Potential contribution of monounsaturated fatty acids to cardiovascular risk in chronic kidney disease

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KEY WORDS

ABSTRACT

INTRODUCTION   Patients with chronic kidney disease (CKD) are particularly susceptible to cardiovascular disease (CVD). Increased synthesis of endogenous monounsaturated fatty acids (MUFAs) by stearoyl-
-CoA desaturase-1 (SCD1) might predispose to cardiovascular complications.
OBJECTIVES   The aim of this study was to examine the serum MUFA content in patients at subsequent stages of CKD, and to evaluate associations between MUFA content and SCD1 activity, patients' diet, and cardiovascular risk.
PATIENTS AND METHODS   Serum fatty acid composition was evaluated in 177 patients with subsequent stages of CKD (1–2, 3a, 3b, 4–5, hemodialysis, peritoneal dialysis, and after kidney transplantation), and in 30 healthy controls. Gas chromatography–mass spectrometry was used for the measurement.
RESULTS   Serum MUFA content was shown to increase with subsequent stages of CKD and to be correlated with various risk factors of CVD, including serum triacylglycerols, HDL cholesterol ($P < 0.01$), and high-sensitivity C-reactive protein ($P < 0.05$). Moreover, also the prevalence of CVD was shown to increase with CKD progression. Estimated SCD1 activity was associated with serum MUFA content ($P < 0.01$), but no association was found between dietary MUFA intake and serum MUFA levels.
CONCLUSIONS   Our results indicate that the elevation of serum MUFA levels in CKD patients may contribute to an increased risk of CVD during CKD progression, mainly due to increased endogenous MUFA synthesis by SCD1.

INTRODUCTION   During the course of chronic kidney disease (CKD), patients are affected by progressive dyslipidemia, which is mainly manifested by elevated concentrations of triacylglycerols (TAGs).[1] Dyslipidemia is a considerable risk factor for cardiovascular disease (CVD) and might affect mortality among CKD patients.[1] Similarly, alterations in renal lipid deposition and metabolism might play an important role in kidney injury.[2] However, lipid disorders at particular stages of the disease progress have not been studied in detail. In CKD patients, the major cardiovascular complication is heart failure, and its occurrence increases with kidney disease progression.[3]

Other CVD risk factors in the course of CKD are low concentrations of high-density lipoprotein cholesterol (HDL-C), and low-grade persistent inflammation.[4] These factors promote atherosclerosis and might contribute to the high cardiovascular mortality rate in CKD patients.

The association between CKD and cardiovascular risk is well established.[5,6] However, the exact contribution of dyslipidemia to CVD in CKD patients remains unclear, and little is known about fatty acid disorders in this population. Recently, we reported disorders of serum omega-3 polyunsaturated fatty acid (PUFA) composition in dialysis patients.[7] However, the main compounds of

TAG are monounsaturated fatty acids (MUFAs),[8,9] and thus their level may be associated with hypertriglyceridemia observed in CKD. Fatty acid composition of TAG is tightly controlled at a cellular level.[8,9] Yet, during the disease progression, alterations of fatty acid metabolism and composition might occur, leading to a cascade of adverse events in the body.[10,11]

In contrast to PUFAs, MUFAs do not belong to essential fatty acids, as they can be synthetized by an endogenous enzyme, stearoyl-coenzyme A desaturase-1 (SCD1).[12] In some countries, consumption of MUFAs accounts for at least one-third of the total fatty acid intake. The main sources of exogenous MUFAs are vegetable oils, high fat fruits such as olives and avocado, red meat, milk products, and nuts.[13] Plant oils, which are rich in MUFAs, have similar beneficial properties to fish oils, which are rich in PUFAs,[14] and might have a beneficial effect on CVD risk reduction in the general population.[15] However, other authors suggested that dietary MUFAs do not provide cardioprotection.[16] By contrast, increased endogenous MUFA synthesis by SCD1 is associated with metabolic diseases, and increased SCD1 activity has been proposed as a marker of CVD risk.[10,12] In previous studies, we found positive correlations between MUFA levels, SCD1 gene expression in adipose tissue, and the body mass index of patients with morbid obesity.[17] Moreover, we observed positive associations between the presence of inflammation in these patients, and serum MUFA levels.[18] This prompted us to determine the significance of serum MUFA content, dietary MUFA intake, and endogenous MUFA synthesis in CKD patients.

In the present study, we investigated serum MUFA levels in patients at subsequent stages of CKD, as well as the potential significance of MUFA for cardiovascular risk during CKD progression. Moreover, we attempted to clarify the major mechanisms that could lead to MUFA disturbances in CKD, by estimating the SCD1 activity and patients' dietary intake.

PATIENTS AND METHODS Study cohort
The study cohort included 207 participants: 40 patients with CKD stage 1–2; 24 patients with CKD stage 3a; 24 patients with CKD stage 3b; 19 patients with CKD stage 4–5; 24 patients on hemodialysis (HD); 22 patients on peritoneal dialysis (PD); 24 kidney transplant (Tx) recipients; and 30 controls matched for age and sex and without any kidney disease. Subjects were recruited from among CKD patients treated at an outpatient clinic and a dialysis unit of a large university clinical center. Controls were recruited from among patients of general medicine clinics. The inclusion criteria for the study group were as follows: age between 18 and 70 years, diagnosed CKD, and consent to participate in the study. Data on comorbidities and the possible use of lipid-lowering drugs were collected. The study conformed to

the principles of the Declaration of Helsinki. All experimental protocols of this study were approved by the Local Bioethics Committee at the Medical University of Gdansk (protocol no. NKEBN/614/2013–2014), and informed consent was obtained from all patients and healthy volunteers before enrollment. All laboratory tests were carried out at the Central Clinical Laboratory of the Medical University of Gdańsk. The CKD stage was based on the estimated glomerular filtration rate (eGFR) calculated with the Chronic Kidney Disease Epidemiology Collaboration equation.[19] Presence of CVD was established on the basis of medical records.

Dietary questionnaire Dietary habits were investigated with the use of the Food Frequency Questionnaire (FFQ). The FFQ is the most common dietary assessment tool used in large epidemiologic studies of diet and health, and validated for the Polish population.[20] It is an advanced tool that enables the evaluation of frequency (times / person / day) and amount (g / person / day) of food consumed during a year. The frequency of product consumption is determined by respondents: by free pointing to habitual intake frequency, during a defined period of time, from a list of 55 line items where each line item is defined by a series of foods or beverages.

Sample collection and storage Fasting blood samples from all subjects were collected into tubes without anticoagulant, kept at room temperature for 30 minutes for clotting, and centrifuged at $3000 \times g$ for 15 minutes at 4°C. After centrifugation, the serum samples were immediately frozen and stored in aliquots at –80°C until the analysis.

Sample preparation and fatty acid methyl ester analysis Total lipids were extracted from serum using the method described by Folch et al.[21] Obtained fatty acids were derivatized to fatty acid methyl esters (FAMEs) and analyzed, as previously described.[22] In brief, total lipids were extracted from serum in a chloroform–methanol mixture (2:1, v/v). After drying, each sample of lipid extracts was hydrolyzed with 1 ml of 0.5 M KOH in methanol at 90°C for 3 hours. The mixture was acidified with 0.2 ml of 6 M HCl, and then 1 ml of water was added. Nonesterified fatty acids were extracted with 1 ml of n-hexane and evaporated to dryness in a stream of nitrogen. FAMEs were prepared using 1 ml of 10% boron trifluoride-methanol solution at 55°C for 90 minutes. Next, FAMEs were extracted with 1 ml of n-hexane, and the solvent was evaporated. FAMEs were analyzed by gas chromatography–mass spectrometry (GC-MS QP-2010 SE, Shimadzu Corporation, Kyoto, Japan). They were separated on a 30 m, 0.25 mm i.d., Rtx-5MS capillary column (film thickness, 0.25 μm). The column temperature was programed from 60°C to 300°C at a rate of 4°C/min with helium as the carrier

Parameter	Controls	CKD stage						
		1–2	3a	3b	4–5	HD	PD	Kidney Tx
Age, y	56.00 (1.28)	53.65 (2.29)	60.12 (2.31)	59.12 (2.68)	61.52 (2.89)	57.12 (2.21)	57.00 (1.53)	52.17 (2.14)
Hemoglobin, g/dl	14.40 (0.19)	14.23 (0.21)	15.53 (0.28)	13.50 (0.38)[a]	12.33 (0.39)[b]	10.75 (0.30)[b]	11.20 (0.36)[b]	13.17 (0.40)[b]
Creatinine, mg/dl	0.85 (0.03)	0.83 (0.03)	1.35 (0.03)[b]	1.72 (0.09)[b]	3.98 (0.96)[b]	7.65 (0.50)[b]	9.00 (0.80)[b]	1.57 (0.17)[b]
GFR, ml/min/1.73 m²	88.4 (2.22)	88.4 (2.47)	50.56 (1.10)[b]	36.62 (1.15)[b]	20.63 (1.35)[b]	7.17 (0.60)[b]	6.86 (0.92)[b]	39.27 (2.65)[b]
BUN, mmol/l	16.09 (0.66)	16.49 (0.64)	22.16 (0.91)[b]	30.53 (1.85)[b]	48.34 (3.49)[b]	54.40 (3.37)[b]	54.51 (4.27)[b]	31.32 (3.29)[b]
Triglycerides, mg/dl	131.10 (10.94)	148.10 (17.07)	145.17 (14.46)	162.91 (12.55)	161.32 (15.32)	195.50 (37.51)	205.86 (17.68)[b]	215.33 (37.46)[a]
Total cholesterol, mg/dl	211.28 (8.33)	212.85 (7.32)	196.61 (11.70)	203.61 (10.78)	212.84 (12.78)	185.33 (10.81)	207.95 (14.18)	217.46 (11.62)
HDL-C, mg/dl	54.00 (2.61)	55.93 (2.20)	46.87 (2.29)	48.96 (3.44)	48.26 (3.53)	41.21 (2.85)[b]	36.62 (2.18)[b]	51.71 (3.92)
LDL-C, mg/dl	131.17 (7.54)	126.29 (6.28)	118.41 (8.88)	122.13 (10.00)	131.79 (10.79)	107.70 (9.69)	132.65 (12.80)	125.80 (10.87)
hs-CRP, mg/l	2.82 (0.92)	3.38 (0.65)	3.61 (0.75)	3.59 (0.72)	4.59 (1.43)	9.67 (1.70)[b]	7.47 (2.83)	8.64 (3.98)
Albumin, g/l	39.52 (0.45)	38.70 (1.06)	39.26 (0.63)	37.83 (0.85)	36.74 (1.06)[a]	31.58 (0.71)[b]	32.13 (0.85)[b]	37.92 (0.86)
Glucose, mg/dl	102.48 (4.45)	102.67 (4.79)	110.33 (4.82)	126.25 (11.84)	125.16 (11.34)[a]	109.62 (13.09)	102.36 (5.52)	116.17 (10.10)
Insulin, μU/ml	11.90 (0.97)	12.99 (1.17)	16.19 (3.30)	16.56 (1.27)[b]	21.05 (6.99)	26.16 (11.07)	16.37 (5.57)	30.18 (15.00)
Na⁺, mmol/l	140.38 (0.41)	140.00 (0.41)	139.95 (0.35)	139.61 (0.68)	140.68 (0.77)	137.17 (0.62)[b]	141.52 (0.56)	139.54 (0.68)
K⁺, mmol/l	4.36 (0.05)	4.24 (0.05)	4.50 (0.08)	4.51 (0.09)	4.81 (0.09)[b]	5.19 (0.16)[b]	4.39 (0.13)	4.26 (0.08)
BMI, kg/m²	27.34 (0.73)	27.52 (0.84)	29.24 (1.13)	31.26 (1.27)[b]	27.54 (1.18)	25.65 (1.29)	27.35 (0.82)	27.04 (0.85)
HOMA	3.15 (0.41)	3.46 (0.38)	4.85 (1.36)	5.14 (0.50)[b]	8.66 (4.13)	6.85 (2.56)	5.37 (0.37)	7.15 (2.65)
Concomitant diseases, n (%)								
Diabetes mellitus	3.33 (3.33)	12.50 (5.30)	26.08 (9.16)[a]	41.67 (10.28)[b]	42,10 (11.63)[b]	29.17 (9.48)[b]	18.18 (8.41)	33.33 (9.83)[b]
Cardiovascular disease	3.33 (3.33)	15.00 (5.72)	34.78 (9.94)[b]	41.67 (10.28)[b]	42.11 (11.64)[b]	50.00 (10.42)[b]	50.00 (10.91)[b]	33.33 (9.83)[b]
Hypertension	33.33 (8.60)	70.00 (7.34)[b]	95.65 (4.26)[b]	95.83 (4.17)[b]	94.74 (5.26)[b]	91.67 (5.76)[b]	100 (0.00)[b]	100.00 (0.00)[b]

Data are presented as mean (SD).

a Significant difference compared with healthy controls at $P < 0.05$

b Significant difference compared with healthy controls at $P < 0.01$

SI conversion factors: to convert glucose to mmol/l, multiply by 0.0555; total cholesterol, LDL-C, and HDL-C to mmol/l, multiply by 0.0259; and triglyecrides to mmol/l, by 0.0113.

Abbreviations: BMI, body mass index; BUN, blood urea nitrogen; CKD, chronic kidney disease; GFR, glomerular filtration rate; HD, hemodialysis; HDL--C, high-density lipoprotein cholesterol; HOMA, Homeostatic Model Assessment; hs-CRP, high-sensitivity C-reactive protein; K⁺, potassium, LDL-C, low-density lipoprotein cholesterol; Na⁺, sodium, NT, not tested; PD, peritoneal dialysis; Tx, transplantation

gas at a column head pressure of 60 kPa. For ionization of FAMEs, the electron energy was 70 eV. Desaturation index (DI), defined as the ratio of oleic acid to stearic acid, was used to estimate the activity of SCD1.

Statistical analysis Statistical analyses were performed with SigmaPlot software (Systat Software, Inc., San Jose, California, United States). The intergroup differences were verified by 1-way analysis of variance, followed by an appropriate post hoc test. The univariate and multivariate regression tests were used to examine the correlations between selected variables. The diagnostic ability of selected parameters to predict CVD was examined, based on the receiver operating characteristic (ROC) curve analysis. A P value of less than 0.05 was considered significant for all analyses.

RESULTS Laboratory and selected anthropometric parameters of all 207 participants were presented in TABLE 1. We detected 8 MUFAs in the examined serum samples. The mean (SD) total MUFA content increased with CKD progression. It was 29.6% (0.6%) in controls, 29.6% (0.5%) in CKD stage 1–2, 30.2% (0.7%) in CKD stage 3a, 31.4% (0.7%) in CKD stage 3b, 32.2% (0.5%) in CKD stage 4–5, 33.2% (1.0%) in HD patients, and 33.6% (0.9%) in PD patients ($P < 0.05$). In Tx recipients, it was slightly lower than in CKD stage 4–5 (mean [SD], 31.7% [0.7%]) (TABLE 2), although

TABLE 2 Content (% of total fatty acids) of the main classes of fatty acids in the serum of patients at subsequent stages of chronic kidney disease

Fatty acids	Controls	CKD stage						
		1–2	3a	3b	4–5	HD	PD	Tx
14:1, %	0.07 (0.01)	0.07 (0.01)	0.08 (0.01)	0.08 (0.01)	0.06 (0.01)	0.07 (0.02)	0.05 (0.01)[a]	0.08 (0.01)
16:1, %	3.04 (0.17)	3.01 (0.12)	3.05 (0.20)	2.98 (0.14)	3.04 (0.19)	2.91 (0.20)	2.84 (0.19)	3.07 (0.17)
18:1, %	26.04 (0.55)	26.04 (0.47)	26.59 (0.61)	27.80 (0.63)[a]	28.57 (0.49)[b]	29.63 (0.83)[b]	30.20 (0.72)[b]	28.00 (0.64)[a]
19:1, %	0.02 (0.002)	0.02 (0.002)	0.02 (0.003)	0.02 (0.002)	0.02 (0.002)	0.02 (0.002)	0.03 (0.002)	0.02 (0.001)
20:1, %	0.16 (0.01)	0.18 (0.01)	0.18 (0.01)	0.18 (0.01)	0.20 (0.02)[a]	0.21 (0.02)[b]	0.21 (0.02)[b]	0.21 (0.01)[b]
22:1, %	0.06 (0.004)	0.05 (0.004)	0.06 (0.007)	0.05 (0.004)	0.06 (0.01)	0.06 (0.005)	0.04 (0.008)[a]	0.04 (0.003)[b]
24:1, %	0.22 (0.01)	0.22 (0.01)	0.20 (0.01)	0.26 (0.02)	0.30 (0.05)	0.26 (0.02)	0.21 (0.02)	0.26 (0.02)
Total MUFA, %	29.63 (0.63)	29.59 (0.54)	30.18 (0.66)	31.36 (0.65)	32.19 (0.54)[b]	33.16 (0.99)[b]	33.59 (0.85)[b]	31.67 (0.73)[a]
Total SFA, %	33.12 (0.35)	33.44 (0.35)	34.14 (0.59)	33.60 (0.46)	33.36 (0.43)	33.54 (0.40)	32.50 (0.67)	34.18 (0.57)
Total n-6 PUFA, %	31.71 (0.64)	31.57 (0.67)	30.54 (0.96)	29.75 (0.73)[a]	29.72 (0.90)	28.19 (0.84)[b]	28.51 (0.95)[b]	29.28 (0.91)[a]
Total n-3 PUFA, %	3.02 (0.22)	2.52 (0.13)[a]	2.44 (0.15)[a]	2.54 (0.14)	2.31 (0.20)[a]	2.13 (0.17)[b]	3.08 (0.16)	2.01 (0.13)[b]
DI (18:1/18:0)	3.65 (0.12)	3.68 (0.10)	3.79 (0.12)	4.09 (0.17)[a]	4.27 (0.14)[b]	4.34 (0.21)[b]	4.61 (0.30)[b]	4.23 (0.16)[b]
16:1/16:0	0.13 (0.007)	0.13 (0.004)	0.13 (0.006)	0.13 (0.005)	0.13 (0.008)	0.13 (0.01)	0.12 (0.008)	0.13 (0.05)

Data are presented as mean (SD).

[a] Significant difference compared with healthy control at $P < 0.05$

[b] Significant difference compared with healthy control at $P < 0.01$

Abbreviations: DI, desaturation index; MUFA, monounsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acid; SFA, saturated fatty acids; others, see **TABLE 1**

the differences were not significant. This pattern of changes in the MUFA content in CKD patients remained similar after exclusion of patients with diabetes. There was no association between the use of lipid-lowering drugs and serum MUFA levels.

We observed a positive correlation of serum MUFA levels with creatinine and blood urea nitrogen (BUN), and a negative correlation with eGFR ($r = 0.23$, $r = 0.25$, and $r = -0.28$, respectively; $P < 0.01$). This analysis was performed only for controls and patients with CKD stages 1–5. Patients treated with renal replacement therapy were excluded from this analysis, because the above parameters are influenced by the dialysis procedure and do not reflect renal function. We also found a strong positive correlation of MUFA levels with serum TAG levels ($r = 0.71$, $P < 0.01$) and a strong negative correlation with HDL-C levels ($r = -0.45$, $P < 0.01$) (Supplementary material, *Table S1*). These data demonstrate that increasing levels of MUFA are associated with CKD progression (**FIGURE 1A–1C**) and dyslipidemia (**FIGURE 1D–1F**). There was a weak correlation between high-sensitivity C-reactive protein (hs-CRP) and MUFA levels ($r = 0.16$, $P < 0.05$) (**FIGURE 1G**; Supplementary material, *Table S1*). We also found a weak negative correlation between low-density lipoprotein cholesterol (LDL-C) and serum MUFA levels ($r = -0.19$, $P < 0.01$) (**FIGURE 1F**; Supplementary material, *Table S1*). However, it should be noted that serum LDL-C concentrations in patients with CKD stages 1–5 did not differ significantly from those in controls, and this negative correlation probably resulted from decreased serum LDL-C concentrations in HD patients (**FIGURE 1F**).

Other, indirect, factors of cardiovascular risk in CKD, albumin and hemoglobin levels, were negatively correlated with the serum MUFA level ($r = -0.28$, $P < 0.01$; $r = -0.20$, $P < 0.01$; respectively), while glucose levels showed a positive correlation ($r = 0.215$, $P < 0.01$) (Supplementary material, *Table S1*, *Figure S1A–S1C*). MUFA levels were strongly associated with the 18:1/18:0 SCD1 DI ($r = 0.79$, $P < 0.01$) (Supplementary material, *Table S1*). In a multiple regression model, CKD remained an independent predictor of MUFA levels after adjustment for age, sex, and comorbidities ($P < 0.01$).

Apart from the correlation between CKD stage and increased MUFA levels, we also observed a trend for a strong positive correlation between the CKD stage and the percentage of patients with CVD ($r = 0.88$, $P < 0.01$; **FIGURE 2**). Tx recipients were not included in this analysis because advanced CVD is a contraindication to kidney Tx, introducing a potential selection bias to the evaluation. To assess if MUFAs may be added to currently known predictors of CVD, we performed the ROC curve analysis for potential predictors of CVD in CKD patients. As presented in **TABLE 3**, MUFA levels, together with age, creatinine, eGFR, BUN, LDL-C, albumin, and hemoglobin levels, were the strongest predictors of CVD in CKD patients ($P < 0.01$).

In order to examine if diet or endogenous MUFA synthesis contributes to an increase in serum MUFA levels in CKD patients, we analyzed their dietary intake as well as serum DI, defined as the ratio of fatty acids 18:1 to 18:0. DI is used to estimate the activity of SCD1 in
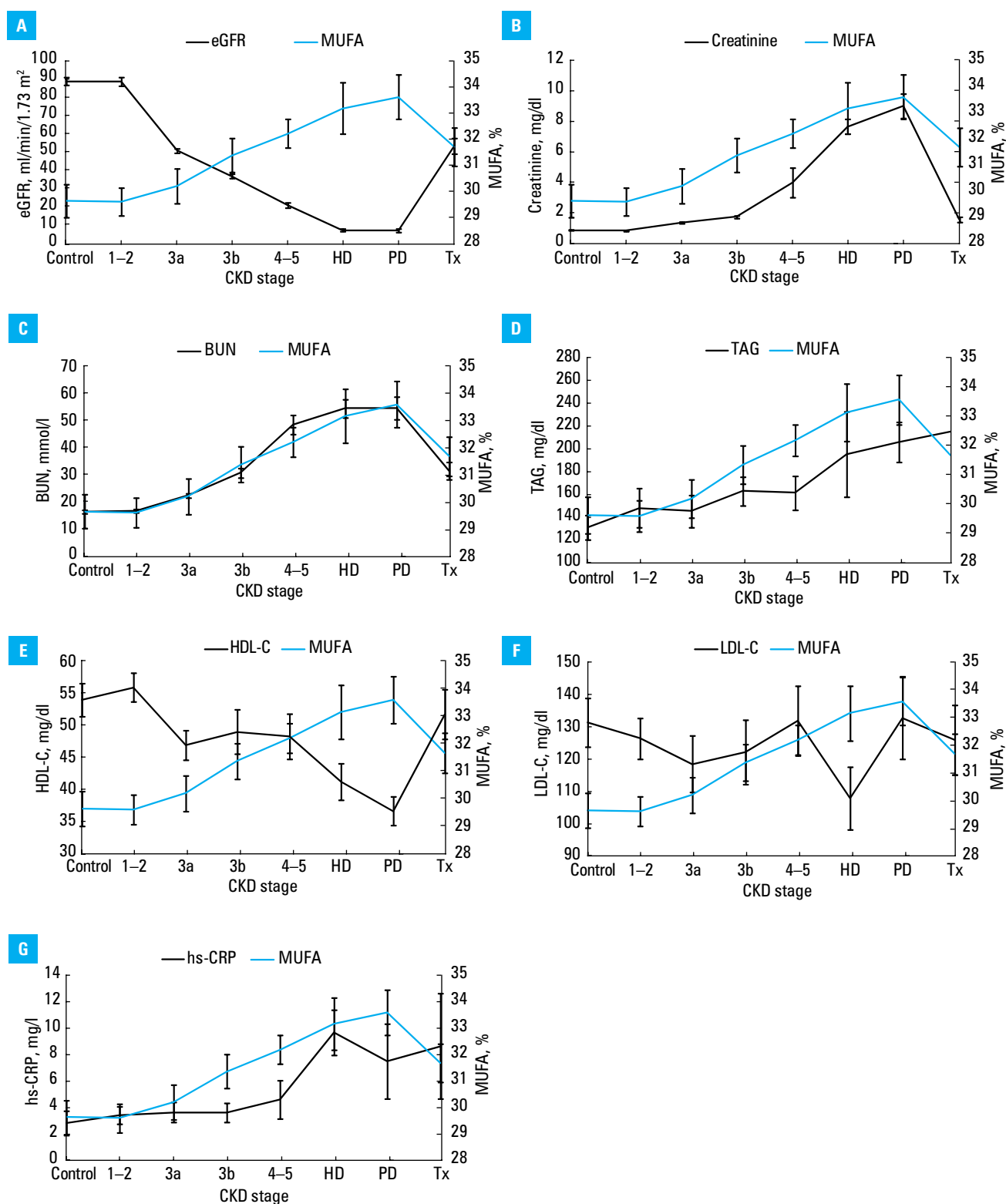
FIGURE 1 Serum level of monounsaturated fatty acids (MUFAs) and concentration of selected markers in controls and patients at subsequent stages of chronic kidney disease (CKD) (1–2; 3a; 4–5; hemodialysis [HD]; peritoneal dialysis [PD]; and transplantation [Tx]): **A** – estimated glomerular filtration rate (eGFR); **B** – creatinine; **C** – blood urea nitrogen (BUN); **D** – triacyloglycerols; **E** – high-density lipoprotein cholesterol (HDL-C); **F** – low-density lipoprotein cholesterol (LDL-C); **G** – high-sensitivity C-reactive protein (hs-CRP). Whiskers represent standard deviation.

lipogenic tissues.[17] Similarly to serum MUFA levels, it was positively correlated with markers of CKD (creatinine and BUN) and serum TAG concentrations (Supplementary material, *Table S1*). A strong negative correlation between DI and serum HDL-C levels was observed ($r = -0.42$, $P < 0.001$). However, in comparison to MUFAs, DI showed a higher correlation with hs-CRP ($r = 0.19$, $P < 0.01$) (Supplementary material, *Table S1*). Serum DI as a measure of SCD1 activity in liver and adipose tissues at subsequent stages of CKD is presented in **FIGURE 3**. DI increased with CKD progression and was the highest in dialysis patients. In contrast, in every group of CKD

FIGURE 2 Serum level of monounsaturated fatty acids (MUFAs) and prevalence of cardiovascular disease (CVD) in controls and patients at subsequent stages of chronic kidney disease (CKD) (1–2; 3a; 4–5; hemodialysis [HD]; peritoneal dialysis [PD]; and transplantation [Tx]). Whiskers represent standard deviation.

a $P <0.01$ compared with controls
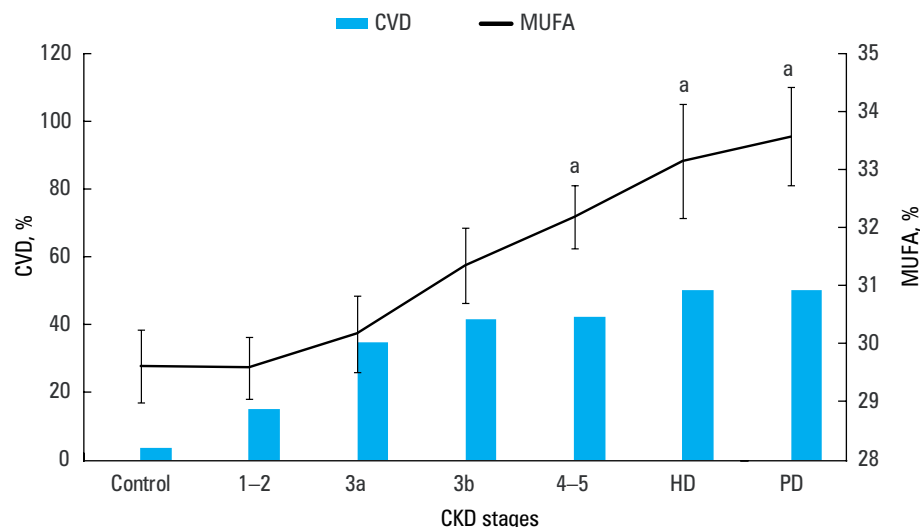
TABLE 3 Receiver operating characteristic curve analysis for potential predictors of cardiovascular disease in patients with chronic kidney disease

Parameter	AUC	*P* value
Creatinine	0.69	<0.001
BUN	0.70	<0.001
Age	0.72	<0.001
Hemoglobin	0.68	<0.001
Albumin	0.65	0.001
MUFA	0.63	0.004
LDL-C	0.63	0.008
Glucose	0.61	0.01
Total cholesterol	0.60	0.03
HDL-C	0.59	0.06
hs-CRP	0.56	0.19
Triacylglycerols	0.55	0.27
Insulin	0.54	0.37
HOMA	0.54	0.42

Abbreviations: AUC, area under the receiver operating characteristic curve; others, see **TABLES 1** and **2**

patients, consumption of products rich in MUFAs was lower than in controls (Supplementary material, *Table S2*). Moreover, the analysis of data from the FFQ did not reveal any considerable correlations between consumption of various food products and serum content of total MUFAs or the major fatty acid from this group (18:1 and 16:1) in CKD patients (Supplementary material, *Table S3*).

DISCUSSION **Serum monounsaturated fatty acid content is associated with cardiovascular risk and inflammation in cardiovascular disease** The major finding of the present study is the positive correlation between the increased prevalence of CVD and serum MUFA content with subsequent CKD stages, as well as significant correlations between serum MUFA content and various markers or risk factors of CVD. CVD has enormous clinical importance in the course of CKD because cardiovascular mortality in CKD patients is several times higher compared with the general population.[5] There are numerous risk factors for development and progression of CVD in CKD patients. These factors are typically divided into 2 groups: traditional and nontraditional.[23] The first group includes dyslipidemia, arterial hypertension, and obesity, while the second one, chronic inflammation, malnutrition, anemia, oxidative stress, and insulin resistance. Increased MUFA content seems to be associated with both these groups.

Dyslipidemia, characterized by abnormal levels of HDL-C and TAGs, is a common complication of CKD.[24] Hypertriglyceridemia is a recognized risk factor for CVD in the general population.[25] It was also reported that it might increase cardiovascular mortality in patients with CKD.[26] Moreover, as CKD progression is associated with the development of hypertriglyceridemia, it is also possible that high TAG concentrations themselves contribute to CKD.[27] One of the reasons for increased TAG in CKD patients may be decreased serum levels of irisin that reduces lipid synthesis in the liver.[28,29] Our results suggest that the elevated TAG levels could, at least in part, be a consequence of the increased content of endogenous MUFAs. Since the SCD1, the rate-limiting enzyme in the biosynthesis of MUFAs, is closely colocalized to diacylglycerol acyltransferase-2 in the endoplasmic reticulum membrane, it is supposed that endogenously synthesized MUFAs are the main substrates for the synthesis of hepatic TAGs.[30] It was reported that the SCD1 overexpression leads to hypertriglyceridemia.[31] This is supported by the strong correlations between serum TAG, MUFA, and 18:1/18:0 DI found in our patients. On the other hand, the increase in serum MUFA levels in CKD patients may be also considered as reflecting increased TAG levels. Anyway, increased MUFA levels can be added to the list of lipid alterations characteristic for CKD.

Chronic low-grade inflammation represents another typical complication in patients with CKD.[32] Concurrent infections, comorbidities, uremia, and dialysis procedure in end-stage renal disease
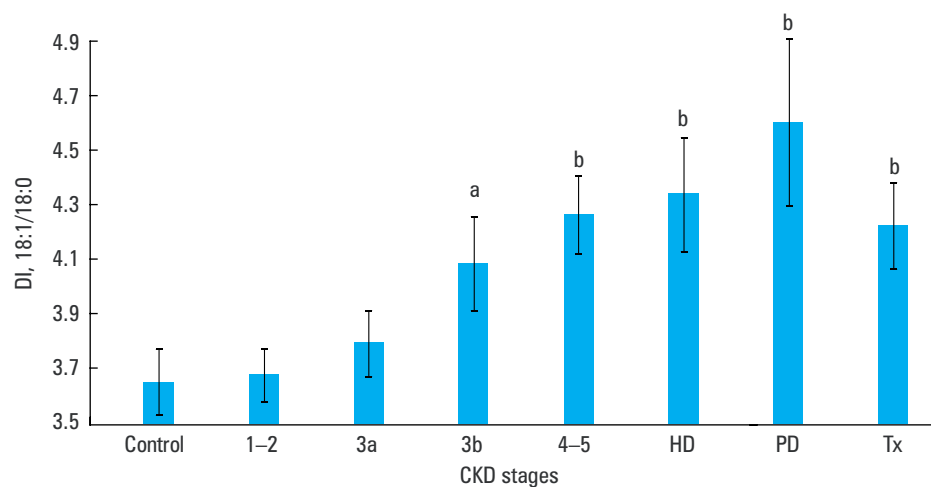
play an important role in the pathogenesis of an inflammatory state in the course of CKD.[32] It is currently widely acknowledged that inflammation significantly contributes to atherosclerotic progression and to CVD risk in the general population.[33] In CKD patients, a similar association has been documented.[34] The major mechanisms include endothelial dysfunction and vascular calcification.[34]

One of the most acknowledged inflammatory biomarkers is hs-CRP. Numerous studies have shown its strong relationship with cardiovascular outcome, both in the general population and in CKD patients.[33,34] In the present study, a gradual increase in hs-CRP levels was observed across the CKD stages, reaching the highest values in patients on dialysis. The association between SCD1 index and inflammation was previously suggested by Petersson et al.[35] In HD patients, some authors observed that estimated SCD1 activity was positively linked to inflammation, both in liver and adipose tissue.[10] Inhibition of SCD1 modulates cellular inflammation and stress in different cell types and tissues including adipocytes, β cells, macrophages, as well as liver, aorta, skin, myocyte, and endothelial cells.[9]

In our study, the correlation between MUFA, DI, and hs-CRP was significant, but weak. A much stronger association was observed between the MUFA content and the low albumin concentration. This is interesting because, in CKD patients, albumin is thought to reflect not only the nutritional status but also the chronic inflammatory state.[36] Indeed, MUFAs were associated with low albumin levels despite a significant correlation with increasing body mass index, suggesting that decreased albumin concentrations were related to chronic inflammation rather than to poor nutritional status. According to some authors, low albumin levels are an even more potent risk factor for CVD than hs-CRP in CKD patients.[37]

We previously documented strong associations between MUFAs and an inflammatory state in other patient groups.[18] In the present study, significant links between MUFAs and inflammation indicate yet another mechanism

behind the relationship of MUFA with CVD in CKD patients. The ROC curve analysis of our results showed that serum MUFA levels are among the most significant predictors of CVD in CKD. Thus, our results suggest that the elevation of serum MUFA levels might significantly contribute to CVD risk in this patient group.

Increased serum levels of monounsaturated fatty acids in chronic kidney disease patients are related to elevated endogenous synthesis rather than diet modifications It is important to define the major mechanisms leading to an increase in MUFA levels during CKD. To solve this problem, we estimated the activity of SCD1 during CKD progression and assessed the consumption of MUFA-rich food products. The highest SCD1 activity is present in liver and adipose tissue.[17] The increased activity is implicated in various disorders, including diabetes, obesity, and atherosclerosis.[30] According to some authors, SCD1 activity in liver and adipose tissue is an independent predictive factor of mortality in dialysis patients.[8] Since liver or adipose tissue were not available in our study, we estimated SCD1 activity indirectly by calculating 18:1/18:0 DI.[17,38] DI presented a similar pattern of changes at subsequent stages of CKD as MUFAs (**TABLE 2**). In addition, it showed a strong correlation with MUFAs themselves (Supplementary material, Table S1). These results suggest that endogenous MUFA synthesis is the key determinant of serum MUFA levels.

The major exogenous MUFA sources include vegetable oils, high-fat fruits such as olives and avocados, red meat, whole milk products, and nuts.[39] In some countries, consumption of MUFA accounts for as much as one-third of the total fatty acid intake.[14] Therefore, to investigate the impact of dietary MUFAs on the changes in serum MUFA levels during CKD progression, the dietary questionnaires of all participants were evaluated. Surprisingly, despite increased serum MUFA levels, the consumption of all MUFA-rich products was lower in CKD patients than in controls (Supplementary material, *Table S2*). Moreover, the results did not show significant correlations between the consumption of MUFA-rich food

products and serum MUFA content in CKD patients. These results indicate that the increase in serum MUFA levels in CKD patients is not due to diet but rather results from increased endogenous synthesis by SCD1.

Our results are in contrast to a previous study by Huang et al[8] in Swedish CKD patients. However, those authors adopted a different approach to determine liver and adipose tissue SCD1 activity based on serum fatty acid composition in dialysis patients. Firstly, they estimated SCD1 activity by calculating 16:1/16:0 DI, since the diet of the Swedish population is rich in oleate that could have impacted the results. Secondly, they estimated liver SCD1 based on fatty acid content in serum phospholipids, and the adipose tissue SCD1 activity based on serum free fatty acid profiles. We did not find significant differences in 16:1/16:0 DI in study groups; however, stearic acid is a preferred SCD1 substrate,[8] and the main product of liver desaturation is oleic acid, which is included in TAGs released from the liver into the blood. Moreover, CKD-related changes among MUFAs were most pronounced in the case of oleic acid. Thus, considering that the serum TAG level is tightly associated with liver SCD1 activity in our study, we determined 18:1/18:0 DI in total serum lipids. The differences in the approach between our study and that by Huang et al[8] could explain the discrepancies in the results and the fact that, in contrast to Huang et al,[8] we observed significant associations between serum DI and TAG, HDL-C, and other CVD risk factors.

Our results, suggesting an association between serum MUFA levels and increased risk of CVD in CKD patients, are also in contrast to studies analyzing the effects of dietary MUFA on human health, showing the potential of MUFAs to reduce the CVD risk. The use of dietary MUFAs reduced blood pressure[40] and modulated the functions of immune system cells in animals.[14] Finucane et al[41] emphasized that dietary MUFA intake, in contrast to saturated fatty acids, increases insulin sensitivity but also reverses disorders of adipose tissue function.[41] The mechanisms of CVD risk reduction with dietary MUFAs are still uncertain but include a simultaneous decrease in saturated fatty acid intake, a decrease in SCD1 activity, or both.[42] However, in our study, the increased CVD risk seems to be associated with increased endogenous MUFA levels resulting from increased SCD1 activity, whereas the consumption of dietary MUFAs was shown to be reduced in CKD patients. Paradoxically, since dietary MUFAs inhibit the activity of SCD1,[30] the increased consumption of MUFAs could prevent the negative effect of increased SCD1 activity in CKD.

Our study has several limitations. First, it was a cross-sectional study, and as such cannot infer causality. Second, the studied cohort was relatively small. However, even with these sample size, the reported associations are strong and convincing. So far, research has focused mostly on the role of omega-3 PUFAs in CKD patients.

Our study is novel in that it showed that MUFAs can also play an important role in the development of CKD complications.

In conclusion, the present work documents a gradual increase in serum MUFA content across the consecutive stages of CKD. Our results suggest that the major mechanism responsible for increased MUFA content is enhanced endogenous synthesis through the increased activity of SCD1, rather than the effect of dietary MUFA intake. Furthermore, the study demonstrates associations linking increased MUFA content and SCD1 activity with markers of cardiovascular risk and with CVD itself. The measurement of MUFA derived from endogenous SCD1 activity can be a useful marker of CVD risk and perhaps even a target for therapeutic interventions.

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