

# Circulating Levels of Adipocyte and Epidermal Fatty Acid-Binding Proteins in Relation to Nephropathy Staging and Macrovascular Complications in Type 2 Diabetic Patients

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**OBJECTIVE** — To investigate the relationships of serum adipocyte fatty acid-binding protein (A-FABP) and epidermal fatty acid-binding protein (E-FABP) with renal dysfunction and macrovascular complications in type 2 diabetic patients.

**RESEARCH DESIGN AND METHODS** — The associations of serum A-FABP and E-FABP with markers of renal function, nephropathy staging, and macrovascular complications were examined in 237 type 2 diabetic patients.

**RESULTS** — Serum A-FABP and E-FABP correlated significantly with serum creatinine, mean albumin excretion rate, and glomerular filtration rate (all  $P < 0.001$ ) and were independently associated with diabetic nephropathy staging ( $P = 0.001$  and  $P < 0.05$ , respectively). Circulating levels of both types of FABP were increased ( $P < 0.01$ ) in subjects with macrovascular complications. Serum A-FABP was independently associated with macrovascular complications (odds ratio 2.92 [95% CI 1.42–6.01];  $P = 0.004$ ).

**CONCLUSIONS** — Serum A-FABP and E-FABP might be novel serum biomarkers for evaluating the progression of nephropathy and its cardiovascular risk in type 2 diabetic patients.

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**A**dipocyte fatty acid-binding protein (A-FABP) and epidermal fatty acid-binding protein (E-FABP), highly expressed in adipocytes and macrophages, have been shown to mediate obesity-related metabolic disorders and atherosclerosis in animal studies (1). We have recently demonstrated that both types of FABP circulate in the human bloodstream (2,3), with levels closely associated with parameters of adiposity, insulin resistance, the metabolic syndrome,

and carotid atherosclerosis (2–4). This study examined the relationships of circulating A-FABP and E-FABP with diabetic nephropathy and its associated macrovascular complications in 237 type 2 diabetic patients in various stages of nephropathy.

**RESEARCH DESIGN AND METHODS** — This cohort included 237 Chinese type 2 diabetic patients (mean  $\pm$  SD age  $55.8 \pm 14.2$  years, BMI

$25.5 \pm 4.3$  kg/m<sup>2</sup>, 110 male and 127 female) from the Diabetes Clinic, Queen Mary Hospital (Hong Kong, China), with complete sets of renal function assessment data collected as part of two previously published studies (3,5). None of the patients were on thiazolidinediones. Informed consent was obtained, and the protocol was approved by the local ethics committee.

All subjects were assessed after at least 10 h of overnight fasting. Details on the assessment for hypertension, various anthropometric and biochemical parameters, serum high-sensitivity C-reactive protein, A-FABP (intra-assay coefficients of variation [CVs] 3.9–6.6% and interassay CVs 2.6–5.1%) and E-FABP (intra-assay CVs 4.5–4.9% and interassay CVs 5.7–5.9%) (BioVendor Laboratory Medicine, Modrice, Czech Republic) were reported previously (2–6). Serum soluble tumor necrosis factor receptor II (sTNF RII) was measured with ELISA kits from R&D Systems (Minneapolis, MN).

Mean albumin excretion rate (MAER) was estimated from the albumin content of two consecutive 12-h overnight urine samples, which were assayed nephelometrically. Subjects were classified as normoalbuminuric (MAER  $< 20$   $\mu$ g/min), microalbuminuric (MAER 20–200  $\mu$ g/min), or macroalbuminuric (MAER  $> 200$   $\mu$ g/min) (6). Glomerular filtration rate (GFR) was estimated using the formula from the Modification of Diet in Renal Disease (MDRD) study (7). Macrovascular complications included ischemic heart disease, stroke, or peripheral vascular disease, diagnosed clinically and documented on electrocardiogram, myocardial perfusion scan, or coronary angiogram; computed tomography brain scan; or Doppler studies, respectively.

All analyses were performed with SPSS (version 15; SPSS, Chicago, IL). Skewed data were logarithmically transformed. One-way ANOVA or a  $\chi^2$  test was used for comparisons between groups,

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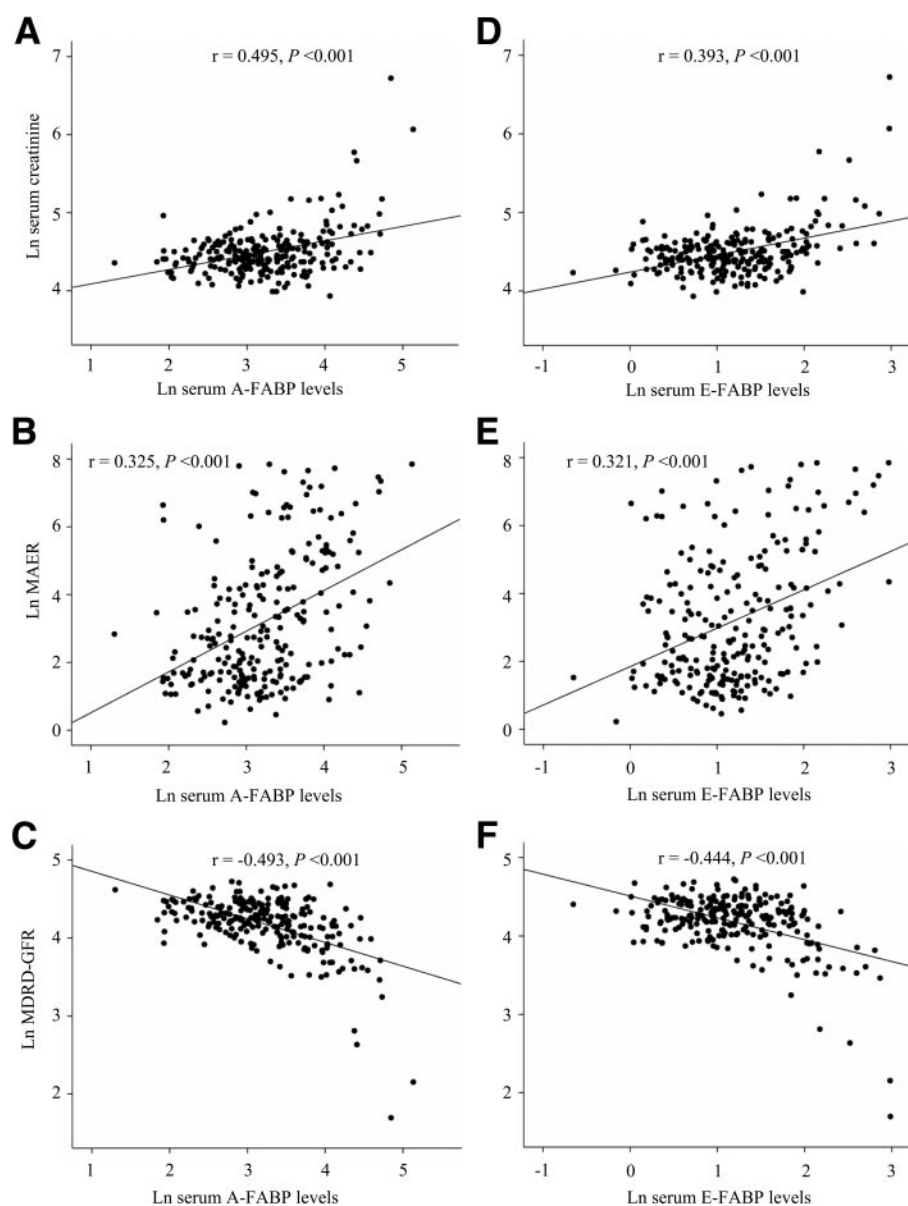
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**Figure 1**—Correlations between serum levels of the two FABPs and indexes of renal function. Serum A-FABP levels correlated significantly with serum creatinine (A), MAER (B), and MDRD-GFR (C). Serum E-FABP also correlated significantly with serum creatinine (D), MAER (E), and MDRD-GFR (F). Ln, natural logarithm.

and correlations between variables were adjusted using partial correlation. Multiple testing was corrected using Bonferroni correction. Multinomial and binary logistic regressions were used to determine the variables with independent significant associations with nephropathy staging and macrovascular complications, respectively.  $P < 0.05$  was considered statistically significant.

**RESULTS**— There were significant and progressive increases in serum A-FABP and E-FABP across the three stages of diabetic nephropathy (for both, with

adjustment for trend,  $P < 0.001$ ) (Table A1 in the online appendix, available at <http://dx.doi.org/10.2337/dc08-1333>). Serum A-FABP correlated positively with serum creatinine and MAER and negatively with MDRD-GFR (all  $P < 0.001$ , adjusted for sex, age, and waist circumference) (Fig. 1A–C). Age- and waist circumference-adjusted serum E-FABP also correlated positively with serum creatinine and MAER and negatively with MDRD-GFR (all  $P < 0.001$ ) (Fig. 1D–F). The correlations of serum A-FABP or E-FABP with serum creatinine and MDRD-GFR re-

mained significant even after adjusting for LDL cholesterol, hypertension, and sTNF RII (adjusted  $P = 0.015$  to  $< 0.001$ ). There was no significant correlation of FABP with either A1C or statin use. Serum E-FABP, but not A-FABP, correlated with adiponectin ( $r = -0.213$ ;  $P = 0.001$ ).

Multinomial regressions, including factors with biological relevance or significant associations (corrected  $P < 0.05$ ) with nephropathy staging, showed that serum A-FABP was independently associated with nephropathy staging for microalbuminuria versus normoalbuminuria (odds ratio [OR] 3.04 [95% CI 1.61–5.74];  $P = 0.001$ ) and for macroalbuminuria versus normoalbuminuria (4.14 [1.83–9.33];  $P = 0.001$ ) together with LDL cholesterol (2.15 [1.23–3.77];  $P = 0.007$ ) (online appendix Table A2). Serum sTNF RII, A-FABP, and E-FABP were input one at a time because of their strong correlations ( $r > 0.4$ ;  $P < 0.001$ ). Repeated analysis yielded similar results for serum E-FABP (OR 1.83,  $P = 0.035$  for microalbuminuria vs. normoalbuminuria, and OR 4.93,  $P < 0.001$  for macroalbuminuria vs. normoalbuminuria).

Thirty-six micro- or macroalbuminuric subjects had macrovascular complications. They had higher serum A-FABP and E-FABP (both  $P < 0.01$ ) than subjects with no macrovascular complications (online appendix Table A3). Binary logistic regression, which included parameters having significant associations or possible biological relevance with macrovascular complications, revealed that serum A-FABP (OR 2.92 [95% CI 1.42–6.01];  $P = 0.004$ ) and fasting glucose (1.15 [1.00–1.33];  $P = 0.046$ ) were independently associated with macrovascular complications (online appendix Table A4). Serum A-FABP, E-FABP, sTNF RII, and MDRD-GFR, being highly correlated, were analyzed one at a time. MDRD-GFR, but not serum E-FABP or sTNF RII, was significantly associated with macrovascular complications (OR 0.24;  $P = 0.006$ ).

**CONCLUSIONS**— This study provides novel evidence that serum A-FABP and E-FABP in type 2 diabetic patients were independently associated with nephropathy staging and correlated positively with serum creatinine and negatively with MDRD-GFR, even after adjustment for age, adiposity, LDL cholesterol, hypertension, and sTNF RII. The elevated levels of these FABPs might have

resulted from both impaired renal clearance and increased production by activated macrophages in diabetic nephropathy. Macrophage accumulation in the kidney, which increases with the progression of nephropathy in diabetes and renal injury (8), is the primary source of inflammation under this pathological condition. Both FABPs are highly expressed in macrophages (1), and several proinflammatory stimuli could induce A-FABP expressions in macrophages (9, 10). On the other hand, both FABPs have been implicated as key mediators of inflammation (1). Taken together, macrophage accumulation in the kidney and the augmented expressions of these FABPs in macrophages would aggravate local inflammation and contribute to the progression of nephropathy in diabetes.

We have also demonstrated the significant increases of serum A-FABP and E-FABP in macrovascular complications and the independent association between serum A-FABP and macrovascular complications. These new findings would support a causative role of these FABPs in the pathogenesis of cardiovascular diseases, suggested by previous animal, genetic, and epidemiological studies (1,3,4,11). In summary, our findings raise the possibility that A-FABP and E-FABP may be used as serum biomarkers for stratifying nephropathy staging and cardiovascular risks in diabetic patients.

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