

A-FABP—A Biomarker Associated With the Metabolic Syndrome and/or an Indicator of Weight Change?

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Objective: Adipocyte fatty acid-binding protein (A-FABP) is a plasma biomarker recently associated with the metabolic syndrome. The aim of these studies was to investigate changes of A-FABP during profound weight loss induced by laparoscopic adjustable gastric banding (LAGB).

Methods and Procedures: In study one, 29 severely obese female subjects were examined before and 1 year after surgical treatment. A subgroup of 10 patients was investigated in 3-month intervals. Metabolic parameters were determined using standard methods, and A-FABP was detected using a commercially available enzyme-linked immunosorbent assay.

Results: Mean weight loss after 1 year was 24.9 kg ($P < 0.001$), mainly due to a decrease in fat mass. Metabolic parameters improved substantially. However, serum A-FABP remained stable. In study two, a subgroup of 10 patients was examined quarterly to determine the time course of A-FABP changes. Quarterly measurements of serum A-FABP were significantly higher than baseline levels with the highest A-FABP value after the first 3 months, where patients had highest weight loss.

Discussion: Our results in study one show that A-FABP serum levels are positively associated with body weight and fat mass. However, 1 year after pronounced weight loss A-FABP levels remained unchanged. In study two, time course analyses revealed maximum increase of serum A-FABP in parallel to highest weight loss, which allows to suppose that A-FABP is not only a biomarker of the metabolic syndrome in the steady state, but also a marker of weight changes in dynamic situations.

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INTRODUCTION

Fatty acid-binding proteins (FABPs) belong to a family of cytosolic chaperones that are involved in systemic regulation of lipid and glucose metabolism (1). FABP4, also known as the adipocyte FABP (A-FABP) is expressed predominantly in adipose tissue and macrophages (2). Ablation of A-FABP in animal studies in apolipoprotein E-deficient mice resulted in a protection against atherosclerosis, which commonly occurs in these genetically deficient mice (3,4) and previous studies showed a protection of A-FABP-deficient mice from developing hyperinsulinemia, hyperglycemia, and insulin resistance (5,6). In macrophages, it has been demonstrated that A-FABP regulates two important functions involved in atherosclerosis, cholesterol trafficking, and inflammatory activity (7).

Recently, A-FABP was found to be also present in human plasma and elevated in metabolic syndrome patients (8). A-FABP levels were significantly higher in overweight/obese than in lean subjects. Age- and sex-adjusted serum A-FABP correlated positively with waist circumference, blood pressure, dyslipidemia, fasting insulin, and the homeostasis model assessment (HOMA) insulin resistance (IR) index. The more components of the metabolic syndrome the patients had, the higher were the A-FABP concentrations (8). These results were confirmed recently by a study in a white population (9).

In this study, we investigated the influence of pronounced weight loss induced by laparoscopic adjustable gastric banding (LAGB) on the serum concentrations of A-FABP. We hypothesized that in this model system of pronounced weight loss, serum

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levels of A-FABP may decrease in parallel with the amelioration of many components of the metabolic syndrome (10–16).

METHODS AND PROCEDURES

Subjects

In study one, 29 women with a BMI >35 kg/m² with comorbidities or a BMI >40 kg/m² participated. Exclusion criteria were secondary causes of obesity, diabetes mellitus, pregnancy, or other medically significant illness. The study subjects were examined within 2 months before LAGB and 1 year after the surgical procedure.

In study two, a subgroup of 10 patients was examined in 3-month intervals. Informed consent was obtained from all subjects, the procedures were performed in accordance with institutional guidelines at the Department of Internal Medicine, Clinical Division of General Internal Medicine, Medical University Innsbruck, and the local ethical committee at the Medical University Innsbruck approved the study.

Surgical procedure

The surgical procedures were performed as previously described by Forsell (17) at the Department of Surgery, Medical University Innsbruck. The Swedish Adjustable Gastric Band was inserted in all the study patients (SAGB Obtech Medical AG, Zug, Switzerland) (18–20).

Body composition

BMI was calculated as body weight in kilograms divided by height in meters squared. Body composition was determined by impedance analysis using InBody 3.0 Body Composition Analyzer from Biospace Europe (Dietzenbach, Germany). Measurements were taken in the morning in the fasted state.

Analyses

Blood was drawn after an overnight fast from an antecubital vein into EDTA tubes (1.6 mg/ml). Plasma was separated from erythrocytes by centrifugation at 3,000 r.p.m. for 10 min at 4°C immediately after collection. Plasma samples were stored at –80°C until assayed.

Plasma triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol were quantified using a commercially available enzymatic kit (Roche Diagnostic Systems, Basel, Switzerland). Low-density lipoprotein cholesterol was calculated using the Friedewald formula (21). Plasma glucose was measured by the hexokinase method on Cobas MIRA analyzer. Plasma insulin was measured using a micro particle enzyme immunoassay from Abbott (Wiesbaden, Germany). Serum A-FABP was determined using a commercially available enzyme-linked immunosorbent assay (BioVendor Laboratory Medicine, Brno, Czech Republic) and the assay was conducted according the manufacturer's instructions. Quality controls of the A-FABP enzyme-linked immunosorbent assay were performed and the coefficient of variation of the assay in our laboratory was <5%. Serum IR was calculated using the HOMA index as follows: HOMA-IR = fasting insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5 (22).

Statistical analysis

Data are expressed as mean ± SD unless stated otherwise. The Shapiro–Wilk test was used to determine normal distribution of the data. As most data were not normally distributed, the Wilcoxon test for paired samples was used to determine significant changes before and after LAGB. Significance of overall changes in A-FABP levels obtained from the 3-month evaluations in study two were calculated using the Friedman test for global interaction. To assess correlations between data, the Spearman Rho correlation coefficient was used. Additionally, linear regression analyses were performed, where applicable. A two-sided *P* value ≤0.05 was considered statistically significant. All analyses were performed using SPSS 11.5 for Windows (SPSS, Chicago, USA).

RESULTS

In study one, mean weight loss was 24.9 kg (*P* < 0.001) and the mean BMI decreased by 8.8 kg/m² (*P* < 0.001) 1 year after LAGB. Weight loss was mainly due to a decrease in fat mass (20.4 kg, *P* < 0.001) (Table 1). Triglycerides decreased significantly by 36.6% (*P* = 0.001) and nonesterified fatty acids (NEFAs) by 25.0%. In parallel, glucose metabolism improved after pronounced weight loss: mean serum insulin decrease was 7.7 μU/ml (*P* < 0.001) and mean HOMA-IR decreased from 4.1 to 2.4 (*P* = 0.001) (Table 2). However in study one, we could not find any significant changes in serum A-FABP. Further statistical analyses were performed, where most of the previous results were confirmed: before surgical intervention, serum A-FABP correlated positively with weight (*r* = 0.372, *P* = 0.047) and fat mass (*r* = 0.415, *P* = 0.025) and negatively with HDL cholesterol (*r* = –0.414, *P* = 0.026). We found positive correlations of Δ A-FABP with Δ fat mass (*r* = 0.402, *P* = 0.031) and Δ BMI (*r* = 0.412, *P* = 0.026) and a negative correlation of Δ A-FABP with Δ HDL (*r* = –0.475, *P* = 0.009).

Table 1 Body composition of study population one at baseline and after pronounced weight loss

	Pre-LAGB (<i>n</i> = 29)	Post-LAGB (<i>n</i> = 29)	Test probability
Age (years)	39.3 ± 10.4	—	—
Height (cm)	167.3 ± 6.8	—	—
Weight (kg)	121.0 ± 18.5	96.1 ± 15.5	<0.001
BMI (kg/m ²)	43.0 ± 4.7	34.2 ± 4.3	<0.001
Fat mass (kg)	57.7 ± 10.2	37.3 ± 9.3	<0.001
Lean mass (kg)	62.1 ± 7.8	57.6 ± 8.4	<0.001

Data are expressed as means ± s.d.
LAGB, laparoscopic adjustable gastric banding.

Table 2 A-FABP, lipid and glucose parameters at baseline and after weight loss (study 1)

	Pre-LAGB	Post-LAGB	Test probability	<i>n</i>
A-FABP (μg/l)	48.9 ± 14.5	50.0 ± 12.5	0.770	29
Total cholesterol (mg/dl)	203.0 ± 43.9	187.1 ± 36.6	0.103	29
LDL cholesterol (mg/dl)	119.5 ± 26.5	112.5 ± 30.8	0.716	29
HDL cholesterol (mg/dl)	53.2 ± 13.0	55.7 ± 11.4	0.122	29
Triglycerides (mg/dl)	148.5 ± 125.0	94.2 ± 40.0	0.001	29
NEFA (mmol/l)	0.8 ± 0.2	0.6 ± 0.2	0.001	29
Glucose (mg/dl)	95.3 ± 15.8	97.0 ± 18.4	0.736	26
Insulin (μU/ml)	17.1 ± 13.2	9.4 ± 6.9	<0.001	26
HOMA	4.1 ± 3.5	2.4 ± 2.7	0.001	26

A-FABP, adipocyte fatty acid-binding protein; LAGB, laparoscopic adjustable gastric banding; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; NEFA, nonesterified fatty acid.
Data are expressed as means ± s.d.

In study two, a subgroup of 10 patients was examined in shorter time periods, i.e., 3 months, to obtain a more detailed impression on the time course of A-FABP during weight loss. Interestingly, levels of serum A-FABP changed significantly over the period of 1 year as determined using the Friedman test for global interaction ($P = 0.003$). Quarterly measurement of serum A-FABP were significantly higher than baseline levels with the highest A-FABP value after the first 3 months (Figure 1), during which patients had maximum weight changes (Figure 2). Measurements of NEFAs over the time course revealed a significant decrease after 12 months ($P = 0.037$). No significant changes were found between the quarterly measurements.

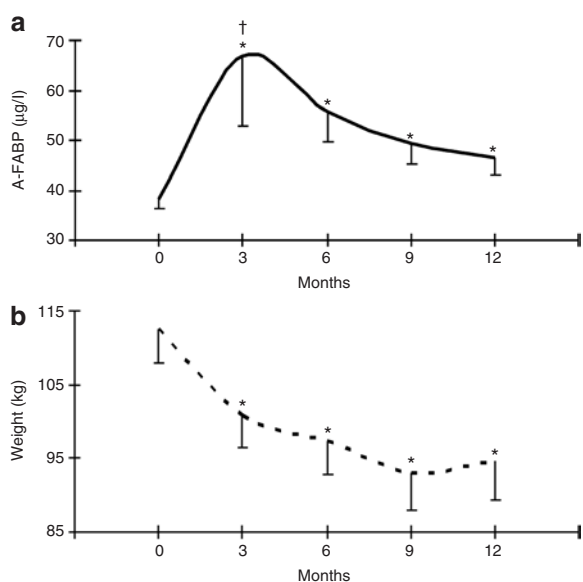


Figure 1 Time course of (a) adipocyte fatty acid-binding protein (A-FABP) and (b) weight loss. * $P \leq 0.05$ vs. baseline; [†] $P \leq 0.05$ vs. all other points in time. Data are expressed as mean \pm s.e.m.

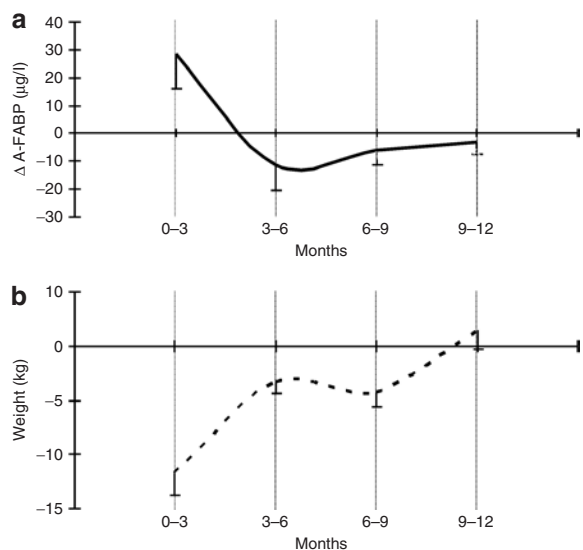


Figure 2 Time course of Δ values of (a) adipocyte fatty acid-binding protein (A-FABP) and (b) Δ values of weight loss. Data are expressed as mean \pm s.e.m.

Statistical analyses revealed negative correlations for Δ A-FABP with Δ insulin ($r = -0.857$, $P = 0.007$) and the HOMA-IR ($r = -0.690$, $P = 0.058$). Similar correlations were found in the second quarter. Δ A-FABP correlated negatively with Δ insulin ($r = -0.667$, $P = 0.050$) and the Δ HOMA ($r = -0.850$, $P = 0.004$). Both these correlations disappeared in the following two quarters.

DISCUSSION

A-FABP, known as a cytoplasmatic protein, has been shown to be released from the adipocytes and to be present in the bloodstream. Circulating A-FABP is markedly increased in overweight and obese subjects, with higher A-FABP levels in women, and a correlation with the components of the metabolic syndrome (8). Aware of the gender difference, we restricted our prospective investigations to obese women.

In study one, we focused on weight loss induced by bariatric surgery and changes in A-FABP serum levels after 1 year. We hypothesized that in parallel with improvements in metabolism induced by weight loss, serum A-FABP levels also decrease in this model system of pronounced weight loss.

Weight loss was mainly due to a decrease in fat mass. Triglycerides and NEFAs decreased significantly by 36.6 and 25.0%, respectively. Glucose metabolism amelioration was due to a significant decrease in mean serum insulin and in the HOMA-IR. In our obese study population, serum A-FABP levels were 48.9 ± 14.5 $\mu\text{g/l}$ before surgical treatment and were positively associated with weight and fat mass, and negatively with HDL cholesterol, similar to results reported by Xu *et al.* (8). This study noted a significant increase in A-FABP, which increased with the number of components of the metabolic syndrome (8).

It is widely accepted that pronounced weight loss as a result of bariatric surgery ameliorates components of the metabolic syndrome (23). Although parameters of lipid and glucose metabolism ameliorated in our study population, we could not find the expected changes in serum A-FABP after 1 year of pronounced weight loss.

A subgroup of 10 patients was investigated in shorter time periods to obtain a more detailed view on the time course of A-FABP. Interestingly, in this study, the A-FABP levels in serum changed significantly over the period of 1 year ($P = 0.003$). In the first quarter, we observed maximum increase in A-FABP (Figure 1), concomitant with the highest weight loss in this period (Figure 2). Quarterly measurements of serum A-FABP were significantly higher than baseline levels. The decrease in A-FABP levels after the third month could be explained due to the lesser extent of weight loss in this period, suggesting that the amount of serum A-FABP is an indicator of weight change due to lipolysis. Mobilization of stored fat is mediated by lipolytic enzymes. Adipose triglyceride lipase was recently described to predominantly perform the initial step in triglyceride hydrolysis and therefore seems to play a pivotal role in the lipolytic catabolism of stored fat in adipose tissue (24). Hormone-sensitive lipase also hydrolyzes triglycerides, and with a higher specificity diglycerides (24). Cytoplasmatic

A-FABP increases the hydrolytic activity of hormone-sensitive lipase and both together constitute a functionally important lipolytic complex (25,26). During weight maintenance and an IR state, it is reasonable that the increased circulating fraction of A-FABP marks the paradox lipolytic activity due to the lacking inhibition of insulin (27). Weight loss in general improves insulin sensitivity (23). The high lipolytic activity during massive weight loss in the first quarter could explain the high A-FABP serum levels and the negative correlations between Δ A-FABP and Δ insulin ($r = -0.857$, $P = 0.007$) in the first and between Δ A-FABP and Δ insulin ($r = -0.667$, $P = 0.050$) and the Δ HOMA ($r = -0.850$, $P = 0.004$) in the second quarter.

Weight loss after bariatric surgery resulted in decreased NEFA levels after 1 year, whereas the quarterly measurements revealed no significant changes of NEFA concentrations over time. We did not find a correlation between A-FABP and NEFA levels over time, the latter could be, on the one hand, due to the small study population, on the other hand because NEFA levels do not reflect only adipose tissue lipolysis, but also lipolysis of plasma lipoproteins.

It is well documented that different FABPs are markers for tissue necrosis. B-AFBP and H-FABP concentrations differ among brain tissues and both are sensitive markers for brain injury (28), and H-FABP is a established marker for cardiac injury (29). Recently it was found that 3T3-L1 adipocytes *in vitro* secrete A-FABP, where unspecific cell lysis was excluded by staining for β -tubulin as a control for intracellular components (8). However, these *in vitro* data cannot be applied directly to *in vivo* findings. In human serum A-FABP was discovered by subjecting serum to two-dimensional gel electrophoresis followed by chromatographic purification and mass spectroscopy. Thus, currently, the origin of A-FABP found in serum is unclear. In addition, there are no data in the literature to indicate that A-FABP possesses a secretion sequence, and further studies are needed to elucidate the molecular mechanism behind A-FABP's appearance in human serum.

The major limitation of this study is the small number of patients ($n = 10$) in study two. However, patients were investigated five times during the observation period of 1 year to achieve a detailed impression of the A-FABP serum levels during pronounced weight loss.

In summary, our results in study one confirm that A-FABP serum levels are positively associated with weight and fat mass and negatively with HDL cholesterol. However 1 year after pronounced weight loss, A-FABP levels remained unchanged. In study two, time course analyses revealed a maximum increase in serum A-FABP in parallel to highest weight loss. We conclude that A-FABP serum levels are a biomarker of metabolic syndrome and additionally an indicator of weight loss, demonstrating the current lipolytic activity in adipose tissue.

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DISCLOSURE

The authors declared no conflict of interest.

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