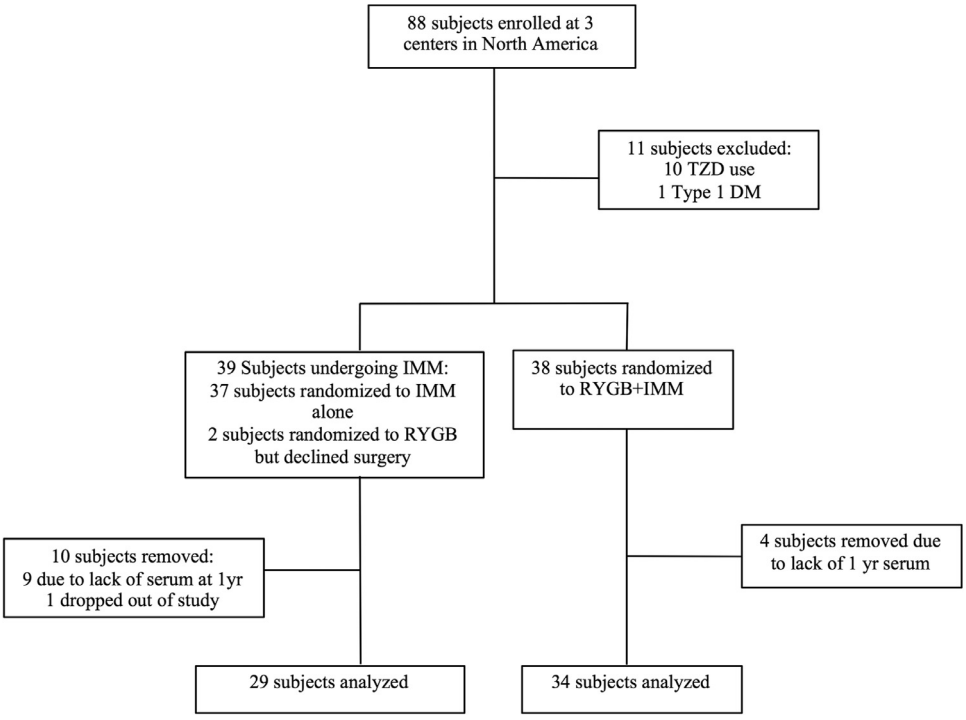

Figure 1. Study flow diagram. *DM,* diabetes mellitus; *IMM,* intensive medical management; *RYGB,* Roux-en-Y gastric bypass; *TZD,* thiazolidinedione

Measurement and data collection

Data were collected at baseline and at scheduled medical visits. Collected data included height, weight, medications used, and laboratory measurements, including HbA1c, fasting and 90-minutes postmeal glucose, and C-peptide concentrations. Serum FABP4 was measured with the human FABP4 Quantikine ELISA kit from R&D Systems (Minneapolis, MN; #DFBP40) according to the manufacturer's instructions.

Statistical design and analysis

Baseline characteristics and 1-year outcomes for this cohort were reported using means (95% confidence interval [CI] or ± standard deviation) for continuous data and percentages for categorical data. Comparisons were made using Student's t test, χ^2 statistics, and exact binomial models where appropriate. Univariate linear regressions were used to analyze variables potentially associated with serum FABP4, including age, sex, race/ethnicity, time since diagnosis of T2DM, BMI, percentage weight change, HbA1c, postchallenge C peptide, fasting and postchallenge glucose, fasting insulin, homeostatic model assessment of insulin resistance (HOMA-IR), Matsuda Index (calculated as 10,000 / [$\text{glucose}_0 \times \text{insulin}_0 \times$ mean glucose $\times$ mean insulin]$^{0.5}$), and use of exogenous insulin, other antihyperglycemics, or statins.[8] Variables with P values $<.15$ in univariate regression analyses were considered in developing optimal multivariate models. Because this is an exploratory analysis, many hypothesis tests were carried out without prior hypotheses specified; thus no adjustments for multiple comparisons were imposed. Univariate models were used to identify determinants of 12-month levels of FABP4 and then used to construct a multivariate model for prediction of FABP4 at 12 months. All statistical analyses were completed using SAS Software, Version 9.3 (SAS Institute, Inc, Cary, NC).

Nonesterified fatty acid assay

Visceral and subcutaneous adipose tissue explants were collected from human participants. Fifty milligrams of tissue samples were used to measure free fatty acid (FFA) release. The explants were treated with either dimethyl sulfoxide (DMSO) vehicle control or 20 μM forskolin (FSK) for 1 and 4 hours. Nonesterified fatty acid assay of tissue explants were performed as described[35] and measured using a NEFA-HR kit (Wako Chemicals USA, Inc, Richmond, VA).

Immunoblot

The tissues were incubated in Krebs-Ringer-HEPES buffer with 1% bovine serum albumin. The incubating media/extracellular material were collected for further analysis. The tissue explants were homogenized on ice in a homogenization buffer (50 mM Tris pH 7.4, 50 mM NaCl and 1 mM EDTA, 1 mM ethylene glycol tetraacetic acid, 1 mM NaP_2O_7, 50 mM NaF supplemented with protease inhibitors [Calbiochem, Billerica, MA]). Homogenates were centrifuged at 1000 g at 4°C for 10 minutes to separate the lipid cake, the infranatant was removed, and sodium dodecyl sulfate was added to a final concentration of 1%. The lysate was then centrifuged at 10,000 g for 20 minutes at 4°C to remove insoluble residue and the supernatant recovered. Western blot analyses were performed on equal volumes of incubating media from all samples and 5% of tissue lysate and incubating media to detect FABP4 and intracellular protein β-actin. FABP4 was assayed by running calculated amounts of purified FABP4 protein samples on respective gels. The immunoblots were imaged using Odyssey infrared imaging (LI-COR Biosciences; Lincoln, NE). The antibodies used were anti-FABP4 and antiactin (Sigma-Aldrich, St. Louis, MO).

Results

Of the 88 possible candidates for review, 25 were excluded: 13 for absent serum samples, 10 because of TZD use at baseline, 1

Table 1
Baseline characteristics of participants randomly assigned to LS/IMM or RYGB.

	LS/IMM ($n=29$)	RYGB ($n=34$)	P
Demographic characteristics			
Age (y)	50 ± 8	51 ± 9	.60
Female	13 (45%)	22 (65%)	.13
Race/ethnicity			1.00
Black or African American	4 (14%)	5 (15%)	
Native American	1 (3%)	2 (6%)	
Hispanic	2 (7%)	2(6%)	
General medical			
BMI (kg/m^2)	35.7 ± 3.2	36.1 ± 2.7	.60
Years since diabetes diagnosis	8.9 ± 5.7	11.1 ± 6.4	.14
Laboratory values			
HbA1c (%)	9.5 ± 1.2	9.5 ± 0.9	.97
Circulating FABP4 (ng/mL)	38 ± 17	38 ± 22	.92
C peptide, 90-min (ng/mL)	5.1 ± 2.6	4.2 ± 1.8	.13
Glucose, fasting (mg/dL)	215 ± 58	224 ± 65	.48
Glucose, 90-min (mg/dL)	273 ± 64	284 ± 55	.47
Insulin, fasting (mU/L)	22 ± 19	27 ± 30	.46
HOMA-IR	11.5 ± 12.1	13.7 ± 13.5	.50
Matsuda Index	2.2 ± 1.6	2.5 ± 2.5	.63
Taking medication (yes/no)			
Insulin	15 (52%)	26 (74%)	.11
Oral antihyperglycemic	28 (97%)	28 (80%)	.06
Any antihyperglycemic	29 (100%)	34 (100%)	1.00
Statin	19 (66%)	24 (69%)	1.00

Values are reported as mean ± standard deviation or n (%).

who was later diagnosed with type 1 diabetes, and 1 patient randomly assigned to LS/IMM patient who later obtained surgery outside the study. Two participants who declined surgery after allocation but participated in the LS/IMM intervention were grouped with the LS/IMM cohort for the present as-treated analysis. The resulting 29 LS/IMM and 34 surgery participants were included in the study (Fig 1).

Baseline characteristics are summarized in Table 1. There were no statistically significant differences between groups. Mean age was 51; mean BMI was 36 kg/m^2; mean HbA1c was 9.5%; and mean circulating FABP4 was 38 ng/mL. Years since diabetes diagnosis and use of insulin were not different between groups.

One-year outcomes are consistent with previous DSS analyses.[2,8,36] Compared with LS/IMM, RYGB participants had significantly greater weight loss and improved glycemic control despite less medication (Table 2). At 1 year, circulating FABP4 was 19 ng/mL (95% CI 15–23) in RYGB participants versus 33 ng/mL (26–40) in LS/IMM participants (42% lower; $P=.002$). Mean FABP4 dropped 50% in the surgery group, with a proportionate reduction in variability. FABP4 decreased in all but 2 RYGB participants, whose concentration did not change. In contrast, in the LS/IMM group, mean FABP4 decreased 13% but was accompanied by an 11% increase in variability; 10 of 29 (34%) of LS/IMM participants exhibited either increased concentrations or no change (Fig 2). In the LS/IMM cohort, no factors (BMI, HbA1c, fasting glucose, fasting insulin) differed significantly between participants who had increased serum FABP4 concentrations compared with those who had decreased concentrations.

Within treatment arm, percentage weight change and 12-month FABP4 were weakly associated in LS/IMM (correlation 0.35; $P=.07$) but not in RYGB (correlation 0.11; $P=.52$; Fig 3). After adjustment for baseline FABP4, percentage weight change was not a significant predictor of 12-month FABP4 in either arm. In the IMM arm, a 10% greater weight loss corresponded to 5.20 ng/mL lower FABP4 in the IMM group (95% CI −3.96 to 14.36; $P=.25$; $R^2=0.34$). In the surgical arm, a 10% greater weight loss was associated with 2.12 ng/mL lower FAPB4 (95% CI −1.12 to 5.37; $P=.19$; $R^2=0.44$). Both cohorts include patients with up to 20% weight loss; although everyone lost weight after RYGB, not everyone lost weight in the IMM co-

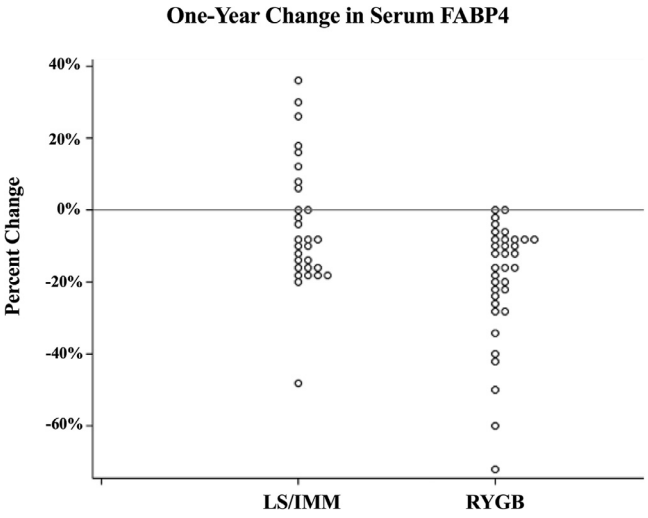

Figure 2. One-year percentage change in serum fatty acid binding protein 4 (FABP4) concentrations in participants after intervention. *LS/IMM*, lifestyle and intensive medical management; *RYGB*, Roux-en-Y gastric bypass.

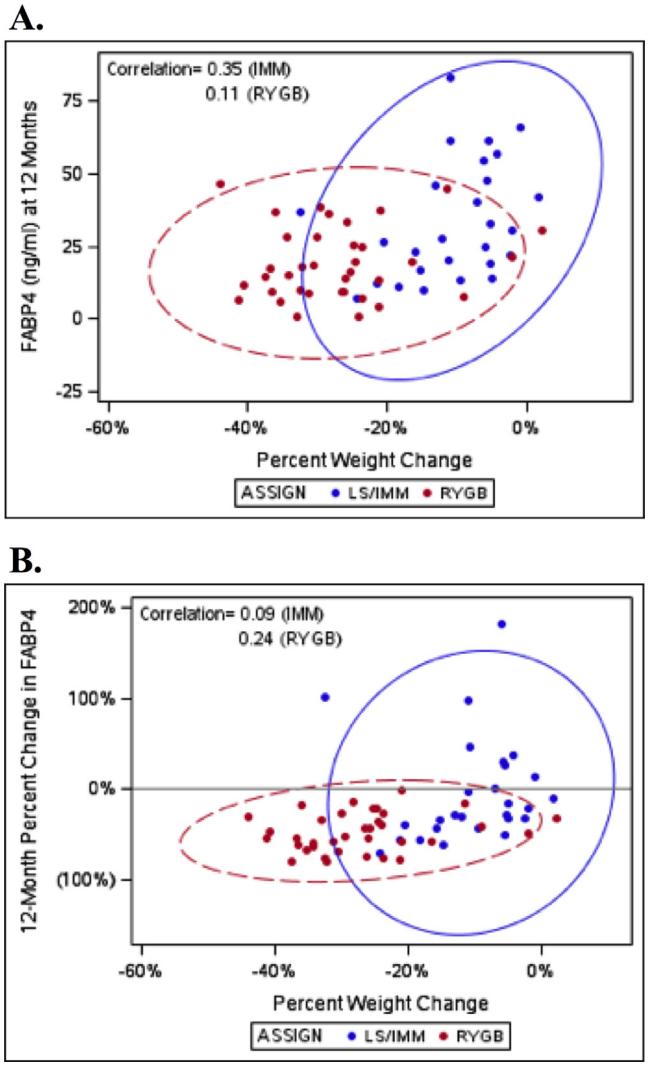

Figure 3. Predictive ellipses of serum fatty acid binding protein 4 (FABP4) versus percent weight change. (A) Cross-sectional 12-month FABP4. (B) The 12-month percentage change in FABP4. *LS/IMM*, lifestyle and intensive medical management; *RYGB*, Roux-en-Y gastric bypass.

Table 2
Characteristics of participants 1 year after intervention began.

	LS/IMM (n = 29)	*RYGB (n = 34)*	*P*
BMI (kg/m^2)	32.2 (30.6,33.7)	25.8 (24.6,26.9)	<.00001
Weight change (percentage of baseline)	−10 (−13, −7%)	−29 (−31, −26)	<.00001
HbA1c (%)	7.1 (6.7, 7.6)	6.3 (6.0, 6.7)	.01
HbA1c < 7.0%	14 (48%)	25 (74%)	.07
HbA1c < 7.0% with no medication	0 (0%)	19 (56%)	<.00001
Circulating FABP4 (ng/mL)	33 (26, 40)	19 (15, 23)	.002
C peptide, 90-min (ng/mL)	5.9 (4.8, 7.0)	3.9 (3.3, 4.4)	.002
Glucose, fasting (mg/dL)	139 (123, 156)	108 (99, 118)	.003
Glucose, 90-min (mg/dL)	180 (156, 205)	129 (116, 142)	.001
Insulin, fasting (mU/L)	16 (10, 21)	7 (3, 10)	.01
HOMA-IR	5.8 (3.2, 8.4)	1.9 (0.8, 3.0)	.01
Matsuda Index	6.0 (3.8, 8.1)	9.3 (7.1,11.4)	.04
Taking medication (yes/no)			
Insulin	13 (45%)	8 (24%)	.11
Oral antihyperglycemic	29 (100%)	13 (38%)	<.00001
Any antihyperglycemic	29 (100%)	14 (41%)	<.00001
Statin	21 (72%)	17 (50%)	.08

Values are reported as mean (95% CI) or *n* (%).

Table 3
Baseline variables as univariate predictors of a 1 ng/mL increase baseline FAPB4.

	Estimate (95% CI)	*P*
Demographic characteristics		
Age (per 10 y)	7.4 (1.5, 13.2)	.01
Female	13.36 (3.91, 22.80)	.01
Race/ethnicity	—	.18
General medical		
BMI (kg/m^2)	−0.41 (−2.14, 1.31)	.63
Years since diabetes diagnosis	0.00 (−0.82, 0.82)	1.00
Laboratory values		
HbA1c (%)	−1.68 (−6.59, 3.24)	.50
C peptide, 90-min (ng/mL)	−0.79 (−3.04, 1.46)	.48
Glucose, fasting (per 10 mg/dL)	−1.1 (−1.9, −0.3)	.01
Glucose, 90-min (per 10 mg/dL)	−0.8 (−1.6, 0.00)	.05
Insulin, fasting (mU/L)	−0.08 (−0.28, 0.12)	.42
HOMA-IR	−0.27 (−0.66, 0.12)	.17
Matsuda Index	0.78 (−1.54, 3.09)	.50
Taking medication (yes/no)		
Insulin	2.56 (−7.79, 12.90)	.62
Oral antihyperglycemic	−0.16 (−15.2, 14.84)	.98
Any antihyperglycemic	—	—
Statin	2.91 (−7.65, 13.47)	.58

(analysis based on all study participants)

hort. FABP4 is much more variable for the IMM patients in this range than for the RYGB patients.

In further exploratory analyses, we examined 15 baseline characteristics as potential univariate predictors of baseline FABP4 (Table 3). Significant associations included age (7.4 ng/mL higher FABP4 per 10-year greater age; 95% CI 1.5–13.2), female sex (13.4 ng/mL higher FABP4; 95% CI 3.9–22.8), fasting glucose (1.1 lower ng/mL FABP4 per 10 mg/dL higher fasting glucose; 95% CI 0.3–1.9), and 90-minute postchallenge glucose (0.8 ng/mL lower FABP4 per 10 mg/mL higher glucose; 95% CI 0–1.6; $P = .05$).

Similarly, individual patient characteristics (including baseline, 12-month, and 12-month change, where appropriate) were examined individually as predictors of 12-month FABP4 (Table 4). Regressions were conducted separately by treatment arm and adjusted for baseline FABP4. At 12 months, percent weight change dominated treatment arm as a predictor of HbA1c, as previously reported [2]. However, percent weight change was not a significant predictor of 12-month FABP4 within either treatment arm, either after adjustment for baseline FABP4 or in potential multivariate models. Similarly, no other baseline value was predictive of 12-month FABP4 after adjustment for baseline FABP4. In the LS/IMM group, the optimal multivariate model for 12-month FABP4 included baseline FABP4, change in fasting insulin, and change in postchallenge C peptide. In the RYGB group, the optimal model included only baseline FABP4. R^2, the proportion of variability explained by the model, was 0.50 in the optimal LS/IMM model and 0.41 in the optimal RYGB model.

Next we used visceral and abdominal subcutaneous adipose tissue explants obtained from obese human participants undergoing bariatric surgery to identify the relative contributions of these depots to the secretion of FABP4 (Supplemental Table I). The ex vivo tissue explants effluxed nonesterified free fatty acids and responded to lipolytic stimuli (Fig 4, *A*), indicating that the tissues were metabolically active. To analyze the secretory capacity of visceral and abdominal subcutaneous depots, we measured the FABP4 secreted from excised samples. The 2 types of adipose samples secreted similar amounts of FABP4 ($P = .47$ and $P = .17$, 1 and 4 hours after DMSO treatment, respectively; Fig 4, *B* and *C*). Intriguingly, the secretion of FABP4 was not stimulated in response to lipolytic signal from human tissue ($P = .19$ and $P = .17$, 1 hour and 4 hours after FSK treatment, respectively). To confirm that the FABP4 in the supernatant was secreted and not present in media as a result of tissue cellular lysis, we evaluated the expression of β-actin in both intracellular and extracellular material. The presence of actin in the cellular lysate but not in the cell culture supernatant fraction is an indicator of intact cells and implies that FABP4 found is the secreted fraction resulting from regulated release rather than lytic release.

Discussion

This study assessed serum FABP4 concentrations in a diabetic population 1 year after random allocation to LS/IMM with or without RYGB. Mean serum FAPB4 decreased 50% in the year after surgery versus a 1-year decrease of just 13% in LS/IMM. Furthermore, no statistically significant correlation was identified between weight loss and 12-month FABP4 levels in either arm. In evaluating the relative contribution of the secretion of FABP4 from visceral and abdominal subcutaneous adipose tissue, both depots secrete approximately similar amounts of FABP4/mg tissue, suggesting that the majority of serum FABP4 is attributable to subcutaneous adipose tissue given its larger volume.

Our observations are consistent with findings from others reporting reductions in serum FABP4 concentrations at 6 and 12 months after RYGB.[30,31] However, the present study described participants with severe T2DM, whereas the previous cohorts were

Table 4
Predictors of 1 ng/mL greater FABP4 at 12 months by treatment arm.

Predictors of FABP4 at 12 months by treatment arm*				
	IMM		*RYGB*	
	Estimate (95% CI)	*P*	*Estimate (95% CI)*	*P*
Baseline FABP4, as a univariate predictor	0.63 (0.24, 1.03)	.003	0.37 (0.22, 0.53)	<.0001
Individual predictors adjusted for baseline FABP4				
At 12 months:				
HbA1c (%)	3.36 (−2.08, 8.8)	.24	3 (−0.31, 6.31)	.09
Glucose, fasting (mg/mL)	−0.01 (−0.16, 0.13)	.88	0.12 (0.01, 0.24)	.04
Insulin, fasting (mU/L)	0.37 (−0.06, 0.8)	.10	0.1 (−0.2, 0.41)	.51
Change from baseline to 12 months				
Percentage weight change (per 10%)	51.98 (−35, 138.95)	.25	21.25 (−10, 52.5)	.19
HbA1c (%)	2.78 (−1.75, 7.31)	.24	2.14 (−0.43, 4.71)	.11
C peptide, 90-min (ng/mL)	−2.88 (−6.5, 0.74)	.13	0.5 (−1.16, 2.17)	.56
Glucose, fasting (per 10 mg/dL)	0.1 (−1.1, 1.3)	.87	0.4 (−0.1, 0.9)	.11
Glucose, 90-min (per 10 mg/dL)	0.0 (−1.0, 1.1)	.94	0.4 (−0.1, 0.8)	.11
Insulin, fasting (mU/L)	0.32 (0.01, 0.63)	.05	−0.01 (−0.13, 0.11)	.87
Optimal multivariate model†				
FABP4 at baseline (ng/mL)	0.44 (0.06, 0.82)	.03	0.37 (0.22, 0.53)	<.0001
Change in fasting insulin (mU/L)	0.36 (0.05, 0.67)	.02		
Change in 90-min C peptide (ng/mL)	−3.4 (−6.92, 0.11)	.06		

* The following potential predictors were also considered: age, sex, years since diabetes diagnosis, BMI, use of insulin, other antihypoglycemic medications or statins, and BMI. Only percentage weight change and predictors with $P < .15$ after adjustment for baseline FABP4 are shown in this table.
† R^2 for the optimal multivariate models was 0.50 (IMM) and 0.41 (RYGB).

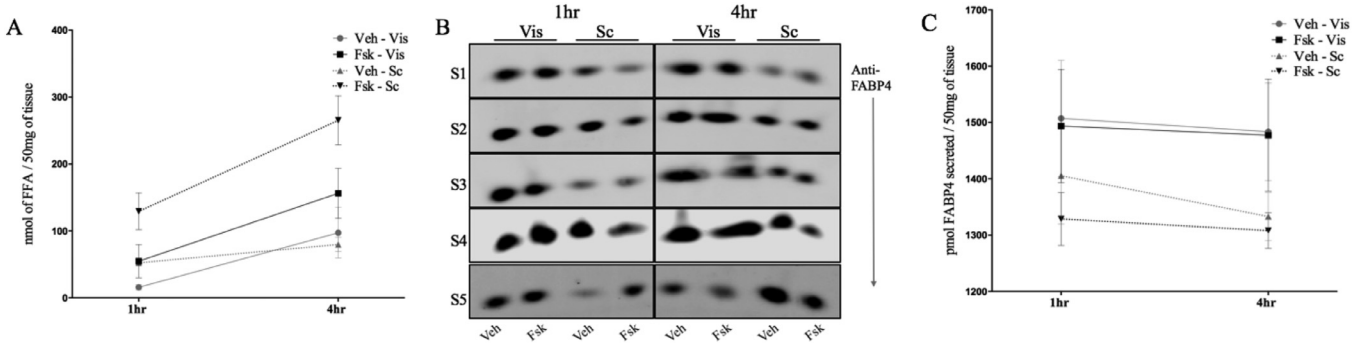

Figure 4. Fatty acid binding protein 4 (FABP4) is secreted from both visceral and subcutaneous adipose tissues. (A) Adipose tissue explants from either the visceral (Vis) or subcutaneous (Sc) depot were treated with dimethyl sulfoxide (DMSO) or forskolin (FSK) for 1 to 4 hours and nonesterified free fatty acids (FFA) released into the incubation medium determined. (B) Immunoblot of FABP4 secreted from tissue explants from human participants (S1–S5) in response to DMSO or FSK treatment. (C) Quantitation of FABP4 secreted into the incubating media. (D) Immunoblot of β-actin from tissue lysates and incubation media of DMSO and FSK-treated tissue explants.

predominantly nondiabetic. It thus appears that RYGB is associated with significant 1-year reductions in serum FABP4, regardless of diabetic status. The reduction of serum concentration of FABP4 may contribute to improved metabolism after RYGB. Numerous animal studies and evaluations of human polymorphisms link FABP4 to insulin resistance, increased production of proinflammatory cytokines, and increased reactive oxygen species.[10,12,20,37] Higher serum FABP4 is associated with higher BMI and worsened dyslipidemia.[18,38] Elevated FABP4 is also linked to hepatic insulin resistance and increased hepatic gluconeogenesis. Furthermore, a genetic polymorphism that decreases FABP4 expression appears to have a protective effect against T2DM and atherosclerosis when controlled for BMI.[20] Although causal pathways have yet to be determined, clinical treatments that reduce serum FABP4 might be beneficial for patients with metabolic syndrome or type 2 diabetes. Animal studies targeting the reduction of FABP4 using monoclonal antibodies have been promising.[39] RYGB is currently the only known treatment that reliably significantly decreases this adipokine, although angiotensin II receptor blockers, atorvastatin, and omega-3 fatty acids have produced modest reductions.[40-42]

Although others have reported a positive correlation between FABP4 concentrations and HOMA-IR after RYGB,[30] in our exploratory analysis we did not identify the same association. We did, however, find a weak negative association between fasting glucose levels and serum FABP4 concentrations at baseline. In the LS/IMM cohort, change in fasting insulin was included in the optimal multivariate model predicting 12-month FABP4. Reasons for these differences are unclear, but they may be related to medication use, extent of weight loss, and diabetic status. Further in-depth metabolic studies are needed to elucidate these relationships. Greater age and female sex were both also associated with higher baseline FABP4. Factors accounting for sex differences include androgen hormone production and body composition.[43]

It is not clear how FABP4 concentrations decline after RYGB. A gene target of the nuclear receptor peroxisome proliferator-activated receptor γ, FABP4 is the major lipid chaperone in adipocytes, facilitates lipolysis, and is secreted into serum by unconventional means.[44,45] Sustained reductions in serum FABP4 at 1 year after RYGB may be due to alterations in adipose tissue metabolism that are specific to RYGB. This reduction may be mediated by the effect of RYGB on other serum factors, like GLP-1, which is increased after RYGB.[8,9] Sitagliptin, a dipeptidyl peptidase 4 inhibitor that increases GLP-1, results in mild reductions of FABP4 concentrations in patients with T2DM.[46] It is possible that

RYGB with significant weight loss itself contributes to reductions in FABP4 in the long term, but how this may occur is also unclear. Long-term studies of FABP4 in medically managed weight loss cohorts after 2 years of treatment have found reductions in FABP4.[47] Baseline FABP4 has even been proposed as a prognostic factor for maintenance of weight loss after a calorically restricted medical intervention.[28] However, these assessments were made in insulin-sensitive populations.

FABP4 lacks a conventional secretion signal sequence and thus uses an unconventional secretion mechanism. Molecular details describing this secretion have been limited. Importantly, the underlying concept of how different adipose depots contribute to circulating FABP4 is yet unexplored. Analyzing the relative contribution of FABP4 secretion from visceral and abdominal subcutaneous depots provides an insight to the relationship of body composition to whole-body FABP4 secretion. Our findings here indicate that both visceral and subcutaneous adipose tissues secrete FABP4. An intriguing finding, however, is that FABP4 secretion was not potentiated in response to lipolytic stimuli, in contrast with studies in mice wherein β-adrenergic signaling stimulated FABP4 secretion.[48] This observation suggests that the subcutaneous depot may be the major contributor to serum concentrations of FABP4 because of its significantly larger volume. Likewise, a substantial loss of subcutaneous adipose tissue after surgery is likely to contribute to reductions in FAPB4 concentrations. However, more studies are needed to confirm our initial observations.

There are limitations of this study that should be highlighted. RYGB participants had greater weight loss than medical management (mean = 29% vs 10% in LS/IMM); the study would be strengthened by weight loss–matched cohorts. Effects of recent weight loss may confound the relationship between adipose tissue and serum FABP4. If the apparent differences in FABP4 metabolism are related to RYGB per se, RYGB participants may be compared with another form of bariatric surgery (eg, banding or sleeve gastrectomy). Other potential confounders include medication use at baseline and differences in medication use at 12 months as well possible changes in dietary fat intake, which has been found to alter the expression of FABP4 in adipose tissue.[49] This study is also limited by the variability in the changes in FABP4 concentrations after each intervention and could be strengthened by a larger sample size. In contrast to the DSS patients providing longitudinal laboratory data, patients providing adipose tissue samples were insulin sensitive and were not taking any antidiabetic medications. The contrast weakens the study, but on the other hand lack of medication use in the patients providing tissue samples may limit confounding. This study would also be strengthened by the evaluation of visceral and subcutaneous adipose depots at additional sites, as well as in a larger study population. Future studies would include evaluating changes in patients who are prediabetic or diabetic off medications, as well as measurements of FABP4 in shorter time intervals after surgical or medical intervention to elucidate potential relationships with weight change. Another cohort of patients to study are those undergoing abdominoplasty, which would provide further insight into the relative contribution of subcutaneous adipose tissue to serum concentrations of FABP4. An important strength of this study is its reliance on data from a randomized clinical trial, eliminating confounding owing to self-selection.

In conclusion, in a randomized cohort of participants with BMI 30.0 to 39.9 and severe T2DM, FABP4 levels decreased markedly 12 months after RYGB. No statistically significant reduction in FABP4 was identified after 12 months of exposure to an intensive lifestyle and medical management protocol. This study also failed to identify a relationship between weight loss and reductions in FABP4. Further in-depth metabolic studies are required to characterize the relationship between serum FABP4 concentrations and tissue-specific insulin resistance and to understand how RYGB contributes to the marked decline in FAPB4.

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Supplementary materials

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References

1. Ogden CLL, Carroll MDD, Kit BKK, Flegal KMM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA*. 2014;311:806–814.
2. Ikramuddin S, Korner J, Lee W-J, Ramesh C, Petrie WK, Bologa CG, et al. Roux-en-Y gastric bypass versus intensive medical management for the control of type 2 diabetes, hypertension and hyperlipidemia: an international, multicenter, randomized trial. *JAMA*. 2012;309:2240–2249.
3. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, et al. Bariatric surgery versus intensive medical therapy for diabetes—3-year outcomes. N Engl J Med 2014:1-12.
4. Westerveld D, Yang D. Through thick and thin: identifying barriers to bariatric surgery, weight loss maintenance, and tailoring obesity treatment for the future. *Surg Res Pract*. 2016;2016.
5. Pitzul KB, Jackson T, Crawford S, Kwong JC, Sockalingam S, Hawa R, et al. Understanding disposition after referral for bariatric surgery: when and why patients referred do not undergo surgery. *Obes Surg*. 2014;24:134–140.
6. Jahansouz C, Xu H, Hertzel A, Kvalheim N, Cole A, Abraham A, et al. Bile acids increase independently from hypocaloric restriction after bariatric surgery. *Ann Surg*. 2016;264:1022–1028.
7. Liou AP, Paziuk M, Luevano J-M, Machineni S, Turnbaugh PJ, Kaplan LM. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Sci Transl Med*. 2013;5 178ra41.
8. Nguyen KT, Billington CJ, Vella A, Wang Q, Ahmed L, Bantle JP, et al. Preserved insulin secretory capacity and weight loss are the predominant predictors of glycemic control in patients with type 2 diabetes randomized to Roux-en-Y gastric bypass. *Diabetes*. 2015;64:3104–3110.
9. Sachdev S, Wang Q, Billington C, Connett J, Ahmed L, Inabnet W, et al. FGF 19 and bile acids increase following Roux-en-Y gastric bypass but not after medical management in patients with type 2 diabetes. *Obes Surg*. 2016;26:957–965.
10. Hotamisligil GS, Bernlohr DA. Metabolic functions of FABPs—mechanisms and therapeutic implications. *Nat Rev Endocrinol*. 2015;11:592–605.
11. Furuhashi M HG. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nat Rev Drug Discov*. 2010;7:489–503.
12. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou E, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science*. 1996;274:1377–1379.
13. Erbay E, Babaev VR, Mayers JR, Makowski L, Charles KN, Snitow ME, et al. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. 2010;15:1383-91.
14. Jahansouz C, Xu H, Hertzel AV, Kizy S, Steen KA, Foncea R, et al. Partitioning of adipose lipid metabolism by altered expression and function of PPAR isoforms after bariatric surgery. *Int J Obes (Lond)*. 2018;42:139–146.
15. Parra S, Cabré A, Marimon F, Ferré R, Ribalta J, Gonzàlez M, et al. Circulating FABP4 is a marker of metabolic and cardiovascular risk in SLE patients. *Lupus*. 2014;23:245–254.
16. Cabré A, Lázaro I, Girona J, Manzanares JM, Marimón F, Plana N, et al. Fatty acid binding protein 4 is increased in metabolic syndrome and with thiazolidinedione treatment in diabetic patients. *Atherosclerosis*. 2007;195 e150-8.
17. Ishimura S, Furuhashi M, Watanabe Y, Hoshina K, Fuseya T, Mita T, et al. Circulating levels of fatty acid-binding protein family and metabolic phenotype in the general population. *PLoS One*. 2013;8 e81318.
18. Kaess BM, Enserro DM, McManus DD, Xanthakis V, Chen MH, Sullivan LM, et al. Cardiometabolic correlates and heritability of fetuin-A, retinol-binding protein 4, and fatty-acid binding protein 4 in the framingham heart study. *J Clin Endocrinol Metab*. 2012;97 E1943-7.
19. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, et al. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem*. 2006;52:405–413.
20. Tuncman G, Erbay E, Hom X, De Vivo I, Campos H, Rimm EB, et al. A genetic variant at the fatty acid-binding protein aP2 locus reduces the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease. *Proc Natl Acad Sci U S A*. 2006;103:6970–6975.

21. Guaita-Esteruelas S, Bosquet A, Saavedra P, Gumà J, Girona J, Lam EW, et al. Exogenous FABP4 increases breast cancer cell proliferation and activates the expression of fatty acid transport proteins. *Mol Carcinog*. 2017;56:208–217.
22. Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R, Zillhardt MR, et al. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat Med*. 2011;17:1498–1503.
23. Uehara H, Takahashi T, Oha M, Ogawa H, Izumi K. Exogenous fatty acid binding protein 4 promotes human prostate cancer cell progression. *Int J Cancer*. 2014;135:2558–2568.
24. Klein S. The case of visceral fat : argument for the defense. *J Clin Invest*. 2004;113:1530–1532.
25. Sam S, Haffner S, Davidson MH, et al. Relationship of abdominal visceral and subcutaneous adipose tissue with lipoprotein particle number and size in type 2 diabetes. *Diabetes*. 2008;57:2022–2027.
26. Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes*. 1997;46:1579–1585.
27. Rosen E, Spiegelman BM. What we talk about when we talk about fat. *Cell*. 2015;156:20–44.
28. Stejskal D, Karpisek M, Bronsky J. Serum adipocyte-fatty acid binding protein discriminates patients with permanent and temporary body weight loss. *J Clin Lab Anal*. 2008;22:380–382.
29. Hotamisligil GS, Bernlohr DA. Metabolic functions of FABPs-mechanisms and therapeutic implications. *Nat Rev Endocrinol*. 2015;11:592–605.
30. Simón I, Escoté X, Vilarrasa N, Gómez J, Fernández-Real JM, Megía A, et al. Adipocyte fatty acid-binding protein as a determinant of insulin sensitivity in morbid-obese women. *Obesity (Silver Spring)*. 2009;17:1124–1128.
31. Terra X, Quintero Y, Auguet T, Porras JA, Hernández M, Sabench F, et al. FABP 4 is associated with inflammatory markers and metabolic syndrome in morbidly obese women. *Eur J Endocrinol*. 2011;164:539–547.
32. Thomas AJ, Bainbridge HA, Schone JL, Chen SC, Connett JE, Ikramuddin S, et al. Recruitment and screening for a randomized trial investigating Roux-en-Y gastric bypass versus intensive medical management for treatment of type 2 diabetes. *Obes Surg*. 2014;24:1875–1880.
33. Wing REA. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes melltitus: four-year results of the look AHEAD trial. *Arch Intern Med*. 2011;170:1566–1575.
34. Group DPPR. The Diabetes Prevention Program: description of lifestyle intervention. *Diabetes Care*. 2002;25:2165–2171.
35. Schweiger M, Eichmann TO, Taschler U, Zimmermann R, Zechner R, Lass A. Europe PMC Funders Group measurement of lipolysis. *Methods Enzym*. 2014;538:171–193.
36. Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med*. 2012;366:1567–1576.
37. Xu H, Hertzel AV, Steen KA, Wang Q, Suttles J, Bernlohr DA. Uncoupling lipid metabolism from inflammation through FABP-dependent expression of UCP2. *Mol Cell Biol*. 2015;35:1055–1065.
38. Djousee L GM. Plasma levels of FABP but not FABP3, are associated with increased risk of diabetes. 2012;47:757-62.
39. Burak MF, Inouye KE, White A, Lee A, Tuncman G, Calay ES, et al. Development of a therapeutic monoclonal antibody that targets secreted fatty acid-binding protein aP2 to treat type 2 diabetes. *Sci Transl Med*. 2015;7 319ra205.
40. Furuhashi M, Hiramitsu S, Mita T, Omori A, Fuseya T, Ishimura S, et al. Reduction of circulating FABP4 level by treatment with omega-3 fatty acid ethyl esters. *Lipids Health Dis*. 2016;15:1–9.
41. Furuhashi M, Mita T, Moniwa N, Hoshina K, Ishimura S, Fuseya T, et al. Angiotensin II receptor blockers decrease serum concentration of fatty acid-binding protein 4 in patients with hypertension. *Hypertens Res*. 2015;38:252–259.
42. Karpisek M, Stejskal D, Kotolova H, Kollar P, Janoutova G, Ochmanova R, et al. Treatment with atorvastatin reduces serum adipocyte-fatty acid binding protein value in patients with hyperlipidaemia. *Eur J Clin Invest*. 2007;37:637–642.
43. Hu X, Ma X, Pan X, Luo Y, Xu Y, Xiong Q, et al. Association of androgen with gender difference in serum adipocyte fatty acid binding protein levels. *Sci Rep*. 2016;6:27762.
44. Cao H, Sekiya M, Ertunc ME, Burak MF, Mayers JR, White A, et al. Adipocyte lipid chaperone aP2 Is a secreted adipokine regulating hepatic glucose production. *Cell Metab*. 2013;17:768–778.
45. Ertunc ME, Sikkeland J, Fenaroli F, Griffiths G, Daniels MP, Cao H, et al. Secretion of fatty acid binding protein aP2 from adipocytes through a nonclassical pathway in response to adipocyte lipase activity. *J Lipid Res*. 2015;56:423–434.
46. Furuhashi M, Hiramitsu S, Mita T, Fuseya T, Ishimura S, Omori A, et al. Reduction of serum FABP4 level by sitagliptin, a DPP-4 inhibitor, in patients with type 2 diabetes mellitus. *J Lipid Res*. 2015;56:2372–2380.
47. Corripio R, Gónzalez-Clemente JM, Pérez-Sánchez J, Näf S, Gallart L, Nosàs R, et al. Weight loss in prepubertal obese children is associated with a decrease in adipocyte fatty-acid-binding protein without changes in lipocalin-2: a 2-year longitudinal study. *Eur J Endocrinol*. 2010;163:887–893.
48. Engl J, Ciardi C, Tatarczyk T, Kaser S, Laimer M, Laimer E, et al. A-FABP—a biomarker associated with the metabolic syndrome and/or an indicator of weight change? Obesity (Silver Spring) 2008;16:1838-42.
49. Camargo A, Meneses ME, Perez-Martinez P, Delgado-Lista J, Jimenez-Gomez Y, Cruz-Teno C, et al. Dietary fat differentially influences the lipids storage on the adipose tissue in metabolic syndrome patients. *Eur J Nutr*. 2014;53:617–626.