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Causal association pathways between fetuin-A and kidney function: a mediation analysis

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Abstract

Objective: Body mass index (BMI), uric acid, diabetes mellitus, and hypertension are risk factors for reduced kidney function and are associated with fetuin-A levels, but their causal pathways remain unclear. The objective of this study was to investigate this knowledge gap.

Methods: A repeated cross-sectional design was used to assess causal pathway effects of fetuin-A on the estimated glomerular filtration rate (eGFR), which is mediated through BMI, uric acid, diabetes mellitus, and hypertension.

Results: Among 2305 participants, the mean eGFR at baseline decreased from 98.7 ± 23.6 mL/minute/1.73 m^2 in 2009 to 92.4 ± 22.9 mL/minute/1.73 m^2 in 2014. Fetuin-A was significantly associated with eGFR , suggesting that increasing fetuin-A levels predict a decrease in eGFR. Additionally, the indirect effect of fetuin-A on eGFR, as assessed through BMI, was also significant. The effects of fetuin-A on eGFR through other mediation pathways showed variable results.

Conclusions: Our study revealed a possible role of fetuin-A in the etiology of declining renal function through mediating body mass index, uric acid, diabetes mellitus, and hypertension via complex causal pathways. Further studies to clarify these mediated effects are recommended.

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Introduction

Chronic kidney disease (CKD) is an irreversible progressive deterioration in renal function[1] that leads to renal insufficiency.[2,3] CKD is a global public health problem associated with an increasing number of patients with renal failure.[4] The report of the 2015 Global Burden of Disease Study indicated that kidney disease was the 12th most common cause of death, which accounted for 1.1 million deaths worldwide.[4] Known risk factors for decreased kidney function have been reported including body mass index (BMI),[5] uric acid (UA),[6] diabetes mellitus (DM),[7] and hypertension (HT).[8] CKD can be characterized by inflammation markers including high-sensitivity C-reactive protein (hsCRP),[9] interleukin-1 (IL-1), endothelin-1 (ET -1), and tumor necrosis factor-α (TNF-α).[10] These markers were found to be associated with a decline in the estimated glomerular filtration rate (eGFR). Additionally, recent evidence from observational studies has shown that low serum fetuin-A serum levels are also associated with CKD morbidity[11] and mortality.[12]

Fetuin-A, also known as alpha 2-Heremans–Schmid glycoprotein (AHSG), is a phosphorylated glycoprotein that has been identified in both animals and humans.[13] The human homolog of fetuin-A consists of 349 amino acids, and fetuin-A comprises a long A chain (282 amino acids) and a short B chain (27 amino acids), which are connected by a short 40-amino acid peptide link.[14] The serum fetuin-A concentration ranges between 0.5 and 1.0 g/L, and its molecular weight varies between 52 and 60 kDa.[14,15]

As a multifunctional protein, fetuin-A modulates some important processes related to energy homeostasis, adipocyte metabolism, cell growth, and inflammation.[16–18] It plays important roles in CKD risk factors such as BMI,[19] DM,[20] HT,[21] and coronary artery disease through different mechanisms.[22–25]

The causal pathways of these disease phenotypes in CKD etiology are complex and not fully understood. Fetuin-A might be directly associated with decreased eGFR, or it might affect eGFR through causal pathways that are mediated by these risk factors (BMI, UA, DM, and HT). On the basis of fetuin-A's association with the above-mentioned disease phenotypes, we conducted a study using data from a prospective cohort from the Electricity Generating Authority of Thailand (EGAT)[26] to explore the causal association pathways of these diseases with fetuin-A and its relationship with declining kidney function.

Materials and methods

Setting

We used cross-sectional data from the EGAT prospective cohort[26] that included 2564 participants who were recruited in 2009 and had their first 5-year follow-up assessment in 2014. The cohort was

designed to assess risk factors for cardiovascular diseases (CVD), health status, functional status, and health-related quality of life. All the participants aged 18 years or older provided written informed consent to voluntarily participate in the cohort study. The participants completed a self-administered questionnaire about their lifestyle behavior and family history of disease, and they underwent baseline medical examinations including laboratory tests. The study was approved by the Institutional Review Board of Ramathibodi Hospital, Bangkok, Thailand Ethical Committee (approval number MURA 2018/932, approved on 28 November 2018).

The main study factor, serum fetuin-A, was assessed using specimens that were collected during the survey in 2009 and analyzed using a sandwich enzyme immunoassay (R&D Systems, Inc., Minneapolis, MN, USA). The intra- and inter-assay precision was 4.9% and 7.3%, respectively.[19] Our clinical outcome of interest was eGFR, which was calculated using the CKD-EPI Creatinine Equation (2009),[27] as well as serum creatinine, age, and sex parameters. Our intermediate outcomes BMI, UA, DM, and HT were considered to be mediators. The outcome and mediators were measured during the surveys in 2009 and 2014. BMI was calculated as weight (kg)/height (m^2). UA was assessed using spectrophotometric absorption after treating the specimen with the enzyme uricase. The normal range for UA was 2.5 to 7.5 mg/dL for women and 4.0 to 8.5 mg/dL for men. DM was diagnosed if the fasting blood glucose level was $\geq$7.0 mmol/L (or $\geq$126 mg/dL) with or without a history of DM or the use of an antidiabetic medication. Participants were considered to have HT if they took any antihypertensive medications or had a systolic blood pressure (SBP) $\geq$140 mmHg or a diastolic blood pressure (DBP) $\geq$90 mmHg. Blood pressure was measured twice, 5 minutes apart with a 5-minute rest period after the first measurement. Covariables that were considered included demographic variables (i.e., age and sex), smoking history, alcohol consumption, and triglyceride and low-density lipoprotein (LDL) levels.

Statistical analysis

Baseline and follow-up demographic variables were analyzed and reported as the mean $\pm$ standard deviation (SD) for continuous data or as the frequency and percentage for categorical data. Multiple mediation analysis was performed on the basis of the causal association pathways (Figure 1), in which fetuin-A was considered as the independent variable, while BMI, UA, DM, and HT were mediators and eGFR was the outcome. All possible serial multiple mediation models were constructed (Supplementary Table 1).

Mediation and outcome models were constructed by fitting fetuin-A on each of the four mediators (i.e., BMI, UA, DM, and HT) using generalized linear structural equation models (GSEMs) with a logit link for DM and HT and an identity link for BMI, UA, and eGFR.

A univariate GSEM model was used to screen the covariables (i.e., age, sex, smoking, alcohol, triglycerides, and LDL) that might be associated with each mediator (i.e., BMI, UA, DM, and HT). Forward selection was applied to identify significant variables in the mediation and outcome models that already contained fetuin-A. Mediated effects were then estimated using the product coefficients of each pathway (Supplementary Table 1). Finally, bias was corrected using bootstrapping with 1000 replications to estimate the average mediation effects.[28] All analyses were performed using STATA (version 15; StataCorp, College Station, TX, USA),[29] and a p-value $<$0.05 was considered statistically

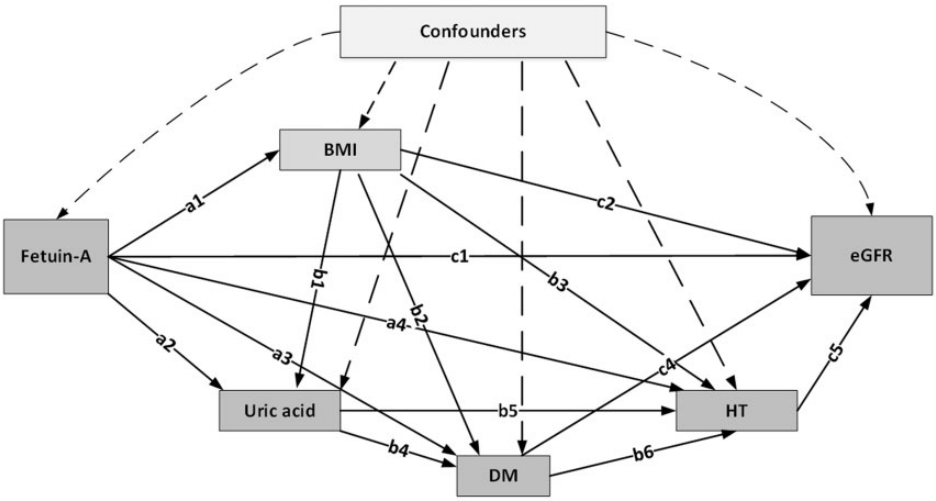

Figure 1. Causal association pathways between fetuin-A and eGFR.
The letter and number on the lines represent corresponding coefficients for the pathway (Supplementary Table 1).
BMI, body mass index; DM, diabetes mellitus; HT, hypertension; eGFR, estimated glomerular filtration rate.

significant. The reporting of this study complied with STROBE guidelines.[30]

Results

Baseline characteristics are described in Table 1. There were 2564 participants enrolled in 2009, but only 2305 participants were available at the follow-up survey in 2014. Among these 2305 participants, mean age, BMI, UA, and eGFR at baseline were 46 ± 7 years, $24.8 \pm 3.8\,kg/m^2$, $5.6 \pm 1.5\,mg/dL$, and $98.7 \pm 23.6\,mL/minute/1.73\,m^2$, respectively, and the prevalence of DM and HT was 4.6% and 22.6%.

The mean eGFR at baseline decreased from $98.7 \pm 23.6\,mL/minute/1.73\,m^2$ in 2009 to $92.4 \pm 22.9\,mL/minute/1.73\,m^2$ in 2014, while the mean BMI increased marginally from $24.0 \pm 3.7\,kg/m^2$ at baseline to $24.8 \pm 3.6\,kg/m^2$ in the follow-up period (Table 1).

The univariate GSEM analysis showed that fetuin-A was significantly associated with all mediators (BMI, UA, DM, and HT) and the eGFR outcome (Supplemental Table 2). Additionally, all covariables including age, sex, smoking, alcohol, triglycerides, and LDL were also significantly associated with each mediator and eGFR. The multivariate GSEMs constructed included all six covariables for the BMI, UA, DM, HT, and eGFR models (Table 2). After adjusting for the covariables, fetuin-A was significantly associated with only BMI and DM but not with UA and HT. Additionally, fetuin-A was also significantly associated with eGFR.

Bootstrapping with 1000 replications was applied to estimate the average causal mediation effects (ACMEs; Table 3). Most fetuin-A effects on eGFR through the BMI pathway were significant. For example, ACME of the fetuin-A→BMI→eGFR pathway was 0.000864 (0.00025, 0.00163), meaning that increasing fetuin-A by one unit would increase the BMI, which could be predictive of an increase in eGFR by $0.000864\,mL/minute/1.73\,m^2$. The indirect effects of fetuin-A through BMI and UA

Table 1. Participant baseline characteristics.

Characteristics	Survey 2009	Survey 2014
Number of participants	2564	2305
Age, years, mean (SD)	41 ± 7	46 ± 7
Sex, number (%)		
Male	1882 (73.4)	1656 (71.8)
Female	682 (26.6)	649 (28.2)
Marital Status, number (%)		
Single	745 (29.1)	461 (20.0)
Married	1705 (66.5)	1641 (71.2)
Divorced/Widowed	114 (4.4)	203 (8.8)
Income, Baht/month, number (%)		
<20,000	130 (5.1)	–
20,000–49,999	970 (38.1)	–
≥50,000	1446 (56.8)	–
Education, number (%)		
≤Secondary school	115 (4.5)	75 (3.3)
Vocational	584 (22.8)	473 (20.5)
≥Bachelors	1865 (72.7)	1757 (76.2)
Smoking, number (%)		
Never smoker	1683 (65.7)	1499 (65.0)
Ex-smoker	450 (17.6)	475 (20.6)
Current smoker	428 (16.7)	331 (14.4)
Alcohol, number (%)		
Never drinkers	981(39.1)	906 (39.3)
Quit drinkers	171 (6.8)	–
Current drinkers	1358 (54.1)	1399 (60.7)
Exercise, times/week, number (%)		
None	1503 (58.7)	743 (32.2)
1–2	406 (15.8)	446 (19.4)
≥3	652 (25.5)	1116 (48.4)
Diabetes		
Yes	117 (4.6)	236 (10.2)
No	2440 (95.4)	2069 (89.8)
Hypertension		
Yes	500 (22.6)	809 (35.1)
No	1984 (77.4)	1496 (64.9)
BMI, kg/m^2, mean (SD)	24.0 ± 3.7	24.8 ± 3.8
Fetuin-A, mg/dL, mean (SD)	558.9 ± 110.5	–
Uric acid, mg/dL, mean (SD)	5.6 ± 1.5	5.8 ± 1.5
Cholesterol, mg/dL, mean (SD)	216.8 ± 39.3	217.4 ± 40.3
HDL, mg/dL, mean (SD)	51.7 ± 12.3	58.3 ± 15.5
LDL, mg/dL, mean (SD)	148.4 ± 36.9	148.6 ± 37.7
Triglyceride, mg/dL, mean (SD)	129.1 ± 90	131.8 ± 84.1
eGFR, mL/minute/1.73 m^2, mean (SD)	98.7 ± 23.6	92.4 ± 22.9

SD, standard deviation; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate.

Table 2. Causal associations between fetuin-A and eGFR: multivariate GSEM models.

Equation	Factors	b	SE	z	p	95% CI
Fetuin-A → BMI	Fetuin-A	0.0039	0.0006	7.12	<0.001	0.0029, 0.0051
	Age	0.0368	0.0088	4.17	<0.001	0.0195, 0.0541
	Male vs. Female	1.4058	0.1549	9.08	<0.001	1.1022, 1.7093
	Smoking					
	Ex-smoker	0.4306	0.1718	2.51	0.012	0.0939, 0.7674
	Current smoker	–0.3115	0.1890	–1.65	0.099	–0.6820, 0.0589
	Alcohol drinking					
	Ex-drinker	0.5371	0.2417	2.22	0.026	0.0634, 1.0108
	Current drinker	0.3329	0.1463	2.28	0.023	0.0463, 0.6196
	Triglycerides	0.0109	0.0008	14.19	<0.001	0.0094, 0.0124
	LDL	0.0062	0.0017	3.66	<0.001	0.0029, 0.0096
Fetuin-A → Uric acid	Fetuin-A	0.0002	0.0002	1.29	0.196	−0.0001, 0.0006
	BMI	0.0849	0.0055	15.51	<0.001	0.0742, 0.0957
	Male vs. Female	1.5952	0.0493	32.33	<0.001	1.4984, 1.6919
	Smoking					
	Ex-smoker	–0.0095	0.0543	–0.17	0.862	–0.1159, 0.0969
	Current smoker	–0.1707	0.0597	–2.86	0.004	–0.2877, –0.0537
	Alcohol drinking					
	Ex-drinker	0.0859	0.0764	1.13	0.261	–0.0638, 0.2356
	Current drinker	0.2249	0.0462	4.87	<0.001	0.1343, 0.3155
	Triglycerides	0.0025	0.0002	10.19	<0.001	0.0020, 0.0030
Fetuin-A → DM	Fetuin-A	0.0015	0.0005	2.75	0.006	0.0004, 0.0026
	BMI	0.1870	0.0159	11.71	<0.001	0.1557, 0.2183
	Uric acid	–0.2350	0.0527	–4.46	<0.001	–0.3383, –0.1317
	Age	0.0639	0.0092	6.96	<0.001	0.0459, 0.0820
	Male vs. Female	0.5310	0.1725	3.08	0.002	0.1930, 0.8690
	Triglycerides	–0.0102	0.0017	–5.86	<0.001	–0.0136, –0.0068
	LDL	0.0025	0.0006	4.03	<0.001	0.0013, 0.0037
Fetuin-A → HT	Fetuin-A	0.0008	0.0004	1.89	0.059	–0.00003, 0.0016
	BMI	0.1389	0.0132	10.54	<0.001	0.1131, 0.1647
	Uric acid	0.1759	0.03333	5.28	<0.001	0.1106, 0.2413
	DM	0.5955	0.1338	4.45	<0.001	0.3333, 0.8576
	Age	0.0919	0.0069	13.39	<0.001	0.0784, 0.1053
	Triglycerides	–0.0066	0.0013	–5.28	<0.001	–0.0091, –0.0042
	LDL	0.0031	0.0005	5.71	<0.001	0.0020, 0.0042
Fetuin-A → BMI → UA → DM → HT →eGFR	Fetuin-A	–0.0077	0.0022	–3.49	<0.001	–0.0119, –0.0034
	BMI	0.1889	0.0724	2.61	0.009	0.0471, 0.3308
	Uric acid	–3.9997	0.1866	–21.43	<0.001	–4.3655, –3.6339
	DM	–0.3346	0.8087	–0.41	0.679	–1.9196, 1.2503
	HT	–2.6717	0.5706	–4.68	<0.001	–3.7901, –1.5534
	Alcohol drinking					
	Ex-drinker	–5.1275	0.9180	–5.59	<0.001	–6.9268, –3.3283
	Current drinker	–1.2136	0.5342	–2.27	0.023	–2.2606, –0.1665
	Triglycerides	0.0077	0.0031	2.50	0.012	0.0017, 0.0138

SE, standard error; 95% CI, 95% confidence interval; LDL, low-density lipoprotein; BMI, body mass index; DM, diabetes mellitus; HT, hypertension.

Table 3. Estimation of average causal mediation effects using bootstrapping.

Paths	Effect	SE	Z	P-value	95% CI
Fetuin-A → eGFR (direct effect)	−0.00721	0.00260	−2.77	0.006	−0.01190, −0.00247
BMI-mediator					
Fetuin-A → BMI → eGFR	0.000864	0.000353	2.45	0.014	0.00025, 0.00163
Fetuin-A → BMI → UA → eGFR	−0.00132	0.00022	−5.99	<0.001	−0.00177, −0.00092
Fetuin-A → BMI → DM → eGFR	−0.000165	0.000674	−0.24	0.807	−0.00149, 0.00116
Fetuin-A → BMI → HT →eGFR	−0.00139	0.000416	−3.35	0.001	−0.00237, −0.00069
Fetuin-A → BMI → UA → DM → eGFR	0.000018	0.000076	0.23	0.817	−0.00005, 0.00028
Fetuin-A → BMI → UA → HT →eGFR	−0.00015	0.000054	−2.77	0.006	−0.00028, −0.00007
Fetuin-A → BMI → UA → DM → HT →eGFR	0.000119	0.000055	2.16	0.031	0.00005, 0.00028
UA mediator					
Fetuin-A → UA → eGFR	−0.00089	0.00067	−1.35	0.177	−0.00216, 0.00039
Fetuin-A → UA → DM → eGFR	0.00001	0.00007	0.17	0.863	−0.00006, 0.00024
Fetuin-A → UA → HT →eGFR	−0.00010	0.00009	−1.18	0.237	−0.00031, 0.00003
Fetuin-A → UA → DM → HT →eGFR	0.00008	0.00007	1.11	0.269	−0.00002, 0.00032
DM mediator					
Fetuin-A → DM → eGFR	−0.00033	0.00139	−0.24	0.812	−0.00332, 0.00230
Fetuin-A → DM → HT →eGFR	−0.00223	0.00113	−1.97	0.049	−0.00535, −0.00066
HT mediator					
Fetuin-A → HT →eGFR	−0.00192	0.00118	−1.64	0.102	−0.00459, 0.00008

SE, standard error; 95% CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; BMI, body mass index; UA, uric acid; DM, diabetes mellitus; HT, hypertension.

(fetuin-A → BMI → UA → eGFR) and then through HT (fetuin-A → BMI → HT → eGFR) showed significantly negative effects, which means that the eGFR was lowered by −0.00132 (−0.00177, −0.00092) and −0.00139 (−0.00237, −0.00069) for an ACME associated with BMI and UA or HT, respectively. Additionally, the fetuin-A → DM → HT → eGFR pathway was also statistically significant, with an ACME of −0.00223 (−0.00535, −0.00066). The other three single-mediator pathways fetuin-A → UA, fetuin-A → DM, and fetuin-A → HT were not statistically significant.

Discussion and conclusions

We explored the causal pathway for fetuin-A and kidney function through BMI, UA, DM, and HT using multiple mediation analysis. The result of our mediation analysis indicated that fetuin-A was negatively related to eGFR in the direct fetuin-A → eGFR pathway, which means that lower fetuin-A levels are predictive of declining eGFR or poor kidney function. This is in agreement with epidemiological studies,[9,11,31] which showed that low fetuin-A levels are associated with declining kidney function.

Cottone and colleagues[11] noted that the functions and regulatory mechanisms of fetuin-A are complex and may seem to differ on the basis of the pathophysiologic characteristics of the population being studied. Some studies have demonstrated that inflammatory processes are increased in CKD, even in the early stages of CKD, and that the inflammatory processes triggered by inflammatory markers such as CRP and adiponectin are linked to endothelial dysfunction (ED).[11,32] Moreover, the deleterious pro-oxidative effects of UA can worsen endothelial function.[33,34]

Human fetuin-A, also known as AHSG, is encoded by the AHSG gene, which is located on chromosome 3 (3q27),[35] and it is mainly secreted by hepatocytes and adipose tissues. As a multi-functional circulating glycoprotein, fetuin-A has both pro-inflammatory and anti-inflammatory functions as well as anti-calcifying properties.[36] Thus, its effects may sometimes appear contradictory in different body systems.[37] As an acute phase anti-inflammatory protein, fetuin-A acts as an acute phase reactant in the extra-cellular space to attenuate inflammatory responses. Therefore, in patients with early stages of kidney disease, fetuin-A levels may be normal or slightly elevated. However, when the inflammatory process is prolonged, pro-inflammatory cytokines such as CRP, downregulate or inhibit fetuin-A synthesis, thereby attenuating the protective effect of fetuin-A.[38] This may explain the low levels of circulating fetuin-A, which is observed in CKD. Moreover, although not consistently demonstrated, variations in fetuin-A levels may also be determined by genetic polymorphisms independent of inflammation.[39]

Previous evidence showed higher fetuin-A levels in obese compared with non-obese people regardless of their DM status,[40] and this relationship may be explained through several mechanisms including generating insulin resistance through the endogenous inhibition of the insulin receptor tyrosine kinase,[20,41] reducing hepatic insulin sensitivity,[42] and inhibiting glucose transporter type 4 translocation, glucose uptake, and glycogen synthesis in skeletal muscle cells.[43,44] Additionally, fetuin-A might reduce peroxisome proliferator-activated receptor γ and adiponectin release through a mechanism that causes disruption of the silent information regulator 1 and AMP-activated protein kinase sensors, which play essential roles in adipocyte metabolism.[18]

Being overweight or obese is a known risk factor for CVD[45] and declining kidney function.[46] Additionally, overweight or obesity are highly associated with other CVD risks such as UA,[47] DM,[48] and HT.[49] For our EGAT cohort, the indirect effect of fetuin-A on eGFR that was mediated through BMI, i.e., the fetuin-A $\rightarrow$ BMI $\rightarrow$ eGFR pathway, was significant, and it had a coefficient of 0.00086 (0.00025, 0.0016). This suggests that for every one unit increase in BMI that results from increasing fetuin-A, eGFR is significantly increased by 0.00086 mL/minute/1.73 m^2.

Moreover, obesity is associated with several major CKD risk factors such as DM, HT, and atherosclerosis,[49,50] and evidence from both epidemiological and pathological investigations have shown that obesity invariably contributes to CKD directly or indirectly.[46,51] Subjects with severe obesity have been found to develop proteinuria with evidence of renal pathologic changes such as podocyte hypertrophy, mesangial expansion, glomerular enlargement, and focal segmental glomerular sclerosis even in the absence of DM and HT.[51] It is hypothesized that obesity contributes to CKD development through different mechanistic pathways that include glomerular hyperfiltration, activation of the renin–angiotensin system, insulin resistance, and direct lipotoxicity.[52]

There has been no general consensus on the relationship between BMI and fetuin-A, and in our EGAT study population, we recorded only a slight increase in the mean BMI from a baseline value of 24.0 ± 3.7 kg/m^2 to 24.8 ± 3.8 kg/m^2 at the 5-year follow up survey. Thus, our findings, which are specific to our EGAT cohort, are consistent with findings by Stefan et al.[53] and Kaushik et al.[54] This may result from genetic factors or other socio-demographic variables that we may not have explored in our study. It is equally plausible that the marginal

increase in BMI over the 5-year follow-up period is not sufficient to cause a significant decline in kidney function, but rather, it shows that BMI in our context may play a protective role instead. This requires further exploration with a longer follow-up period for our cohort.

The result of our mediation analysis involving the BMI, UA, and HT pathways showed a significant effect of fetuin-A on eGFR through the fetuin-A → BMI → UA → eGFR and fetuin-A → BMI → HT → eGFR pathways, respectively. This suggests that for the fetuin-A → BMI → UA → eGFR pathway, every unit of fetuin-A increase would increase the BMI and UA risk resulting in a decrease in the eGFR of 0.00132 mL/minute/1.73 m^2. Similarly, for the fetuin-A → BMI → HT → eGFR pathway, increasing fetuin-A would increase the BMI and HT risk, causing a decrease in the eGFR of 0.00139 mL/minute/1.73 m^2. The effects of fetuin-A could also be mediated through DM and HT, causing a decrease in the eGFR.

DM and HT are comorbid conditions that are frequently associated with poor kidney function.[55] The role of DM in the pathogenesis of kidney disease has been established by epidemiological studies, which showed that older subjects with a longer duration of DM have a higher risk of developing CKD and that approximately 40% of patients with DM developed impaired kidney function, albuminuria, or both.[56]

Fetuin-A has been implicated in the vascular inflammatory processes related to the etiology of complex diseases such as CVDs[57] and DM.[20] Although some studies have been conducted to assess the relationship of fetuin-A with CKD morbidity and progression,[11] and mortality,[58] its role in the etiology of kidney disease remains unclear.

Some observational studies have shown that increasing fetuin-A levels are associated with both improvements in the CKD status[59,60] and ED.[31] ED is considered to be a major causal pathological mechanism of CKD.[11,61] ED has also been implicated in the pathophysiology of different forms of complex phenotypes such as HT/coronary artery disease,[62] DM,[63] and CKD,[59] which might be associated with the ED vascular inflammatory processes. Additionally, fetuin-A levels were significantly increased after kidney transplantation compared with the pre-transplantation levels,[64] but this was not found in the study by Schaible et al.[65] Further study is required to explore a causal association between fetuin-A, ED, and CKD.

Our study has some strengths. We assessed both the direct causal effect of fetuin-A on kidney function and the effects that were mediated through known risk factors of kidney function including BMI, UA, DM, and HT. We applied a multiple-mediation analysis to determine possible causal pathways and effects of fetuin-A on eGFR. We used the EGAT cohort to demonstrate the causal pathways that fetuin-A could have on kidney function through multiple mediator pathways, adjusting for covariables, which were obtained during the baseline and follow-up visits.

A few limitations of this study should be noted. We used longitudinal data from the EGAT cohort, but fetuin-A was measured only once because of budget limitations. We considered intermediate mediators and also used eGFR as a surrogate outcome of declining kidney function instead of end-clinical outcomes because the 2009 EGAT cohort had been followed-up for only 5 years (i.e., the first follow-up visit). We assessed a causal pathway of fetuin-A and eGFR on the basis of data from 2305 participants in the EGAT cohort. We did not calculate the sample size before the study, but we used a rule-of-thumb of 30 participants for each predictor and for each causal

pathway. Thus, at least 450 participants were required for at least 15 predictors that were included in each causal equation. However, a causal effect was a product of coefficients that required a large sample size to detect a causal effect through each pathway. For example, over 10 million participants may be required to detect a causal effect of 0.000864 via the fetuin-A → BMI → eGFR pathway to achieve 80% power of a test, given a type I error of 0.05 and eight predictors in the equation.[66] The sample size in our study (2305 participants) can be justified because our study was not intended to detect a causal association, but was rather, it aimed to estimate a causal effect.[67] Last, other factors such as nutritional intake and exercise might influence the serum fetuin-A level,[68] but we did not consider them because of a lack of data.

In conclusion, fetuin-A might have a direct effect on declining kidney function, where increasing the serum fetuin-A might reduce the kidney function. Additionally, its effects might be mediated through a BMI mediator, which might increase the eGFR, or alternatively, it may decrease the eGFR through the UA, DM, and HT mediators. However, these findings need to be further assessed in a cohort with a longer follow-up period.

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Supplemental material

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