

# Serum Adipocyte Fatty Acid-Binding Protein as a New Biomarker Predicting the Development of Type 2 Diabetes

A 10-year prospective study in a Chinese cohort

ANNETTE W.K. TSO, MD<sup>1</sup>  
AIMIN XU, PHD<sup>1,2</sup>  
PAK C. SHAM, MD<sup>3</sup>  
NELSON M.S. WAT, MD<sup>1</sup>  
YU WANG, PHD<sup>3</sup>

CAROL H.Y. FONG, BSC<sup>1</sup>  
BERNARD M.Y. CHEUNG, MD<sup>1,2</sup>  
EDWARD D. JANUS, PHD<sup>4</sup>  
KAREN S.L. LAM, MD<sup>1,2</sup>

**OBJECTIVE** — Adipocyte fatty acid-binding protein (A-FABP) is abundantly expressed in adipocytes and plays a role in glucose homeostasis in experimental animals. We have previously shown that circulating A-FABP levels are associated with the metabolic syndrome, which confers an increased risk of type 2 diabetes. Here we investigated whether serum A-FABP levels could predict the development of diabetes in a 10-year prospective study.

**RESEARCH DESIGN AND METHODS** — Baseline serum A-FABP levels were measured with an enzyme-linked immunosorbent assay in 544 nondiabetic subjects, recruited from the Hong Kong Cardiovascular Risk Factor Prevalence Study cohort, who were followed prospectively to assess the development of type 2 diabetes. The role of A-FABP in predicting the development of type 2 diabetes over 10 years was investigated using Cox regression analysis.

**RESULTS** — At baseline, serum sex-adjusted A-FABP levels were higher in subjects with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) ( $P < 0.00001$  versus normal glucose tolerance) and correlated positively with adverse cardiometabolic risk factors. Over 10 years, 96 subjects had developed type 2 diabetes. High baseline A-FABP was predictive of type 2 diabetes, independent of obesity, insulin resistance, or glycemic indexes (relative risk [RR] 2.25 [95% CI 1.40–3.65];  $P = 0.001$ ; above versus below sex-specific median). High A-FABP levels remained an independent predictor of type 2 diabetes in the high-risk IGT/IFG subgroup (adjusted RR 1.87 [1.12–3.15];  $P = 0.018$ ).

**CONCLUSIONS** — Serum A-FABP was associated with glucose dysregulation and predicted the development of type 2 diabetes in a Chinese cohort.

*Diabetes Care* 30:2667–2672, 2007

From the <sup>1</sup>Department of Medicine, University of Hong Kong, Hong Kong, China; the <sup>2</sup>Research Centre of Heart, Brain, Hormone and Healthy Aging, University of Hong Kong, Hong Kong, China; the <sup>3</sup>Genome Research Centre, University of Hong Kong, Hong Kong, China; and the <sup>4</sup>Department of Clinical Biochemistry, Queen Mary Hospital, Hong Kong, China.

Address correspondence and reprint requests to Prof. Karen S.L. Lam, Department of Medicine, University of Hong Kong, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong. E-mail: kslam@hkucc.hku.hk.

Received for publication 28 February 2007 and accepted in revised form 1 July 2007.

Published ahead of print at <http://care.diabetesjournals.org> on 9 July 2007. DOI: 10.2337/dc07-0413.

A. Tso and A. Xu contributed equally to this work and should be considered as co-first authors.

E.D.J. is currently affiliated with the Department of Medicine, University of Melbourne, Western Hospital, Melbourne, Victoria, Australia.

**Abbreviations:** A-FABP, adipocyte fatty acid-binding protein; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment index of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

© 2007 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Adipocyte fatty acid-binding protein (A-FABP), also known as aP2 or FABP4, is one of the most abundant proteins in mature adipocytes (1). It belongs to a family of fatty acid-binding proteins, which are small cytoplasmic proteins expressed in a highly tissue-specific manner, thought to be important in mediating intracellular fatty acid trafficking and energy metabolism (2,3). Recent studies in animal models suggested that A-FABP may be important in glucose homeostasis. Deletion of the A-FABP gene protected mice from insulin resistance and hyperinsulinemia associated with both diet-induced obesity (4) and genetic obesity (5). In humans, a promoter polymorphism, T-87C, of the A-FABP gene that resulted in reduced adipose tissue A-FABP mRNA expression was found to be associated with reduced risk for type 2 diabetes and cardiovascular disease (6).

We have previously demonstrated that A-FABP, although traditionally considered to be an intracellular cytosolic protein, is present in the circulation (7). We have also reported the positive association between serum A-FABP levels and parameters of adiposity, hyperglycemia, insulin resistance, and the metabolic syndrome in cross-sectional (7,8) and longitudinal studies (9). As the metabolic syndrome is known to confer a more than threefold risk of development of type 2 diabetes (10), we set out to examine the role of serum A-FABP in predicting the risk of type 2 diabetes using the data of subjects from the Hong Kong Cardiovascular Risk Factor Study who had just completed their 10-year follow-up.

## RESEARCH DESIGN AND METHODS

Subjects were recruited from the population-based Hong Kong Cardiovascular Risk Factor Prevalence Study conducted in 1995–1996 (11), in which unrelated individuals were randomly invited to undergo a comprehensive assessment of cardiovascular risks, including a 75-g oral glucose tolerance test (OGTT). In 1997, 322 subjects with

impaired glucose tolerance (IGT) and 322 age- and sex-matched subjects with normal glucose tolerance (NGT) were invited to participate in a prospective study examining the progression to type 2 diabetes. All subjects with IGT were given similar dietary and exercise advice without initiating active medical therapy at baseline, and all were under the care of their primary care physicians between assessments.

Subjects returned at years 2, 5, and 10 after an overnight 10-h fast for a repeat OGTT assessment. For subjects who had a diagnosis of type 2 diabetes and started treatment before the follow-up assessment, the date of type 2 diabetes diagnosis was ascertained and an OGTT not performed. Results from the year 2 and year 5 studies were reported previously (9,12–14). For this report, subjects were classified as having NGT, IGT, impaired fasting glucose (IFG), or type 2 diabetes according to the World Health Organization 1998 diagnostic criteria (15). Only subjects who had baseline fasting plasma glucose (FPG) <7 mmol/l and 2-h post-OGTT glucose (2-h glucose) <11.1 mmol/l (nondiabetic according to the World Health Organization 1998 criteria) and who had complete baseline anthropometric and biochemical data were included in this report. (Five subjects with baseline FPG 7–7.8 mmol/l were reclassified as type 2 diabetic and were excluded from analysis; subjects without baseline stored serum were also excluded.) Altogether, 544 subjects were included in this report, of whom 286 had NGT, 252 had IGT, and 6 had IFG at baseline, after reclassification. No significant differences in baseline anthropometric parameters were found between the study cohort and subjects without available stored baseline serum. Metabolic syndrome was defined according to the U.S. National Cholesterol Education Program Adult Treatment Panel III guidelines (16) and modified as recommended in the latest American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement (17) by adopting the Asian criteria for abdominal obesity (waist circumference  $\geq 90$  cm in men or  $\geq 80$  cm in women) and a lower cutoff for elevated fasting glucose (FPG  $\geq 5.6$  mmol/l). Metabolic syndrome would be diagnosed in the presence of three or more of five adverse cardiometabolic factors: abdominal obesity, hypertriglyceridemia, reduced HDL, hypertension, or elevated FPG.

At each visit, medical histories were

obtained. A family history of diabetes referred to first-degree relatives only. Alcohol drinking referred to any frequency of alcohol consumption including social drinking. Subjects were considered physically active when they had sessions of more than one-half an hour of continuous exercise at least once per week. Anthropometric (body weight, height, BMI, waist circumference, and resting blood pressure) and biochemical parameters (FPG, 2-h glucose, insulin, total cholesterol, triglycerides, LDL, and HDL) were measured as described previously (9,11–14). The presence of hypertension was defined as blood pressure  $\geq 130/85$  mmHg or receiving regular antihypertensive treatment. Insulin resistance was estimated using the homeostasis model assessment index of insulin resistance (HOMA-IR), calculated as (FPG [in millimoles per liter]  $\times$  fasting insulin [in milli-International Units per liter])/22.5). Serum high-sensitivity C-reactive protein (hsCRP) was measured using a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). Serum adiponectin was measured using an in-house sandwich ELISA established in our laboratory (14). A-FABP was measured using an ELISA (BioVendor Laboratory Medicine, Modrice, Czech Republic). Briefly, diluted serum samples, calibrators, and quality control samples were applied to 96-well microtiter plates coated with affinity-purified goat anti-human A-FABP antibody, and the absorbance values were measured at 450 nm. The intra- and interassay coefficients of variance were 3.7–6.4% and 2.6–5.3%, respectively (7). All subjects gave informed consent, and the study was approved by the ethics committee of the Faculty of Medicine, University of Hong Kong.

### Statistical analyses

All statistical calculations were performed with SPSS (version 12.0; SPSS, Chicago, IL). Results are presented as means  $\pm$  SD or median (interquartile range) as appropriate. Data with skewed distributions, as determined using the Kolmogorov-Smirnov test, were logarithmically transformed before analysis. Differences in baseline characteristics with glycemic status were compared using  $\chi^2$  tests for categorical variables and one-way ANOVA for continuous variables. The Bonferroni correction was used for multiple testing. Correlations between A-FABP and anthropometric and biochemical variables

were analyzed using Pearson's correlation. Stepwise multiple logistic regression analysis was used to examine the association (odds ratio [OR]) of baseline A-FABP with IGT/IFG at baseline. The population median of A-FABP was determined for each sex using the baseline data of the entire cohort of 544 subjects. The optimal sensitivity and specificity of using various cutoff values of A-FABP to predict type 2 diabetes was examined by receiver operating characteristic curve analysis. Survival was calculated from the date of the visit at baseline to the date of diagnosis of type 2 diabetes or year 10 follow-up. Survivals were estimated by the Kaplan-Meier method and compared by the log-rank test. Subjects who were lost to follow-up were assumed to be free from type 2 diabetes at 10 years. To identify independent predictors of the development of type 2 diabetes, baseline variables that were significantly different between subjects with and without type 2 diabetes (after correction for multiple testing) and that were biologically likely to affect glycemic status were analyzed using a multiple Cox proportional hazard regression model with a stepwise elimination procedure. For parameters that were highly correlated, such as BMI and waist circumference ( $r = 0.87$  in men and  $0.79$  in women;  $P < 0.001$ ), only one was entered into the regression analysis. Two-sided  $P$  values  $< 0.05$  were considered significant.

**RESULTS** — There were 544 nondiabetic subjects with complete baseline demographic and biochemical data. Of these, 286 had NGT and 258 had IGT or IFG at baseline. As expected, when compared with subjects with NGT, those with IGT/IFG were more obese, were more hyperinsulinemic and insulin resistant as estimated by HOMA-IR, were more hypertensive, and had more adverse lipid profiles and a higher prevalence of metabolic syndrome. They also had lower serum adiponectin but higher hsCRP (all  $P < 0.001$ , IGT/IFG vs. NGT). Consistent with our previous reports (7,9), A-FABP levels were significantly higher in women ( $P < 0.001$ ). The sex-adjusted A-FABP concentrations were significantly higher in the IGT/IFG group (IGT/IFG vs. NGT: medians of 18.2 vs. 12.5 ng/ml in men and 22.4 vs. 18.5 ng/ml in women,  $P < 0.00001$ ). There were no significant differences between the groups in smoking status, alcohol consumption, physical

**Table 1—Baseline clinical parameters of subjects with or without development of type 2 diabetes by 10 years and the RR of each parameter in the prediction of the development of type 2 diabetes over a median of 10.0 years using univariate Cox regression analysis**

	Type 2 diabetic	Nondiabetic	P	RR (95% CI)	P
n	96	448	—	—	—
Age (years)	51.2 ± 11.8	50.7 ± 12.5	0.745	1.02 (1.00–1.03)	0.037
Men/women	51/45	183/265	0.027	1.33 (1.10–1.63)	0.005
BMI (kg/m <sup>2</sup> )	26.4 ± 3.0	24.3 ± 3.7	<0.001	1.14 (1.08–1.19)	<0.001
Waist circumference (cm)					
Men	89.5 ± 7.8	83.4 ± 9.5	<0.001‡	1.06 (1.04–1.08)	<0.001‡
Women	82.2 ± 8.8	76.6 ± 8.8			
Systolic blood pressure (mmHg)*	128 ± 18.8	119 ± 17.7	<0.001	1.03 (1.02–1.04)	<0.001
Diastolic blood pressure (mmHg)*	80 ± 10.2	74 ± 9.6	<0.001	1.05 (1.03–1.08)	<0.001
Mean arterial pressure (mmHg)*	96 ± 12.4	89 ± 11.5	<0.001	1.05 (1.03–1.07)	<0.001
Hypertension (%)	54.2	33.7	<0.001	2.49 (1.66–3.73)	<0.001
Fasting glucose (mmol/l)	5.5 ± 0.5	5.1 ± 0.5	<0.001	3.38 (2.31–4.95)	<0.001
2-h post-OGTT glucose (mmol/l)	8.8 ± 1.5	6.8 ± 1.8	<0.001	2.00 (1.72–2.33)	<0.001
Insulin (mIU/l)†	6.9 (4.6–10.5)	5.0 (3.3–7.3)	<0.001	1.96 (1.40–2.73)	<0.001
HOMA-IR†	1.7 (1.1–2.7)	1.1 (0.7–1.7)	<0.001	2.09 (1.51–2.85)	<0.001
LDL cholesterol (mmol/l)	3.6 ± 1.0	3.3 ± 0.9	0.004	1.39 (1.12–1.72)	0.003
HDL cholesterol (mmol/l)	1.1 ± 0.3	1.3 ± 0.3	<0.001	0.22 (0.11–0.44)	<0.001
Triglyceride (mmol/l)†	1.4 (1.0–1.9)	1.0 (0.7–1.5)	<0.001	2.51 (1.83–3.44)	<0.001
Adiponectin (μg/ml)†					
Men	4.2 (2.5–6.3)	5.5 (3.7–7.4)	<0.001‡	0.50 (0.35–0.71)	<0.001‡
Women	5.0 (3.7–7.3)	6.6 (4.9–9.3)			
hsCRP (mg/l)†	1.4 (0.7–2.6)	0.8 (0.3–1.8)	0.001	1.56 (1.29–1.87)	<0.001
A-FABP (ng/ml)†					
Men	19.3 (13.5–24.4)	13.8 (9.3–19.9)	<0.00001‡	3.03 (2.00–4.60)	<0.00001‡
Women	24.9 (19.0–28.9)	19.2 (12.7–27.0)			
Presence of IGT/IFG (%)	85.4	39.3	<0.001	8.37 (4.74–14.8)	<0.001
Presence of metabolic syndrome (%)	43.8	19.2	<0.001	2.95 (1.97–4.42)	<0.001

Data are means ± SD or median (interquartile range). \*Excluded 56 subjects taking antihypertensive medication. †Log transformed before analyses. ‡Sex-adjusted P value.

activity, and family history of type 2 diabetes.

Baseline sex-adjusted A-FABP levels correlated positively with increasing age, BMI, waist circumference, systolic and diastolic blood pressure, FPG, 2-h glucose, fasting insulin, HOMA-IR, LDL cholesterol, triglycerides, and hsCRP but negatively with HDL (all  $P < 0.001$ ; data not shown). At baseline, the positive association of sex-adjusted A-FABP levels with the IGT/IFG group remained significant (OR 2.05 [95% CI 1.38–3.04],  $P < 0.001$ ) even after adjustment for age, BMI, HOMA-IR, adiponectin, and hsCRP (data not shown).

Of the 544 subjects, 72 were lost to follow-up at 5 years and 64 were lost to follow-up at 10 years. The main reasons for their loss to follow-up were withdrawal of consent, emigration, change of address, disability, or death. There were no significant differences in any baseline parameters between subjects who returned for follow-up and those who did not, except for older age in those lost to

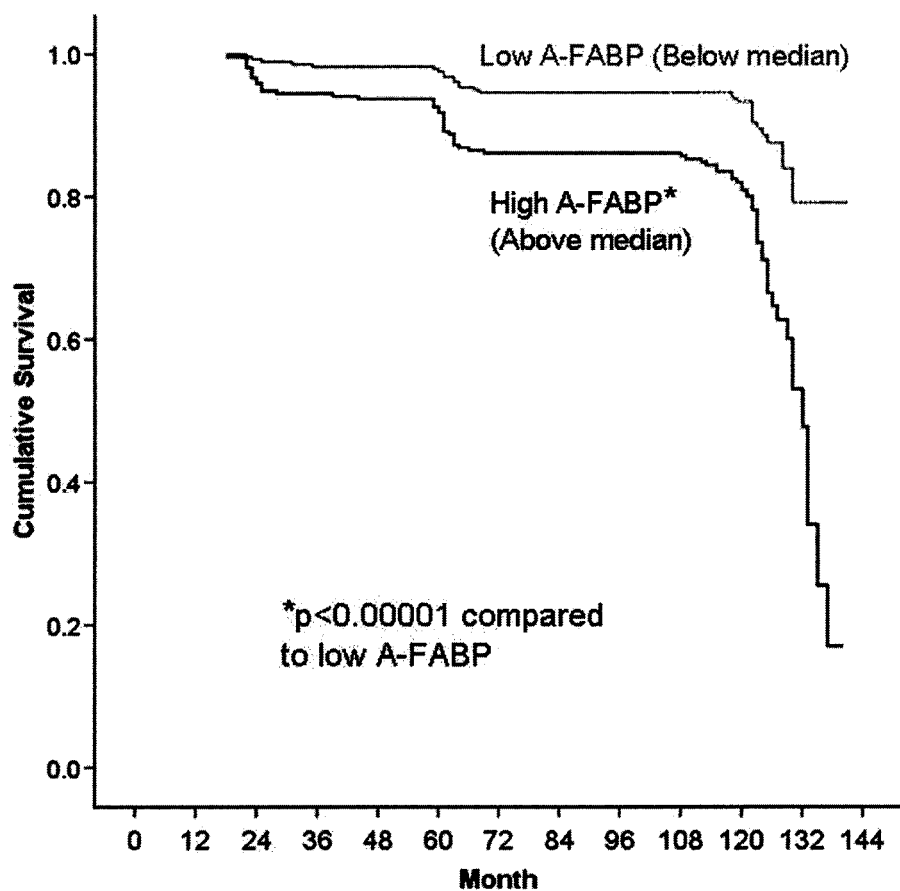
follow-up. Over the 10 years, a total of 96 subjects (17.6%) had developed type 2 diabetes (21 new cases by 2 years, 26 by 5 years, and 49 by 10 years). The cumulative 10-year type 2 diabetes incidence rate was 31.8% for those with baseline IGT/IFG and 4.9% for those with NGT.

The clinical characteristics of subjects who subsequently developed type 2 diabetes are shown in Table 1. More men than women had developed type 2 diabetes in this cohort. As expected, those who had progressed to type 2 diabetes had more adverse cardiometabolic risk factors at baseline, being more obese, insulin resistant, hyperglycemic, hypertensive, and hyperlipidemic. They also had higher hsCRP, lower adiponectin levels, and a higher prevalence of IGT/IFG and metabolic syndrome at baseline (all  $P < 0.001$ , type 2 diabetic vs. nondiabetic subjects). There was no significant difference between the two groups in the proportion of subjects receiving antihypertensive treatment at baseline. (Details of individual medications were not available.) There

was also no significant difference in interval weight change between the two groups and no difference in crude physical activity assessment.

Baseline A-FABP levels were significantly higher in subjects who had developed type 2 diabetes by year 10 compared with those who did not (type 2 diabetic vs. nondiabetic: medians of 19.3 vs. 13.8 ng/ml in men and 24.9 vs. 19.2 ng/ml in women,  $P < 0.00001$ ). On univariate analysis, the relative risk (RR) of A-FABP in predicting the development of type 2 diabetes was 3.03 (95% CI 2.00–4.60,  $P < 0.00001$ ; Table 1). All of the well-known cardiometabolic risk factors were significantly associated with the development of type 2 diabetes in our cohort, together with high hsCRP and low adiponectin, on univariate analysis (Table 1).

The Kaplan-Meier curve for the development of type 2 diabetes is shown in Fig. 1. Subjects with baseline A-FABP levels above the population median (according to sex) had significantly higher risk



**Figure 1**—Cumulative survival (by the Kaplan-Meier method) for the development of type 2 diabetes over a median follow-up of 10.0 years for subjects with A-FABP above and below sex-specific medians.

( $P < 0.00001$ ) of developing type 2 diabetes than those with A-FABP levels below the median. According to receiver operating characteristic curve analysis, using the population median of 15.30 ng/ml for men and 20.36 ng/ml for women as cutoffs offered sensitivities of 71 and 76% and specificities of 56 and 54%, respectively, which approximated to the optimal cutoff level of A-FABP in the prediction of type 2 diabetes.

The independent predictors for the development of type 2 diabetes were identified using a stepwise multiple Cox proportional hazard regression model. As shown in Table 2, baseline serum A-FABP was a significant independent predictor of development of type 2 diabetes, together with male sex and baseline 2-h glucose, in a model that also included age, baseline BMI, HOMA-IR, adiponectin, and hsCRP. Subjects with A-FABP above the median had a RR of 2.25 (95%CI 1.40–3.65,  $P = 0.001$ ) of developing type 2 diabetes compared with those with A-FABP below the median at baseline. Identical results were

obtained if waist circumference was entered into the model instead of BMI or if the presence of metabolic syndrome was added to the model. As the presence of IGT/IFG at baseline is known to be strongly associated with the development of type 2 diabetes, we also performed a subgroup analysis of this IGT/IFG cohort, and A-FABP remained a significant independent predictor of the development of type 2 diabetes ( $P = 0.018$ ), together with 2-h glucose (RR 1.75,  $P < 0.001$ ) and adiponectin (0.63,  $P < 0.05$ ), in a model

that also included age, sex, BMI, HOMA-IR, and hsCRP. For subjects with IGT/IFG who had serum A-FABP levels above the median at baseline, the adjusted RR of developing type 2 diabetes over the course of 10 years was 1.87 (1.12–3.15) compared with those with A-FABP below the median.

**CONCLUSIONS**— A-FABP, one of the most abundant proteins expressed in adipocytes, has recently been suggested to play an important role in energy metabolism. In this study, we have demonstrated that circulating A-FABP levels were associated with dysglycemia and predicted the development of type 2 diabetes in a 10-year prospective study in Chinese. This finding is of potential clinical importance as the prevalence of type 2 diabetes is increasing worldwide and is reaching epidemic dimensions in Asian countries (18).

The adverse effects of A-FABP on glucose metabolism in mice have been well demonstrated. In A-FABP-deficient mice, the improvement in glucose metabolism (4,5) was in part offset by the compensatory upregulation of *mal1*, a minor isoform of FABP in adipocytes (4,19,20). On the other hand, mice with targeted disruption of both A-FABP and *mal1* (*aP2-mal1<sup>-/-</sup>*) had better glucose tolerance and were more insulin sensitive than wild-type mice even when they were fed an ordinary chow diet and were protected from high-fat diet-induced insulin resistance and type 2 diabetes (21). In addition, simultaneous ablation of A-FABP and *mal1* in *ob/ob* mice led to normalization of hyperglycemia and associated metabolic abnormalities, even in the presence of obesity (22).

In humans, A-FABP expression was also found to affect glucose metabolism. A functional promoter polymorphism of the A-FABP gene, T-87C, which resulted in impaired transcriptional activation by CAAT box/enhancer-binding protein

**Table 2**—Baseline predictors of the development of type 2 diabetes over a median 10.0 years of follow-up, examined using multiple stepwise Cox regression analysis (final model)

Baseline parameters	RR (95% CI)	P
Sex (male)	1.30 (1.05–1.61)	0.017
2-h post-OGTT glucose	1.84 (1.57–2.15)	<0.001
Adiponectin*	0.69 (0.45–1.05)	0.081
Sex-specific A-FABP†	2.25 (1.40–3.65)	0.001

\*Log transformed before analysis. †Above versus below sex-specific median, with the latter as reference with RR = 1. Model included sex, age, baseline BMI, 2-h post-OGTT glucose, HOMA-IR, adiponectin, hsCRP, and A-FABP in sex-specific median.



and hence reduced adipose A-FABP mRNA expression, was shown to be associated with a reduced risk for type 2 diabetes, especially in obese individuals (6). Our finding that high baseline serum A-FABP levels could predict the development of type 2 diabetes in our study subjects is in keeping with an adverse effect of increased A-FABP expression on glucose metabolism, as the serum A-FABP level has been shown to correlate strongly with its protein expression in adipose tissues (9). The 10-year cumulative incidence rate of type 2 diabetes in our cohort was 17.6% (96 of 544), whereas those with A-FABP above and below the median at baseline had cumulative incidence rates of 25.7% (70 of 272) and 9.6% (26 of 272), respectively. Assuming that the association between A-FABP and type 2 diabetes was causal, then lowering the A-FABP level, if this were possible, would have a substantial impact on the population incidence of type 2 diabetes. The dysregulated production of various adipokines, such as adiponectin and plasminogen activator inhibitor 1, in obese individuals, has been shown to improve with weight reduction and various medications (23). The effect of weight reduction and other therapeutic approaches on A-FABP expression is currently being investigated in our laboratory.

The mechanism linking A-FABP with glucose homeostasis is not yet fully understood. It is known that A-FABP can bind various intracellular fatty acids and probably mediates intracellular lipid trafficking between cellular compartments (2). It can also form a 1:1 complex with hormone-sensitive lipase and enhance the efficiency of the lipase, hence facilitating lipolysis and efflux of fatty acids from adipocytes (24,25). It might also modulate the availability and composition of fatty acids in muscles and adipose tissues (26). In mice, improved glucose homeostasis in A-FABP ablation was shown to be associated with preferential accumulation of shorter chain fatty acids in myocytes and adipose tissues. This was accompanied by increased AMP-activated protein kinase activity, Akt phosphorylation (26), and glucose oxidation in muscles (27), increased basal glucose conversion into lipids (28), and reduced lipolysis-induced insulin resistance and pancreatic dysfunction (20). Although these observations might partly account for the enhanced insulin sensitivity observed in mice with A-FABP ablation, their physiolog-

ical relevance in humans awaits further clarification.

We have recently demonstrated by proteomic analysis that A-FABP, traditionally considered a cytoplasmic protein, was released from cultured mature adipocytes in the absence of any obvious cell damage (7). In humans, A-FABP circulates at concentrations of ~10–50 ng/ml, comparable to or higher than that of most adipokines, and its level was elevated in obese subjects and correlated with BMI, waist circumference, fat percentage, and insulin resistance (7–9). In both Chinese (7–9) and Caucasians (29), close associations are found between serum A-FABP levels and various cardiometabolic risk factors. We have also demonstrated that high serum A-FABP predicts the development of the metabolic syndrome (9), well known to be associated with an increased risk of type 2 diabetes and cardiovascular diseases. In this 10-year prospective study, we have explored the association of circulating A-FABP levels with hyperglycemia and found that baseline fasting A-FABP levels were elevated in subjects with IGT/IFG, compared with those with NGT. Furthermore, the serum A-FABP level was predictive of the progression to type 2 diabetes, independent of the influence of established risk factors of type 2 diabetes, including excess adiposity (12), the presence of the metabolic syndrome (10) or IGT/IFG (10), and the more recently recognized predictors, such as hsCRP (13) and hypo adiponectinemia (30). Nevertheless, it is currently unclear whether circulating A-FABP functions as a lipid-hormone transporter or in a hormone-like fashion to mediate the effects on muscle glucose metabolism where no local A-FABP expression is present. Factors influencing the balance between serum and tissue A-FABP also remain to be determined. Further work investigating the possible physiological role of circulating A-FABP is in progress.

The current study population included only the subjects with IGT/IFG who consented to participate in long-term follow-up and their age- and sex-matched control subjects, instead of the entire population-based cohort of the Hong Kong Cardiovascular Risk Factor Prevalence Study (11). Our findings may therefore not be directly applicable to the general population because of the sampling bias introduced by the study design. Our study is also limited by the relatively small number of subjects with incident type 2 diabetes and the relatively high rate of loss

to follow-up due to high rate of emigration, a common problem in prospective studies in Hong Kong. Whether serum A-FABP can be useful in the prediction of type 2 diabetes needs to be confirmed in other populations and preferably with larger cohorts randomly recruited from the general population. As not all patients in Hong Kong see primary care physicians regularly, the documentation of the timing of incident type 2 diabetes in this cohort might have been delayed, and diabetes may only have been diagnosed at the scheduled study follow-up (at 2, 5, or 10 years). More detailed data on diet, exercise, and activity scores, which can affect glycemic outcome, will also be helpful, although we did not observe a significant difference in the crude assessment of baseline physical activity level or interval weight changes between groups in the subjects studied. The lack of detailed data on concomitant medications is another limitation. The differential use of drugs that may affect glucose metabolism, such as ACE inhibitors or  $\beta$ -blockers, cannot be excluded, although the proportion of subjects receiving antihypertensive medications was similar for those who did or did not develop type 2 diabetes. Despite these limitations, our data suggest that the measurement of serum A-FABP level may be of potential clinical relevance in identifying subjects at risk of developing diabetes, independent of the well-established predictors including BMI, waist circumference, glucose, or metabolic syndrome.

In summary, we have demonstrated that circulating A-FABP levels were increased in subjects with glucose dysregulation and high A-FABP levels predicted the development of type 2 diabetes in this Chinese cohort. Further studies are warranted to examine the role of A-FABP in the pathogenesis of type 2 diabetes and to investigate the potential application of this new biomarker for the identification of at-risk individuals for targeted lifestyle intervention, the benefit of which is well documented (31).

**Acknowledgments**—This study was supported by the Hong Kong Research Grant Council (HKU 7404/04M and HKU7590/06M) and the Hong Kong Innovation and Technology Fund (GHP/027/05).

## References

1. Makowski L, Hotamisligil GS: Fatty acid binding proteins—the evolutionary

- crossroads of inflammatory and metabolic responses. *J Nutr* 134:2464S–2468S, 2004
2. Coe NR, Bernlohr DA: Physiological properties and functions of intracellular fatty acid-binding proteins. *Biochim Biophys Acta* 1391:287–306, 1998
  3. Hertzler AV, Bernlohr DA: The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. *Trends Endocrinol Metab* 11: 175–180, 2000
  4. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM: Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science* 274:1377–1379, 1996
  5. Uysal KT, Scheja L, Wiesbrock SM, Bonner-Weir S, Hotamisligil GS: Improved glucose and lipid metabolism in genetically obese mice lacking aP2. *Endocrinology* 141:3388–3396, 2000
  6. Tuncman G, Erbay E, Hom X, De Vivo I, Campos H, Rimm EB, Hotamisligil GS: 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* 103:6970–6975, 2006
  7. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, Wat NM, Wong WK, Lam KS: Adipocyte fatty acid binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem* 52:405–413, 2006
  8. Yeung DCY, Xu A, Cheung CWS, Wat NMS, Yau MH, Fong CHY, Chau MT, Lam KSL: Serum adipocyte fatty acid-binding protein levels were independently associated with carotid atherosclerosis in Chinese women. *Arterioscler Thromb Vasc Biol* 27:1796–1802, 2007
  9. Xu A, Tso AWK, Cheung BMY, Yu W, Wat NMS, Fong CHY, Janus ED, Lam KSL: Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. *Circulation* 115:1537–1543, 2007
  10. Lorenzo C, Okoloise M, Williams K, Stern MP, Haffner SM: The metabolic syndrome as predictor of type 2 diabetes: the San Antonio Heart Study. *Diabetes Care* 26: 3153–3159, 2003
  11. Janus ED, Wat NMS, Lam KS, Cockram C, Siu S, Liu L, Lam TH, Hong Kong Cardiovascular Risk Factor Steering Committee: The prevalence of diabetes, association with cardiovascular risk factors and implications of diagnostic criteria (ADA 1997 and WHO 1998) in a 1996 community-based population study in Hong Kong Chinese. *Diabet Med* 17:741–745, 2000
  12. Wat NM, Lam TH, Janus ED, Lam KS: Central obesity predicts the worsening of glycemia in southern Chinese. *Int J Obes Relat Metab Disord* 25:1789–1793, 2001
  13. Tan KC, Wat NM, Tam SC, Janus ED, Lam TH, Lam KS: C-reactive protein predicts the deterioration of glycemia in Chinese subjects with impaired glucose tolerance. *Diabetes Care* 26:2323–2328, 2003
  14. Tso AW, Sham PC, Wat NM, Xu A, Cheung BM, Rong R, Fong CH, Xu JY, Cheung KK, Janus ED, Lam KS: Polymorphisms of the gene encoding adiponectin and glycaemic outcome of Chinese subjects with impaired glucose tolerance: a 5-year follow-up study. *Diabetologia* 49: 1806–1815, 2006
  15. Alberti KGMM, Zimmet P: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus: provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
  16. Executive summary of the Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 285:2486–2497, 2001
  17. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Bordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F: Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 112:2735–2752, 2005
  18. Yoon KH, Lee JH, Kim JW, Cho JH, Choi YH, Ko SH, Zimmet P, Son HY: Epidemic obesity and type 2 diabetes in Asia. *Lancet* 368:1681–1688, 2006
  19. Shaughnessy S, Smith ER, Kodukula S, Storch J, Fried SK: Adipocyte metabolism in adipocyte fatty acid binding protein knockout (aP2<sup>-/-</sup>) mice after short-term high-fat feeding—functional compensation by the keratinocyte fatty acid binding protein. *Diabetes* 49:904–911, 2000
  20. Scheja L, Malowski L, Uysal KT, Wiesbrock SM, Shimshek DR, Meyers DS, Morgan M, Parker RA, Hotamisligil GS: Altered insulin secretion associated with reduced lipolytic efficiency in aP2<sup>-/-</sup> mice. *Diabetes* 48:1987–1994, 1999
  21. Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, Hotamisligil GS: Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. *Circulation* 110:1492–1498, 2004
  22. Cao H, Maeda K, Gorgun CZ, Kim HJ, Park SY, Shulman GL, Kim JK, Hotamisligil GS: Regulation of metabolic responses by adipocyte/macrophage fatty acid-binding proteins in leptin-deficient mice. *Diabetes* 55:1915–1922, 2006
  23. Xavier PSF: The relationship of adipose tissue to cardiometabolic risk. *Clin Cornerstone* 8 (Suppl. 4):S14–S23, 2006
  24. Smith AJ, Sanders MA, Thompson BR, Londos C, Kraemer FB, Bernlohr DA: Physical association between the adipocyte fatty acid-binding protein and hormone-sensitive lipase: a fluorescence resonance energy transfer analysis. *J Biol Chem* 279:52399–52405, 2004
  25. Shen WJ, Sridhar K, Bernlohr DA, Kraemer FB: Interaction of rat hormone-sensitive lipase with adipocyte lipid-binding protein. *Proc Natl Acad Sci U S A* 96: 5528–5532, 1999
  26. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, Cao Q, Atsumi G, Malone H, Krishnan B, Minokoshi Y, Kahn BB, Parker RA, Hotamisligil GS: Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab* 1:107–119, 2005.
  27. Baar RA, Dingfelder CS, Smith LA, Bernlohr DA, Wu C, Lange AJ, Parks EJ: Investigation of in vivo fatty acid metabolism in AFABP/aP2<sup>-/-</sup> mice. *Am J Physiol* 288: E187–E193, 2005
  28. Coe NR, Simpson MA, Bernlohr DA: Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J Lipid Res* 40:967–972, 1999
  29. Stejskal D, Karpisek M: Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome? *Eur J Clin Invest* 36:621–625, 2006
  30. Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J: Adiponectin and development of type 2 diabetes in the Pima Indian populations. *Lancet* 360:57–58, 2002
  31. Lindstrom J, Ilanne-Parikka P, Peltonen M, Aunola S, Eriksson JG, Hemio K, Hämäläinen H, Harkonen P, Keinanen-Kiukkaanniemi S, Laakso M, Louheranta A, Mannelin M, Paturi M, Sundvall J, Valle TT, Uusitupa M, Tuomilehto J, Finnish Diabetes Prevention Study Group: Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. *Lancet* 368:1673–1679, 2006