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ARTICLE

Urinary liver-type fatty acid binding protein is an independent predictor of stroke and mortality in individuals with type 1 diabetes

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Abstract

Aims/hypothesis In type 1 diabetes, cardiovascular disease (CVD) and diabetic nephropathy progress in parallel, thereby potentiating the risk of premature death during their development. Since urinary liver-type fatty acid binding protein (L-FABP) predicts the progression of diabetic nephropathy, the aim of this study was to investigate whether urinary L-FABP also predicts cardiovascular outcomes and mortality.

Methods We tested our hypothesis in a Finnish cohort of 2329 individuals with type 1 diabetes and a median follow-up of 14.1 years. The L-FABP to creatinine ratio was determined from baseline urine samples. The predictive value of urinary L-FABP was evaluated using Cox regression models, while its added predictive benefit for cardiovascular outcomes and mortality was evaluated using a panel of statistical indexes.

Results Urinary L-FABP predicted incident stroke independently of traditional risk factors (HR 1.33 [95% CI 1.20, 1.49]) and after further adjustment for eGFR (HR 1.28 [95% CI 1.14, 1.44]) or AER (HR 1.24 [95% CI 1.06, 1.44]). In addition, it predicted mortality independently of traditional risk factors (HR 1.34 [95% CI 1.24, 1.45]), and after adjustment for eGFR (HR 1.29 [95% CI 1.18, 1.39]) or AER (HR 1.22 [95% CI 1.09, 1.36]). Urinary L-FABP was as good a predictor as eGFR or AER, and improved the AUC for both outcomes on top of traditional risk factors, with no reclassification benefit (integrated discrimination improvement/net reclassification improvement) for stroke or mortality when AER or eGFR were added to traditional risk factors. However, urinary L-FABP was not a predictor of other cardiovascular endpoints (coronary artery disease, peripheral vascular disease and overall CVD events) when adjusted for the AER.

Conclusions/interpretation Urinary L-FABP is an independent predictor of stroke and mortality in individuals with type 1 diabetes.

Angelika Bierhaus died during the course of this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-017-4328-x) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

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Keywords Cardiovascular disease · Diabetic complications · Liver-type fatty acid binding protein · Mortality · Stroke · Type 1 diabetes · Urinary biomarkers · Urinary L-FABP

Abbreviations

CAD	Coronary artery disease
CVD	Cardiovascular disease
ESRD	End-stage renal disease
IDI	Integrated discrimination improvement
L-FABP	Liver-type fatty acid binding protein
NRI	Net reclassification improvement
PVD	Peripheral vascular disease
ROC	Receiver operating characteristic

Introduction

Individuals with type 1 diabetes have a higher risk of cardiovascular disease (CVD) compared with the general population [1, 2]. In addition, the risk of death from cardiovascular causes is already higher by early adulthood [3]. Furthermore, diabetic nephropathy increases the risk of CVD and mortality along with the progression of kidney damage [4, 5]. Diabetic nephropathy and CVD thus progress in parallel and share many common risk factors such as hypertension, dyslipidaemia, hyperglycaemia and smoking [6]. Therefore, the use of biomarkers to identify kidney and vascular dysfunction at an early stage would be an attractive approach to detecting these complications.

AER is the most widely used predictor of diabetic nephropathy, mortality and CVD [7]. Its predictive power derives from the fact that it reflects glomerular damage and generalised endothelial dysfunction [8, 9].

Similar to AER, urinary L-FABP reflects proximal tubular damage and was shown to be as good a predictor of diabetic nephropathy progression as AER [10]. L-FABP is a member of the FABP family of intracellular lipid chaperones that coordinate lipid responses in cells and are strongly linked to metabolic and inflammatory pathways [11, 12]. L-FABP expression is highly increased in cells exposed to NEFAs or hypoxia, where it promotes fatty acid metabolism and acts as a powerful endogenous antioxidant that mitigates the effects of hypoxia and high lipid levels [13, 14]. In tubular cells, L-FABP responds to NEFA overload and to decreased peritubular capillary blood flow [13, 15]. However, these pathological changes reflect more than just tubular injury because NEFA overload depends on the serum NEFA concentrations, while peritubular capillary blood flow is an expression of the general microcirculation and hypoxia. Thus, urinary L-FABP may represent a surrogate marker of general lipid metabolism, microvascular injury and chronic hypoxia [11, 15]. Although there is some suggestion that urinary L-FABP may be related to CVD in individuals with type 2 diabetes and related to mortality in type 1 diabetes, no study has extensively evaluated the role of urinary L-FABP as a predictor of CVD outcomes and mortality in type 1 diabetes [16–19]. Therefore, the aim of our study was to investigate the prognostic value and potential added clinical benefit of urinary L-FABP for prediction of the most important cardiovascular outcomes and mortality in individuals with type 1 diabetes.

Methods

Study participants This study is part of the Finnish Diabetic Nephropathy Study (FinnDiane)—a nationwide cohort of individuals with type 1 diabetes followed prospectively at more than 80 centres. The FinnDiane protocol, described in detail elsewhere, was approved by the local ethics committees at all centres [20]. Individuals recruited to the study provided written informed consent in accordance with the revised Declaration of Helsinki. Those with a previous history of CVD (stroke, coronary artery disease [CAD] or peripheral vascular disease [PVD]) or baseline end-stage renal disease (ESRD) were excluded from the study.

Baseline data and sample collection All relevant medical history and data on cardiovascular endpoints, drug treatment and diabetes complications were collected at enrolment using a standard questionnaire. Anthropometric characteristics and BP were recorded. Blood samples were drawn at baseline. Glycated HbA_{1c} and the following biochemical measurements were measured in fresh serum samples: total cholesterol, HDL-cholesterol, triacylglycerol, creatinine and C-reactive protein. The LDL-cholesterol level was calculated using the Friedewald formula [20]. Based on serum creatinine values, eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula [21]. AER levels were measured in 24 h urine collections. [10]. Additional blood and urine samples were collected at baseline in 1998 and stored at −20°C. In 2008, these additional urine samples were used for L-FABP measurements.

Assays Common biochemical tests were performed using standard laboratory methods [20]. Urinary L-FABP was evaluated in single 24 h urine samples using an Elecsys Immunoassay Kit (Roche Diagnostics, Mannheim, Germany), with a coefficient of variation of <7% and a recovery in serial measurements of ~100 ± 10% [10]. Urinary L-FABP concentrations were normalised to urinary creatinine concentrations.

Follow-up data and ascertainment of outcomes Participants were followed prospectively and information on

cardiovascular outcomes and all-cause mortality were documented until the end of 2014 by linking the FinnDiane data with two high quality registries (the Finnish Hospital Discharge Registry and Finnish Cause of Death Registry), as previously described [22]. Cardiovascular outcomes were documented from the Hospital Discharge Registry using hospital admission, discharge diagnoses and treatment procedure data, based on ICD-8, ICD-9 (www.icd9data.com/2007/Volume1) and ICD-10 (www.who.int/classifications/icd/en/)., together with Nordic Classifications of Surgical Procedures (http://www.nordclass.se/ncsp_e.htm).

CAD was defined as a history of acute myocardial infarction (ICD8 or ICD9 code 410; ICD10 codes I21, I22) or of a coronary artery procedure (bypass grafting surgery or angioplasty). PVD was considered only when a limb amputation or peripheral artery procedure (bypass grafting surgery or angioplasty) was performed. Stroke (ischaemic or haemorrhagic) was considered if the corresponding ICD codes (ICD8 or ICD9 codes 430–434; ICD10 codes I60–I64) were documented in the Hospital Discharge Registry as a history of ischaemic, haemorrhagic or unspecified stroke. CVD events were defined as a composite outcome including all three endpoints (CAD, PVD and stroke). A full description of the cardiovascular outcomes used in the FinnDiane Study is presented elsewhere [23].

Statistical analysis All continuous variables were tested for normal distribution. A generalised extreme Studentised deviate test ($\alpha = 0.05$ and $n_{outliers} < 15$) was used for outlier detection and exclusion. Normally distributed variables are presented as the mean ± SD, while non-normally distributed variables are presented as the median and interquartile range. Comparison between groups was performed using one-way ANOVA or the Kruskal–Wallis test, depending on the distribution of variables. Categorical variables are presented as percentage and compared using the χ^2 test.

Prediction of cardiovascular outcomes and mortality was performed using Cox proportional hazard models only in individuals with first-ever cardiovascular events. When required for the Cox analysis, variables were loge transformed. All HRs were calculated per 1 $\log_e$ unit change in L-FABP concentration.

We first tested urinary L-FABP in a simple Cox analysis, without any adjustment; then we adjusted the Cox analysis with a model comprising well-known traditional predictors of cardiovascular risk (sex, age, diabetes duration, BMI, ever smoking, HDL-cholesterol, triacylglycerol, systolic BP, HbA_{1c} and proliferative retinopathy). Next, eGFR and AER were sequentially included in these models to further test the independence of urinary L-FABP. The Cox model fit was assessed by cumulative Cox–Snell residuals to (−log) Kaplan–Meier estimates and the validity of the model assumption was tested by checking the normal distribution of the residuals, using the D'Agostino–Pearson test [22]. Colinearity was estimated using the variance inflation factor, tolerance and R values. Variance inflation factor values of <10, tolerance values of >0.5 and R values of <0.7 were considered acceptable.

The discrimination ability of urinary L-FABP was compared with those of eGFR or AER was tested using Harrell's C-index. In the statistical analysis, we used the estat concordance package in STATA (version 13, StataCorp, College Station, TX, USA) for postestimation after Cox regression. AUCs were compared using the Delong method [24]. We first compared the receiver operating characteristic (ROC) curves of urinary L-FABP, eGFR and AER alone, and then compared the ROC curve of the model formed by urinary L-FABP with either AER or eGFR with that of L-FABP, AER or eGFR alone. In addition, we compared the ROC curves of urinary L-FABP, LDL-cholesterol, HbA_{1c} and systolic BP.

The added predictive (reclassification) benefit obtained by adding urinary L-FABP to the traditional risk factor models plus eGFR, AER or both was assessed by calculating the net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) using the NRI3 and IDI packages implemented for STATA [25, 26]. For NRI calculation, we used three relevant thresholds (5%, 10% and 20%) for cardiovascular risk decision-making [27]. The same cut-offs were used also for the survival analysis. In addition, we calculated NRI3 and IDI generalised for survival data using the nricens and survIDINRI packages implemented in RStudio (version 1.0.136, Boston, MA, USA).

As we tested urinary L-FABP for predicting five outcomes, Bonferroni correction was applied and a p value for outcome prediction of <0.01 was considered significant ($\alpha_{Bonferroni} = \alpha/m = 0.05/5$, where m = the number of tested hypotheses). For all other tests, a p value of <0.05 was considered statistically significant.

Results

Cohort characteristics The clinical characteristics of all participants are presented in Table 1 and in electronic supplementary material (ESM) Tables 1–5. At baseline, there were 2329 individuals with type 1 diabetes without ESRD or a previous cardiovascular event for whom data on urinary L-FABP concentrations were available. The median follow-up time was 14.1 years (interquartile range 12.5–16.0). The percentages of incident macrovascular complications and mortality increased with the urinary L-FABP quartile at baseline.

Urinary L-FABP and incident CAD events During follow-up, 240 participants developed CAD, and urinary L-FABP concentrations were higher in those who had a CAD event. Urinary L-FABP predicted incident CAD events in the

Table 1 Clinical baseline and prospective data for individuals enrolled in the study, stratified by urinary L-FABP quartile

Variable	L-FABP quartile at baseline				*p*
	1st	2nd	3rd	4th	
L-FABP (μg/μmol)	0.01 (0.00–0.02)	0.03 (0.02–0.04)	0.09 (0.07–0.11)	0.34 (0.19–0.92)	<0.001
Baseline clinical data					
Sex (male/female)	258/302	262/308	295/301	359/244	<0.001
Age (years)	38.2 ± 12.0	37.5 ± 12.1	36.4 ± 12.9	38.2 ± 12.2	0.04
Age of diabetes onset (years)	17.9 ± 9.1	17.2 ± 9.2	16.3 ± 9.6	13.2 ± 8.8	<0.001
Diabetes duration (years)	20.2 ± 12.2	20.2 ± 11.8	20.1 ± 11.8	24.9 ± 10.9	<0.001
BMI (kg/m^2)	25.3 ± 3.6	25.2 ± 3.6	25.1 ± 3.4	25.2 ± 3.9	0.58
WHR					
Men	0.90 ± 0.07	0.89 ± 0.07	0.90 ± 0.07	0.91 ± 0.08	<0.05
Women	0.81 ± 0.06	0.81 ± 0.06	0.81 ± 0.06	0.82 ± 0.07	<0.05
Insulin dose (IU/kg)	0.68 ± 0.24	0.69 ± 0.22	0.74 ± 0.24	0.76 ± 0.25	<0.001
Smoking (%)	41.96	39.9	46.5	53.4	<0.001
Systolic BP (mmHg)	130 ± 16	131 ± 15	131 ± 17	139 ± 19	<0.001
Diastolic BP (mmHg)	79 ± 9	79 ± 9	79 ± 10	81 ± 10	<0.001
HbA_{1c} (%)	8.0 ± 1.2	8.2 ± 1.4	8.6 ± 1.4	9.0 ± 1.6	<0.001
HbA_{1c} (mmol/mol)	63.9 ± 6.0	66.1 ± 7.1	70.5 ± 7.1	74.9 ± 8.1	<0.001
Serum total cholesterol (mmol/l)	4.87 ± 0.84	4.80 ± 0.88	4.86 ± 0.95	5.10 ± 1.11	<0.001
Serum LDL-cholesterol (mmol/l)	2.97 ± 0.77	2.98 ± 0.82	3.02 ± 0.81	3.16 ± 0.88	<0.001
Serum HDL-cholesterol (mmol/l)	1.37 ± 0.37	1.34 ± 0.37	1.32 ± 0.37	1.27 ± 0.38	<0.001
Serum triacylglycerol (mmol/l)	0.94 (0.74–1.24)	0.95 (0.73–1.32)	0.98 (0.77–1.36)	1.19 (0.86–1.75)	<0.001
Urinary AER (mg/24 h)	7 (4–12)	8 (5–15)	10 (7–23)	115 (16–510)	<0.001
eGFR (ml min^{-1} 1.73 m^{-2})	85 (70–105)	87 (71–106)	87 (69–107)	69 (45–97)	<0.001
Serum C-reactive protein (mg/l)	1.76 (1.05–3.53)	1.86 (1.00–3.19)	2.03 (1.26–4.11)	2.40 (1.40–4.89)	<0.001
Diabetic nephropathy (%)[a]	2.1	3.3	6.4	46.0	<0.001
Proliferative retinopathy (%)	16.5	21.1	22.1	45.5	<0.001
Antihypertensive medication (%)	19.7	24.8	25.7	52.3	<0.001
Prospective outcome					
CAD (%)	7.1	7.7	7.9	19.3	<0.001
PVD (%)	9.4	10.4	12.3	30.3	<0.001
Stroke (%)	3.6	3.5	3.7	12.4	<0.001
CVD (%)	9.4	10.4	12.3	30.3	<0.001
Mortality (%)	4.1	7.7	8.7	24.8	<0.001

Categorical data are presented as numbers or percentages (%). Continuous data are presented as means ± SD if they are normally distributed or as median (interquartile range) for non-normally distributed variables. Comparison between groups was performed using one-way ANOVA or the Kruskal–Wallis test depending on the distribution of variables. Categorical variables were compared using the χ^2 test

[a] Individuals with severely increased albuminuria (macroalbuminuria)

unadjusted analysis and also when adjusted for traditional risk factors or eGFR, but not when it further adjusted for AER (Table 2).

Urinary L-FABP and incident PVD Over the study duration, 116 participants developed PVD and L-FABP concentrations were higher in those who experienced a PVD event. Urinary L-FABP predicted an incident PVD event in the unadjusted analysis as well as after adjustment for traditional risk factors or eGFR, but after adjustment for AER (Table 2).

Urinary L-FABP and incident stroke During follow-up, 133 participants suffered a stroke; urinary L-FABP was higher in individuals who suffered a stroke than in those who did not. Urinary L-FABP independently predicted stroke in the unadjusted analysis, as well as after adjustment for traditional risk factors and after further adjustment for eGFR and AER

Table 2 Prediction of cardiovascular outcomes using proportional hazard models with baseline cohort data

Outcome	Variable tested	Unadjusted (univariate)			Adjusted for traditional risk factors			Further adjusted for eGFR			Further adjusted for AER		
		HR	95% CI	*p*	HR	95% CI	*p*	HR	95% CI	*p*	HR	95% CI	*p*
CAD	AER (mg/24 h)	1.41	1.33, 1.49	<0.0001	1.28	1.15, 1.41	<0.0001	1.24	1.11, 1.40	0.0002	NA	NA	NA
	L-FABP (μg/μmol)	1.45	1.35, 1.55	<0.0001	1.21	1.11, 1.32	<0.0001	1.12	1.02, 1.23	0.02	1.00	0.89, 1.12	0.97
PVD	AER (mg/24 h)	1.66	1.53, 1.80	<0.0001	1.43	1.28, 1.59	<0.0001	1.36	1.20, 1.54	<0.0001	NA	NA	NA
	L-FABP (μg/μmol)	1.61	1.48, 1.76	<0.0001	1.30	1.16, 1.45	<0.0001	1.19	1.05, 1.35	0.006	1.10	0.94, 1.29	0.25
Stroke	AER (mg/24 h)	1.45	1.34, 1.56	<0.0001	1.27	1.15, 1.42	<0.0001	1.24	1.11, 1.40	0.0002	NA	NA	NA
	L-FABP (μg/μmol)	1.55	1.42, 1.68	<0.0001	1.33	1.20, 1.49	<0.0001	1.28	1.14, 1.44	<0.0001	1.24	1.06, 1.44	0.009
CVD	AER (mg/24 h)	1.49	1.42, 1.56	<0.0001	1.27	1.19, 1.36	<0.0001	1.23	1.14, 1.32	<0.0001	NA	NA	NA
	L-FABP (μg/μmol)	1.53	1.45, 1.62	<0.0001	1.30	1.21, 1.39	<0.0001	1.23	1.14, 1.32	<0.0001	1.11	1.00, 1.23	0.05
Mortality	AER (mg/24 h)	1.50	1.42, 1.58	<0.0001	1.32	1.23, 1.42	<0.0001	1.28	1.18, 1.39	<0.0001	NA	NA	NA
	L-FABP (μg/μmol)	1.56	1.47, 1.66	<0.0001	1.34	1.24, 1.45	<0.0001	1.29	1.18, 1.39	<0.0001	1.22	1.09, 1.36	0.0003

In this analysis, urinary L-FABP concentrations normalised with urinary creatinine were logarithmically transformed because the values were non-normally distributed; AER values were logarithmically transformed for the same reason. The traditional Cox proportional hazard models for predicting every outcome comprised the following variables: age, sex, diabetes duration, HDL-cholesterol, triacylglycerol, HbA_{1c}, ever smoking, mean systolic BP, BMI and proliferative retinopathy. In addition to this, traditional risk factor further adjustments for eGFR and AER were performed. A full description of the statistical analysis is provided in the "Methods" section

L-FABP, urinary liver-type fatty acid binding protein; NA, not available

(Table 2). There was no difference between the AUC for L-FABP ($AUC_{L\text{-}FABP}$) and the AUC_{eGFR} ($p = 0.94$) or AUC_{AER} ($p = 0.96$), but the AUC for L-FABP was superior to other well-known cardiovascular predictors for predicting incident stroke (ESM Table 6 and ESM Table 7). Notably, when urinary L-FABP was added to the model comprising the traditional risk factors, the increment in AUC was similar to that for AER but significantly different from that for the traditional risk factors ($p = 0.04$). However, there was no increment in the AUC compared with models comprising the AER or eGFR (Table 3). When the NRI and IDI generalised for survival data were calculated, the largest reclassification benefit on top of traditional risk factors was seen at 5 years ($NRI_{3\text{-}S5} = 14.6\%$). However, there was no predictive benefit of adding urinary L-FABP on top of AER and/or eGFR (Table 4).

Urinary L-FABP and incident CVD Throughout the study, a total of 343 individuals had an incident CVD event, and L-FABP concentrations were higher in those who experienced a CVD event. Urinary L-FABP was a predictor of incident CVD events in a univariate analysis and after adjustment for the traditional risk factors or eGFR, but not after adjustment for AER ($p < 0.05$ but not $p < 0.01$, as required by Bonferroni correction).

Urinary L-FABP and mortality During follow-up, there were 269 deaths of any cause. In deceased individuals, the baseline L-FABP concentration was higher compared with survivors. Urinary L-FABP was an independent predictor of death in the unadjusted analysis and when adjusted for traditional risk factors as well as after adjustment for eGFR and AER (Table 2). The $AUC_{L\text{-}FABP}$ was not different from the AUC_{eGFR} ($p = 0.90$) or the AUC_{AER} ($p = 0.72$). After addition of urinary L-FABP to the model comprising traditional risk factors, the increment in AUC was significant ($p = 0.001$) and was similar to that for AER ($p = 0.76$; Table 3). Notably, the AUC for urinary L-FABP was again much better than that for other traditional cardiovascular risk factors (ESM Table 6 and ESM Table 8). When the NRI and IDI generalised for survival data were calculated, there was no added predictive benefit on top of AER or eGFR (Table 4).

Discussion

In this prospective study comprising 2329 individuals, urinary L-FABP was an independent predictor only for incident stroke and mortality. For these outcomes, urinary L-FABP was as good a predictor as AER or eGFR and improved the AUC for both outcomes on top of traditional risk factors, without a reclassification benefit (IDI/NRI) for stroke when AER or eGFR were added to traditional risk factors. However, urinary L-FABP was not an independent predictor of the other tested cardiovascular endpoints (CAD, PVD and overall CVD events) when adjusted for AER, while AER was a predictor for all outcomes except stroke, independently of L-FABP.

The novel finding that urinary L-FABP is an independent predictor of stroke is interesting because until now only a marginal association of a SNP in the *FABP1* gene with stroke

Table 3 ROC curve analysis of the main comparisons between urinary L-FABP and other cardiovascular risk factors

Variable	AUC	95% CI	Differences between AUCs									
			TRF		TRF&eGFR		TRF&AER		TRF&L-FABP		TRF&eGFR&AER	
			Difference	*p*	Difference	*p*	Difference	*p*	Difference	*p*	Difference	*p*
Stroke												
TRF	0.805	0.786, 0.819	–	–	0.005	0.32	0.018	0.005	0.017	0.04	0.019	0.006
TRF&eGFR	0.810	0.788, 0.821	0.005	0.32	–	–	0.014	0.11	0.011	0.17	0.013	0.03
TRF&AER	0.824	0.799, 0.831	0.018	0.005	0.014	0.11	–	–	0.002	0.74	0.001	0.83
TRF&L-FABP	0.822	0.804, 0.839	0.017	0.04	0.011	0.17	−0.002	0.74	–	–	−0.002	0.74
TRF&eGFR&AER	0.824	0.806, 0.841	0.019	0.006	0.013	0.03	0.001	0.83	0.002	0.74	–	–
TRF&eGFR&L-FABP	0.822	0.804, 0.839	–	–	0.012	0.04	–	–	0.002	0.52	−0.002	0.74
TRF&AER&L-FABP	0.826	0.809, 0.843	–	–	–	–	0.003	0.53	0.004	0.13	0.002	0.57
TRF&eGFR&AER&L-FABP	0.826	0.809, 0.843	–	–	0.016	0.03	0.003	0.47	0.005	0.07	0.003	0.50
Mortality												
TRF	0.833	0.815, 0.849	–	–	0.007	0.12	0.015	0.003	0.017	0.001	0.016	0.003
TRF&eGFR	0.840	0.822, 0.856	0.007	0.12	–	–	0.008	0.09	0.010	0.06	0.009	0.03
TRF&AER	0.848	0.831, 0.864	0.015	0.003	0.008	0.09	–	–	0.001	0.76	0.001	0.69
TRF&L-FABP	0.849	0.832, 0.865	0.017	0.001	0.010	0.06	0.001	0.76	–	–	<0.001	0.86
TRF&eGFR&AER	0.849	0.832, 0.864	–	–	0.009	0.03	0.001	0.69	<0.001	0.86	–	–
TRF&eGFR&L-FABP	0.850	0.834, 0.866	–	–	0.010	0.008	–	–	0.001	0.57	0.002	0.62
TRF&AER&L-FABP	0.851	0.835, 0.867	–	–	–	–	0.003	0.19	0.002	0.28	0.003	0.27
TRF&eGFR&AER&L-FABP	0.852	0.835, 0.867	–	–	0.012	0.02	0.004	0.19	0.002	0.28	0.003	0.21

eGFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula. L-FABP and AER relate to urinary concentrations

TRF, traditional risk factors model; TRF&eGFR, Cox model formed using TRF and eGFR together; TRF&AER, Cox model formed using TRF and AER together; TRF&L-FABP, Cox model formed using TRF and L-FABP together; TRF&eGFR&AER, Cox model formed using TRF with eGFR and AER; TRF&eGFR&L-FABP, Cox model formed using TRF with eGFR and L-FABP; TRF&AER&L-FABP, Cox model formed using TRF with AER and L-FABP; TRF&eGFR&AER&L-FABP, Cox model formed using TRF with eGFR, AER and L-FABP

Table 4 Added predictive benefit of urinary L-FABP

Model	$NRI_{3\text{-}lm}$	SE	*p*	$NRI_{3\text{-}S5}$	$NRI_{3\text{-}S10}$	IDI_{lm}	SE	*p*	IDI_{S5}	IDI_{S10}
Stroke										
TRF&L-FABP vs TRF	0.179	0.050	<0.001	0.146	0.073	0.013	0.004	0.001	0.026	0.041
TRF&eGFR vs TRF	0.010	0.040	0.01	0.040	0.061	0.008	0.002	<0.001	0.006	0.005
TRF&AER vs TRF	0.062	0.028	0.03	0.044	−0.132	0.008	0.001	<0.001	0.011	0.022
TRF&L-FABP&eGFR vs TRF&eGFR	0.076	0.033	0.04	−0.156	−0.027	0.007	0.003	0.02	0.020	0.035
TRF&L-FABP&AER vs TRF&AER	0.073	0.032	0.02	−0.057	−0.100	0.005	0.002	0.01	0.011	0.022
TRF&eGFR&AER&L-FABP vs TRF&eGFR&AER	0.045	0.027	0.10	−0.053	−0.101	0.005	0.002	0.01	0.010	0.022
Mortality										
TRF&L-FABP vs TRF	0.074	0.027	0.005	0.203	0.078	0.043	0.007	<0.001	0.036	0.041
TRF&eGFR vs TRF	0.086	0.040	0.03	−0.026	0.056	0.007	0.002	<0.001	0.003	0.005
TRF&AER vs TRF	0.014	0.020	0.47	−0.064	−0.057	0.006	0.002	<0.001	0.004	0.022
TRF&L-FABP&eGFR vs TRF&eGFR	0.064	0.023	0.006	−0.226	−0.051	0.028	0.005	<0.001	0.031	0.035
TRF&L-FABP&AER vs TRF&AER	0.026	0.020	0.20	−0.218	−0.099	0.010	0.003	0.001	0.024	0.022
TRF&eGFR&AER&L-FABP vs TRF&eGFR&AER	0.027	0.019	0.16	−0.239	−0.089	0.009	0.003	0.004	0.023	0.021

L-FABP and AER relate to urinary concentrations

$NRI_{3\text{-}lm}$, NRI with three thresholds for the logistic model; $NRI_{3\text{-}S5}$, NRI with three thresholds generalised for survival data (calculated for 5 year survival); $NRI_{3\text{-}S10}$, NRI with three thresholds generalised for survival data (calculated for 10 year survival); IDI_{S5}, IDI generalised for survival data (calculated for 5 year survival); IDI_{S10}, IDI generalised for survival data (calculated for 10 year survival); IDI_{lm}, IDI for logistic model; TRF, traditional risk factors model; TRF&eGFR, Cox model formed using TRF and eGFR together; TRF&AER, Cox model formed using TRF and AER together; TRF&L-FABP, Cox model formed using TRF and L-FABP together; TRF&eGFR&AER, Cox model formed using TRF with eGFR and AER; TRF&eGFR&L-FABP, Cox model formed using TRF with eGFR and L-FABP; TRF&AER&L-FABP, Cox model formed using TRF with AER and L-FABP; TRF&eGFR&AER&L-FABP, Cox model formed using TRF with eGFR, AER and L-FABP

has been reported in a single study [28]. In our study, urinary L-FABP predicted stroke independently not only of known risk factors such as age, sex, BMI, diabetes duration, systolic BP, triacylglycerol, HDL-cholesterol, glycaemic control and proliferative diabetic retinopathy but also of eGFR and AER. The potential clinical value of this finding is emphasised by the finding that urinary L-FABP was as good a predictor of stroke as eGFR or AER and better than the traditional risk factors in the ROC curve analysis. When NRI and IDI were calculated, urinary L-FABP did not have an added reclassification benefit to the long-term prediction of stroke on top of eGFR and AER. However, when added to the model of traditional risk factors, urinary L-FABP correctly reclassified 17.9% of participants (compared with 6% or 10% provided by AER or eGFR) when NRI was calculated.

Another important finding was that urinary L-FABP is an independent predictor of death. This was previously suggested by another study that was unfortunately not sufficiently powered to adjust for other risk factors, thus leaving the question open [18]. In our study, urinary L-FABP predicted mortality independently of the most important traditional risk factors and after additional adjustment for eGFR and AER. As in the case of stroke, urinary L-FABP was as good as eGFR or AER in predicting death. When added to the traditional risk factors, urinary L-FABP correctly reclassified about 7.5% of participants compared with 8.6% and 1.4% for eGFR and AER. Since urinary L-FABP predicted only stroke and mortality but not CAD, PVD or total CVD (i.e. two out of five outcomes tested), one can argue that these findings are just a statistical anomaly. However, the most rigorous statistical testing with Bonferroni correction was used for all outcomes. In addition, the results were obtained using the most extended statistical model for adjustment that contained all well-known risk factors for cardiovascular outcomes. Another potential reason for the differential association of CAD or PVD and stroke with urinary L-FABP may be related to how these two outcomes are defined. However, the same definition of outcomes was previously demonstrated to be reliable, while data collection relied on high quality registries and medical documents.

With regard to the other cardiovascular endpoints, it is notable that urinary L-FABP predicted CAD, PVD and CVD independently of the traditional risk factors model and eGFR, but not independently of AER. One interpretation of these results could be that the actions of urinary L-FABP on these cardiovascular endpoints are independent of kidney function but not of endothelial dysfunction. Previous reports suggested that urinary L-FABP is a good discriminator between patients with acute coronary syndrome and those with stable angina, as well as a good predictor of future coronary restenosis after a first acute coronary event [29, 30]. It is possible that the most important triggers of L-FABP expression (hypoxia and NEFA concentration) play smaller roles in the chronic processes that lead to CAD or PVD compared with

acute coronary syndromes, while AER and endothelial dysfunction are more important in these chronic states [15].

From the biological point of view, these independent predictive abilities of urinary L-FABP for stroke but not for the other cardiovascular endpoints are very interesting because NEFAs are metabolised by FABP proteins in all organs and new data have linked NEFAs to BP regulation [31]. However, these results may suggest a new mechanism of L-FABP action on stroke because, when adjusted for AER or BP, urinary L-FABP still predicted stroke but not other cardiovascular outcomes. Since L-FABP is not expressed in the brain but is expressed in proximal tubular cells, increased urinary L-FABP in the case of stroke prediction may represent tubular injury. Mechanisms proposed to explain the increase in L-FABP expression in the proximal tubular cells are NEFA overload, oxidative stress or toxic insults [32]. Thus, NEFA overload of the proximal tubule could mimic the effect of fatty acids on the progression of atherosclerosis in the carotid arteries. Other possible mechanisms linking the brain or carotid atherosclerosis with L-FABP could be oxidised LDL-cholesterol or other modified lipoproteins. This possibility is supported by the role of these lipoproteins in stroke development and tubular dysfunction [33]. In addition, this is based on the similar behaviour of tubular and endothelial cells in response to oxidised LDL-cholesterol exposure as well as on the positive effect of statin therapy toward tubular dysfunction and urinary L-FABP concentrations [34, 35]. Furthermore, local expression of L-FABP in the kidney can be triggered by hypoxia due to decrease peritubular capillary blood flow, which could be a proxy for the brain microcirculation status [15]. Moreover, we cannot exclude the possibility of crosstalk between the brain and kidney through B-type natriuretic peptide, which also predicts stroke and is supposed to increase diuresis and to be cleared by the kidney. Alternatively, there may be a feedback mechanism between L-FABP and other FABPs or signalling molecules expressed in hypoxic areas of the brain [36, 37]. However, it is beyond the scope of this study to further explore these potential underlying mechanisms. Therefore, the possible existence of a specific brain–kidney axis must be elucidated in future studies.

The strength of this study was the large multicentre cohort representative of the Finnish population with type 1 diabetes recruited from 80 centres in Finland. In addition, the study benefited from well-characterised phenotypes, long follow-up and high quality registry data on outcomes [22]. One potential limitation of the study may be that only a single urinary L-FABP measurement was included, although large variability in urinary L-FABP concentrations has never been reported. Another potential limitation could be the lack of data regarding anaemia, since anaemia may influence L-FABP expression. However, this was not an issue in this study because all individuals with ESRD were excluded at baseline and no member of the cohort received treatment with erythropoietin.

In summary, this large prospective study of individuals with type 1 diabetes showed that urinary L-FABP is an independent predictor of stroke and mortality, but did not have an additional predictive benefit over AER and eGFR.

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Data availability The dataset is not publicly available for reasons of patient confidentiality. Please contact the authors for more information.

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Contribution statement P-HG, CF, AB, PMH, MS and NMP conceived and designed the study and designed the data analysis; CF, MS, LMT, DG, NE, VH and P-HG collected and followed up the data; NMP researched data, performed statistical analyses and wrote the manuscript; and CF, MS, LMT, DG, NE, VH, PMH and P-HG researched data, contributed to discussions, and reviewed and edited the manuscript. P-HG is the guarantor of this work and gave final approval for the decision to publish.

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