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Plasma levels of FABP4, but not FABP3, are associated with increased risk of diabetes

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Abstract

Little is known about the association between plasma concentrations of fatty acid binding protein 3 and 4 and the risk of diabetes in population-based cohorts. In a prospective nested case-control design, we studied 149 cases of diabetes and 149 matched controls from the Physicians' Health Study. Plasma fatty acid binding proteins were measured on frozen specimens collected between 1995–2001 by ELISA. Cases of diabetes were self-reported and validated in a subsample via review of medical records. We used conditional logistic regression to estimate multivariable relative risks. The mean age at baseline was 64.9 years and median plasma fatty acid binding protein 3 and 4 were 2.12 ng/ml (IQR 1.62–2.66) and 15.32 ng/ml (IQR: 12.14–18.73), respectively. In separate models, each fatty acid binding protein was positively associated with the risk of diabetes in a conditional logistic regression adjusting for matching variables, smoking, and hypertension. However, upon adjustment for each other, only fatty acid binding protein 4 (but not 3) was positively associated with the risk of diabetes [relative risk (95% CI): 1.0 (reference), 2.73 (1.08–6.89), 2.66 (1.11–6.42), and 6.89 (2.83–16.80) across consecutive quartiles of fatty acid binding protein 4), *p* for trend <0.0001. The FABP4-diabetes association was modified by body mass index (*p* interaction 0.03). Our data showed a positive association between plasma fatty acid binding protein 4 but not 3 and the risk of diabetes in US male physicians. The interaction with body mass index warrants further investigations.

Keywords

Diabetes; epidemiology; fatty acid binding proteins; adipokines; risk factors

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Statistical analysis: Djoussé

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Introduction

Type 2 diabetes (DM) is a major health issue with worldwide health consequences(1–4). It is estimated that more than 350 million people will be diagnosed with DM by 2030(5). Complex secular trends in nutrition and physical activity have led to a dramatic increase in the prevalence of overweight and obesity(6) with resulting increased prevalence of DM(4,7,8). Adipocytes are known to produce various adipokines, which can influence inflammation, lipids, and glucose metabolism, all of which are relevant in the development of DM(9,10). Fatty-acid-binding proteins (FABPs) are a family of carrier proteins for fatty acids and other lipophilic substances such as eicosanoids and retinoids(11–13). Of the 9+ members of FABP(14), FABP4 is mainly expressed by adipocytes and macrophages whereas FABP3 is expressed by skeletal and heart muscle. Mice lacking FABP4 gene gain weight under high-fat diet but do not develop insulin resistance as would be the case in wild type mice(15). This observation suggests that FABP4 may play a role in the obesity-DM association. Such a hypothesis is supported by the fact that expression of FABP4 leads to insulin resistance in animals(16–18). While other studies have reported cross-sectional associations between FABP4 and the prevalence of metabolic syndrome(12,19,20), only one prospective study has suggested a positive association between plasma FABP4 and incident DM(21). No previous study has examined the association between FABP3 and the risk of DM and it is unclear whether FABP3 and FABP4 are independently associated with the risk of DM. Hence, we sought to assess whether plasma concentrations of FABP3 and FABP4 are independently associated with the risk of DM in US male physicians.

Materials and Methods

Study population

Participants in these analyses are members of the Physicians' Health Study I and II who provided blood samples between 1995 and 2001. The Physicians' Health Study I is a completed randomized, double-blind, placebo-controlled trial designed to study low-dose aspirin and beta-carotene for the primary prevention of cardiovascular disease and cancer(22,23). The Physicians' Health Study II started in 2001 and is a randomized trial assessing the effects of vitamins on the risk of cardiovascular disease and cancer(24,25). Current analyses used a prospective nested case-control design with a risk set sampling technique to select subjects. All eligible subjects were free of DM at the time of blood collection. For each case of incident DM occurred during follow up, we randomly selected a control among participants who were alive and free of DM at the time of diagnosis of the index case (risk set) and matched to the case on age at blood collection (within 1 year), race, year of birth (same), and time of blood collection (within 30 days). From the total pairs identified, we randomly selected 150 pairs for the current study. Because one control did not have data on FABP3 and FABP4, our final sample consisted of 149 matched pairs. Each participant signed an informed consent and the Institutional review Board at Brigham and Women's Hospital approved the study protocol.

Measurement of fatty acid binding protein 3 and 4

Plasma FABP4 was measured using the Human Adipocyte FABP ELISA (Human FABP4 ELISA) from BioVendor LLC (Candler, NC). In this assay, samples and standards are added to microplate wells coated with immobilized polyclonal anti-human AFABP antibody. After incubation and washing, biotin-labeled polyclonal anti-human AFABP antibody is added. Following a second wash, streptavidin-HRP conjugate is added. After a final wash, tetramethylbenzidine is added to the wells. An acidic stop solution terminates the reaction. The optical density of the wells is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA) at 450 nm and 630 nm. The intensity of the color formed by the

enzymatic reaction is directly proportional to the concentration of FAPB4 in the sample and concentration is determined from a standard curve. The inter-assay coefficient of variations were 2.6–5.1%.

Plasma FABP3 was measured using the Human H-FABP ELISA (Human FABP3 ELISA) from Cell Sciences, Inc (Canton, MA). In this assay, samples and standards are added to microplate wells coated with immobilized anti-human HFABP antibody. After incubation peroxidase conjugated antibody is added. Following a wash, tetramethylbenzidine is added to the wells. An acidic stop solution terminates the reaction. The optical density of the wells is measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA) at 450 nm and 630 nm. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of FAPB3 in the sample and concentration is determined from a standard curve.

Ascertainment of incident DM in the PHS

Ascertainment of DM and other endpoints in the PHS has been achieved using annual follow-up questionnaires. We have validated the diagnosis of self-reported DM in a sample of men by reviewing their medical records. In the PHS, a systematic request of medical records is available for the trial primary endpoints (myocardial infarction, stroke, cancer, death, and pulmonary embolus). For the validation study of DM, we selected all participants who reported a diagnosis of DM on the follow-up questionnaires and had a subsequent diagnosis of myocardial infarction. The rationale for selecting myocardial infarction was that medical records for this event are more likely to contain pertinent information on past medical history, laboratory measurements, and current medications. In contrast, records to confirm cancer or death events are limited mostly to histological reports or death certificates, respectively. Of the 186 subjects who suffered myocardial infarction after a self-reported diagnosis of type 2 diabetes, we randomly selected half (93 people) for chart review. Medical records were available for 60 subjects. Two physicians independently reviewed these charts. A diagnosis of DM was made if there was sufficient evidence in the chart defined as one of the following: a) diagnosis of DM on the discharge summary, b) current treatment with insulin or oral hypoglycemic agents, or c) fasting glucose above 126 mg/dl or non-fasting glucose above 200 mg/dl. Using these criteria, DM was confirmed in 59 out of 60 cases (98.3%). There was an excellent agreement between the two examiners ($\kappa=100\%$).

Other variables

Demographic information was obtained through self-reports. At baseline, each subject provided information on exercise [how often do you exercise vigorously enough to work up sweat? Possible answers included rarely/never, 1–3/month, 1/week, 2–4/week, 5–6/week, and daily]; smoking (never, former, and current smoker); and alcohol intake (rarely/never, 1–3 per month, 1 per week, 2–4/week, 5–5/week, daily, and 2+/day). Self-reported baseline weight and height were used to compute body mass index (weight in kilograms divided by height in meter squared). Information on prevalent hypertension and coronary heart disease was collected at baseline and through follow-up questionnaires. An endpoint committee of the Physicians' Health Study reviewed medical records to confirm the diagnosis of myocardial infarction and coronary bypass or angioplasty.

Statistical analyses

Due to the non-Gaussian distribution of FABP3/4, we use the natural logarithm to normalize their distributions. We created quartiles of $\log(\text{FABP3})$ and $\log(\text{FABP4})$ using the distribution of these biomarkers in the control series. Means and percentages of baseline characteristics of the study participants are presented according to diabetes status. We used

conditional logistic regression to estimate the relative risk of diabetes using the lowest quartile of each biomarker as the reference category. The multivariable adjusted model controlled for matching variables (race, age, time of blood collection, and year of birth), cigarette smoking, hypertension, and mutual control for FABP3 and FABP4. We also evaluated confounding by prevalent coronary heart disease (myocardial infarction, coronary bypass or angioplasty). Additional control for exercise and alcohol consumption did not alter the conclusions (data not shown). Furthermore, we modeled FABP4 and FABP3 as continuous variables using the logarithmic transformed values and estimated relative risks associated with each standard deviation higher log-FABP4 or log-FABP3 using above described models. We considered effect measure modification by body mass index given our prior findings of interaction between body mass index and FABP4 in older adults(26), by including product terms of both main exposures (FABP3/4) and body mass index and main effects in the regression model. To obtain a p value for linear trend, we created a new variable that was assigned the median value of FABP3/4 in each quartile within the control series and fitted such a variable in the conditional logistic regression. All analyses were performed using SAS (SAS version 9.3, NC) and the alpha level was set at 0.05. All p values were 2-sided.

Results

The mean age was 64.9 years and 84.6% were Caucasians. FABP3 and FABP4 were not normally distributed (skewness and kurtosis were 2.92 and 12.2, respectively, for FABP3; corresponding values for FABP4 were 1.84 and 5.60). Subjects who developed DM had a higher body mass index, higher prevalence of coronary heart disease, higher plasma concentrations of FABP3 and 4, lower prevalence of vigorous exercise and hypertension, and were more likely to be current smokers than controls (Table 1). Body mass index was positively correlated with log-transformed FABP4 (Spearman correlation coefficient =0.44, $p<0.0001$) but not with log(FABP3) (Spearman correlation coefficient =-0.02, $p=0.78$). Spearman correlation coefficient between log(FABP3) and log(FABP4) was 0.23 ($p=0.005$). In a conditional logistic regression model adjusting for matching factors, log(FABP3) was positively associated with diabetes [relative risk (95% CI) of 1.0 (ref), 0.68 (0.30–1.54), 1.11 (0.56–2.23), and 2.24 (1.09–4.59) across consecutive quartiles of log(FABP3), p for trend 0.008]. Additional adjustment for hypertension and smoking had little effect on the results (p for trend 0.012). Likewise, log(FABP4) was positively associated with DM in the basic model [relative risk (95% CI): 1.0 (ref), 1.62 (0.73–3.61), 1.84 (0.85–4.01), and 5.20 (2.40–11.27) across consecutive quartiles of log(FABP4), p for trend <0.0001]. Additional adjustment for hypertension and smoking strengthened the results [RR (95% CI): 1.0 (ref), 2.08 (0.88–4.89), 2.32 (1.01–5.31), and 6.23 (2.75–14.10), respectively]. When both FABP4 and FABP3 were included in the fully adjusted model, FABP4 but not FABP3 was associated with the risk of DM (Table 2). Additional adjustment for prevalent coronary heart disease had minimal effect on the odds ratios (for example, relative risks were 1.0 (ref), 2.70 (1.06–6.87), 2.87 (1.16–7.01), and 6.87 (2.82–16.76) across consecutive quartiles of FABP4). Each standard deviation increment of log(FABP4) was associated with 80% increased risk of DM (95% CI: 37% to 137%), Table 2). There was evidence for interaction between body mass index and FABP4 (p for interaction 0.03) but not FABP3 ($p=0.29$) in the fully adjusted model. Unfortunately, we did not have adequate data (i.e. only 17 matched pairs with body mass index <25 kg/m²) for stratified analyses by body mass index.

Discussion

In this prospective nested case-control study, we found a positive and linear association between plasma FABP4 and FABP3 on the risk of DM among United State male physicians when examined individually. However, only FABP4 (but not FABP3) was independently

associated with the risk of DM upon mutual adjustment for the other FABP. The risk of DM was nearly seven fold higher in the fourth compared to the first quartile of FABP4 adjusting for confounders and FABP3. Lastly, the FABP4-diabetes association was modified by body mass index whereas no interaction was seen between body mass index and FABP3. This is the first study to show that FABP4 is positively related to DM risk, independent of FABP3 in a large sample of humans.

Several animal studies have examined the effects of FABP4 insulin resistance and DM risk(13,15,16,27). However, only limited data are available in humans. In cross-sectional studies, FABP4 was associated with a higher prevalence of metabolic syndrome(10,12,19,20,28). Furthermore, in a small Chinese population, a higher concentration of FABP4 was associated with a two-fold increased risk of DM after ten years of follow up(21). Those findings are consistent with a positive association between plasma FABP4 and incident DM among US older adults (65+ years at baseline)(26). However, neither of the previous studies examined the role of FABP3 on the FABP4-DM association for further comparison. The current study suggests that FABP3 may not be as important as FABP4 in the development of DM. The effect modification observed between FABP4 and body mass index is consistent with data from the Cardiovascular Health Study showing similar interaction(26). We had inadequate data to conduct stratified analyses by body mass index in our study. However, the Cardiovascular Health Study reported a 78% higher risk of diabetes (95% CI: 13% to 181%) per standard deviation higher FABP4 in men with body mass index below 25 kg/m² but no significant relation in overweight [1.03 (0.71–1.49)] or obese [1.43 (0.90–2.28)] men(26). It is possible that overweight/obesity confers a higher background rate for diabetes, thereby making it more difficult to detect a small increment in diabetes risk in such a high-risk population. In contrast, with a lower background risk of diabetes in lean subject, a small increase in diabetes risk conferred by FABP4 might be readily detectable. Our working hypothesis is that FABP4 is downstream of obesity and future work is needed for further investigation.

Data from the Nurse's Health and Health Professionals Follow-up Studies(17) showed that a functional mutation (T-87C) in the FABP4 gene was associated with a reduced expression of FABP4 in adipocytes and a lower risk of DM (especially among obese subjects). Those data also suggest a possible interaction between body composition and FABP4 on DM risk and are consistent with the interaction observed in the current study. In contrast, other investigators did not show a major effect of FABP4 gene on diabetes risk(29). At this point, little is known about potential pathways by which FABP4 may influence the risk of DM in humans.

FABP3 and 4 are transport proteins and play an important role in lipid metabolism and perhaps glucose utilization(30,31). FABP4-knockout mice remain insulin sensitive despite the development of adiposity under a high-fat diet(15). In contrast, wild type mice develop insulin resistance and adiposity under high-fat diet. A working hypotheses could be that FABP4 might mediate obesity-induced insulin resistance and subsequent development of DM. Alternatively, FABP4 may promote lipolysis by removing fatty acids that are inhibiting hepatic sensitive lipase(27,32). Lastly, FABP4 may increase the risk of DM by interfering with key enzymes in the de novo lipogenesis(33,34). Unfortunately, we did not have appropriate data to further explore the role of FABP4 on DM risk in our study. At this point, we cannot eliminate the possibility that elevated plasma FABP4 could just be a marker of metabolic derangement associated with metabolic syndrome, obesity, or diabetes. Consistent with this hypothesis, the PREDIMED data suggest that elevated plasma FABP4 predict atherogenic dyslipidemia in 578 volunteers after a median follow up of four years(35). FABP4 has also been reported as a biomarker of atherogenic dyslipidemia, independent of obesity and insulin resistance(36). Though our subjects were free of DM at the time of blood

collection, we cannot exclude the prevalence of metabolic syndrome or abnormal glucose tolerance at the time of blood collection.

Our study has some limitations. The fact that all participants were male physicians, most of whom were Caucasians, limits the generalizability of the current findings. Our validation of diabetes was limited to a subsample in this cohort, hence, we cannot exclude the possibility of misclassification of DM. Such misclassification of DM cases is more likely to be non-differential with respect to FABP3 and 4 concentrations and would likely have led to an underestimation of the true association. As an observational study, we cannot rule out chance, residual or unmeasured confounding as alternative explanation of our results. On the other hand, the use of risk set technique, matching to minimize confounding, the availability of several potential covariates, and standardized methods for follow-up and cases ascertainment are strengths of the present study.

In summary, our data suggest that FABP4 but not FABP3 is positively associated with a higher risk of DM. Such association appears to be modified by body mass index. If confirmed in other studies, FABP4 may offer a new pharmacological targets in the management of diabetes and/or risk stratification.

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List of abbreviations

CI	confidence interval
DM	type 2 diabetes
ELISA	enzyme-linked immunosorbent assay
FABP	fatty acid binding protein
RR	relative risk
SD	standard deviation

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Table 1

Characteristics of 298 male physicians according to diabetes status

Characteristics	Diabetes cases (N=149)	Controls (N=149)
Age (y) *	64.9±8.1	64.9±8.1
Race (% Caucasian) *	84.6	84.6
Body mass index (kg/m ²)	28.3±4.6	25.5±3.5
Log(FABP3)	0.87±0.58	0.75±0.49
Log(FABP4)	2.98±0.42	2.73±0.38
Current smokers (%)	7.4	3.4
Former smokers (%)	41.6	41.6
Current alcohol drinkers (%)	80.1	84.5
Vigorous exercise (%)	48.3	68.7
Hypertension (%)	29.5	36.9
Prevalent coronary heart disease (%)	13.4	10.1

* Matching variables; FABP= fatty acid binding protein

Data are presented as mean ± standard deviation or percentage

Table 2
Relative risk (95% CI) for diabetes according to quartiles and standard deviation increase of fatty acid binding protein

Quartiles	FABP3			FABP4		
	Cases/N	Model 1*	Model 2 [†]	Quartiles	Cases/N	Model 1* Model 2 [†]
2.485	31/67	1.00 (ref)	1.00 (ref)	0.471	15/50	1.00 (ref)
2.486–2.728	24/61	0.48 (0.20–1.18)	0.37 (0.15–0.96)	0.472–0.745	25/63	1.96 (0.85–4.52)
2.729–2.930	32/70	0.82 (0.38–1.77)	0.85 (0.38–1.89)	0.746–0.978	28/66	2.05 (0.91–4.62)
2.930	62/100	1.31 (0.58–2.95)	1.13 (0.48–2.67)	0.979	81/119	5.31 (2.35–12.00)
P for trend		0.23	0.36			<0.0001
Per SD increase of log(FABP3)		1.07 (0.81–1.41)	1.03 (0.77–1.37)	Per SD increase of log(FABP4)		1.74 (1.34–2.25)
						1.80 (1.37–2.37)

* Model 1: Conditional logistic regression adjusted for matching factors (age, race, time of blood collection, and year of birth) and the fatty acid binding protein; FABP = fatty acid binding protein; SD=standard deviation

[†]Model 2: Additional adjustment for hypertension and cigarette smoking.