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ARTICLE

Dietary intake, clinical-nutritional status, and homocysteine in hemodialysis subjects: the mediating role of inflammation (NUGE-HD study)

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Abstract: The aim of the present study was to test the hypothesis that inflammation mediates the associations among food intake, clinical-nutritional status, and plasma homocysteine (Hcys) in hemodialysis (HD) subjects. This was a cross-sectional analysis of data on 129 subjects undergoing HD (58.9% male, 61.8 ± 15.5 years of age) from the cohort Nutrition and Genetics on HD outcomes (NUGE-HD study). Sociodemographic, anthropometric, and metabolic data were collected, and food intake was assessed using a quantitative food frequency questionnaire. Plasma C-reactive protein (CRP) was used as an inflammatory marker. Data were analyzed by structural equation modeling. Regarding the direct effects, complex B vitamin intake was negatively associated with body mass index, and diabetes mellitus was positively associated with CRP. Plasma CRP also showed a negative association with Hcys, and the ratio of saturated and polyunsaturated fatty acids intake showed a positive association with Hcys. Regarding indirect effects, the results showed that the relationship between the presence of diabetes mellitus and Hcys is mediated by plasma CRP. In conclusion, the ratio of saturated and polyunsaturated fatty acids had a direct effect on plasma Hcys, whereas inflammation had a direct and mediating effect on the relationship between Hcys and diabetes mellitus in HD subjects.

Novelty

- In end-stage renal disease, CRP influences plasma Hcys directly and also indirectly through its mediating effect.
- The quantity and quality of dietary fatty acids influence plasma Hcys concentrations in HD subjects.

Key words: cardiovascular risk, C-reactive protein, end-stage renal disease, polyunsaturated fatty acids, saturated fatty acids, complex B vitamins.

Résumé : Le but de la présente étude est de tester l'hypothèse selon laquelle l'inflammation médie l'association entre l'apport alimentaire, l'état nutritionnel clinique et l'homocystéine plasmatique (« Hcys ») chez les sujets hémodialysés (« HD »). On effectue une analyse transversale de 129 sujets HD (58,9 % hommes, 61,8 ± 15,5 ans) de la cohorte Nutrition et génétique sur les résultats de HD (étude NUGE-HD). Des données sociodémographiques, anthropométriques et métaboliques sont collectées et l'apport alimentaire est évalué à l'aide d'un questionnaire quantitatif sur la fréquence des aliments. La protéine C-réactive plasmatique (« CRP ») est utilisée comme marqueur inflammatoire. Les données sont analysées par modélisation d'équations structurelles. En ce qui concerne les effets directs, l'apport en complexe de vitamines B est négativement associé à l'indice de masse corporelle et le diabète est positivement associé à la CRP. À propos d'Hcys, la CRP plasmatique présente une association négative et le ratio des apports en acides gras saturés et polyinsaturés révèle une association positive. En ce qui concerne les effets indirects, les résultats indiquent que la relation entre la présence de diabète et Hcys est médiée par la CRP plasmatique. En conclusion, le ratio des acides gras saturés et polyinsaturés a un effet direct sur l'Hcys plasmatique tandis que l'inflammation a un effet direct et médiateur sur la relation entre l'Hcys et le diabète chez les sujets HD. [Traduit par la Rédaction]

Les nouveautés

- En présence d'insuffisance rénale terminale, la CRP influence directement Hcys plasmatique et indirectement par son effet médiateur.
- La quantité et la qualité des acides gras alimentaires influencent les concentrations plasmatiques de Hcys chez les sujets HD.

Mots-clés : risque cardiovasculaire, protéine C-réactive, maladie rénale terminale, acides gras polyinsaturés, acides gras saturés, complexe de vitamines B.

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Introduction

The most common cause of death in the dialysis population is cardiovascular disease (CVD); cardiovascular mortality is 10–20 times higher in dialysis patients than in the general population (Wald et al. 2012). Risk factors for CVD in maintenance hemodialysis (HD) patients include both traditional risk factors, such as age, hypertension, diabetes mellitus (DM), and dyslipidemia, and unique nontraditional risk factors, including volume overload, mineral metabolism abnormalities, proteinuria, malnutrition, oxidative stress, hyperhomocysteinemia, and inflammation (Kalantar-Zadeh et al. 2003).

Homocysteine (Hcys), a sulfhydryl group containing amino acid, is an intermediate product of the metabolism of the amino acids methionine and cysteine (Selhub and Troen 2016). Hyperhomocysteinemia occurs in 83% to 91% of HD patients (Manns et al. 2001), probably as a consequence of decreased renal excretion of Hcys, a disturbance or undiagnosed genetic abnormalities in Hcys metabolism, and insufficient intake of Hcys-related vitamins (Tinelli et al. 2019). A high Hcys concentration can decrease the formation of adenosine, an important cardiac vasodilator and constrictor of the renal vascular bed (Chen et al. 2001), which, in addition to enhancing prooxidizing effects, decreases nitric oxide bioavailability (Stuhlinger et al. 2001). In this sense, an increase in Hcys has been positively associated with overall mortality and with mortality as a result of CVD in HD patients (Jamison et al. 2007).

Inflammation is also common in these individuals, and nearly 30%–60% of HD subjects exhibit persistent inflammation caused by multiple factors including vascular access infections, malnutrition, and chronic infections; this inflammation is further aggravated by uremic immune dysfunction, inadequate renal removal of cytokines, and inflammatory response to dialysis (Jankowska et al. 2017). The release of inflammatory mediators during HD treatment has been indicated as an aggravating factor in the increase in morbidity and mortality and in the decline in subjective perception of overall quality of life (Lai et al. 2016).

Furthermore, C-reactive protein (CRP), an acute-phase protein, stands out as an important marker for the identification and control of inflammation. In addition, serum CRP has been shown to be a strong independent risk factor for CVD (Panichi et al. 2008). Elevated levels of serum CRP in HD subjects is linked to the development of coronary artery disease, even in the absence of dyslipidemia (Bazeley et al. 2011).

Overall, we tested the hypothesis that inflammation mediates the associations among dietary intake, clinical-nutritional status, and plasma Hcys in HD subjects.

Materials and methods

Design and study population

This cross-sectional study consisted of 129 HD patients (76 men and 53 women; mean age, 61.8 ± 15.5 years) from the cohort Nutrition and Genetics on HD outcomes (NUGE-HD study). Treatment with HD for at least 1 month and age ≥ 18 years were the inclusion criteria. Patients with auditory deficiency (informed by the HD nursing team), newly implanted catheters, or instability hemodynamics (according to the service protocol) were excluded. All patients included in the study underwent HD (3 to 4 h, 3 times a week) with blood flow of >250 mL/min and dialysate flow of 500 mL/min. The causes of chronic kidney disease were hypertensive nephrosclerosis in 49 patients (38.0%), diabetic nephropathy in 43 patients (33.3%), polycystic kidney disease in 8 patients (6.2%), chronic glomerulonephritis in 5 patients (3.9%), and other, or unknown, etiologies in 24 patients (18.6%). The mean urea clearance divided by the volume of the distribution of urea (Kt/V) was 1.6 ± 0.3.

All patients gave informed consent before participating in the study, in accordance with the principles of the *Declaration of Helsinki*. The ethics committee of our university approved the study (No. 1.956.089/2017).

Blood measurements

Nonfasting blood samples were collected before the initiation of dialysis to measure Hcys and high-sensitivity CRP. The plasma Hcys level was assayed using the ARCHITECT Homocysteine 1L71 assay (Abbott Laboratories, Abbott Park, Ill., USA), which is a chemiluminescent microparticle immunoassay for the quantitative determination of L-Hcys in the ARCHITECT iSystem. We used the Latex CRP Reagent of the Beckman Coulter AU system (Beckman Coulter Inc., Calif., USA) to determine CRP concentrations. Subjects were categorized according to cardiovascular risk on the basis of the following CRP values: high risk (>3 mg/L), medium risk (1–3 mg/L), and low risk (<1 mg/L) (Myers et al. 2004). Subjects were also categorized on the basis of Hcys levels as having hyperhomocysteinemia (Hcys ≥ 15 μmol/L) or not (Hcys < 15 μmol/L) (Manns et al. 2001).

Anthropometric assessment

The anthropometric assessment was performed approximately 30 min after the end of the HD session. Body mass (kg) and height (cm) were measured using standard techniques, as described previously (Balbino et al. 2017). Body mass index (kg/m^2) was then calculated, and the cut-off points of the World Health Organization (2000) for adults, and of Lipschit (1994) for the elderly, were used to classify nutritional status.

Food consumption

To estimate food consumption, we used a quantitative food frequency questionnaire (FFQ) that was based on a questionnaire developed for patients with chronic kidney disease (Kalantar-Zadeh et al. 2011). During routine HD sessions in the dialysis unit, the FFQ was used by trained researchers to interview patients. For patients unable to respond, the questions were addressed to their caretakers. During the interview, a photographic album (Monteiro et al. 2010) with similar portions was used, and the interviewee chose the categories of food portions from the album that corresponded to their habitual intake.

To calculate dietary intake, consumption of each food item was converted to grams per day, and the daily consumption of each nutrient was calculated according to the nutritional composition of Brazilian food tables (Brazilian Institute of Geography and Statistics 2011; Brazilian Table of Food Composition 2011) using a Microsoft Excel spreadsheet especially designed for this. In this study, we evaluated caloric intake (kcal), fatty acid profile (g), and complex B vitamins (mg/μg) quantitatively. To calculate the ratio between saturated (g) and polyunsaturated (g) fatty acids (SFA/PUFA), the consumption of SFA (g) was divided by the consumption of PUFA (g) for each individual assessed. The median value (1.86) of this relationship in the population studied was calculated. From this value, individuals were categorized according to consumption above (>1.86) or below (<1.86) the median.

All the nutrients evaluated in the present study were adjusted for daily caloric intake by the residual method (Willett and Stampfer 1998) before statistical analysis, to minimize the effect of caloric intake on the relationship of consumption variables.

Other variables

The patients were interviewed using a semistructured questionnaire to obtain sociodemographic (sex and age), clinical (underlying kidney disease), and lifestyle (smoking habit) data. The medical records of each patient were also reviewed carefully, and data related to underlying kidney disease or other comorbidities were collected.

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA), for sample characterization through absolute and relative frequency distribution and calculation of the median and interquartile range. Plasma Hcys normality was assessed by

the Shapiro–Wilk test, and Mann–Whitney *U* and Kruskal–Wallis tests were used to compare the Hcys medians according to the study variable categories.

MPlus software, version 5.0, was used to analyze the relationship between plasma Hcys and CRP and to investigate the mediating role in relationships involving food intake and clinical-nutritional status, using structural equation modeling (SEM). SEM is a multivariate technique that allows simultaneous exploration of the complex relationships among the multiple variables studied through statistical procedures that combines multiple regression, confirmatory factor analysis (CFA), and path analysis (Amorim et al. 2010).

The SEM includes both directly observed and latent variables. F1 Figure 1 shows the tested theoretical model, in which the main response variable is plasma Hcys, a continuous variable observed directly. The SEM begins by constructing a measurement model composed of latent variables (or constructs), which are estimated from directly observed variables or indicators; the structural model, which deals with the relationship between all variables, is then estimated (Amorim et al. 2010).

The measurement model is estimated by CFA on the basis of the specification of indicator variables, which should present loads higher than 0.50 and be significant ($p < 0.05$), indicating the presence of convergent validity. In addition, the loads should not be excessively high, and discriminant validity should exist because each indicator must measure a different aspect of the construct (Kline 2004). For the present study, a latent variable was estimated. The indicators of the latent variable, complex B vitamin consumption, were dietary intake of vitamin B_6 (pyridoxine), vitamin B_2 (riboflavin), and folate. In SEM, latent variables are represented by circles or ellipses. The directly observed variables, in turn, are represented by squares or rectangles (Fig. 1).

In SEM, explanatory variables may affect the outcome variable directly or indirectly. Direct effects represent the direct relationships between 2 variables (i.e., relationships that are not mediated by other model variables) and can be interpreted similarly to a regression coefficient. Indirect effects, in turn, express a sequence of paths with at least 1 intermediate or mediating variable and are calculated by multiplying the direct effects between the variables belonging to that path. Finally, the total effect is calculated from the sum of the direct and indirect effects between 2 variables (Kline 2004; Hair et al. 2009). It is important to clarify that, in this study, the term "effect" is used in the sense of association, not causality.

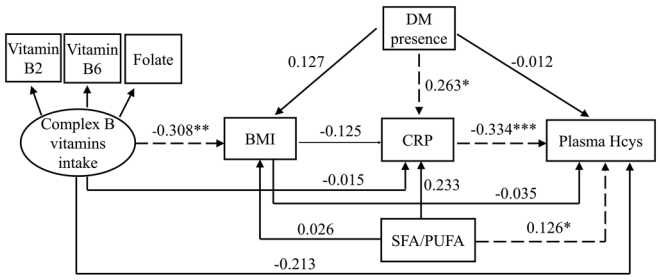
The standardized coefficients (SC) with their respective SE values and the *p* value were estimated. The SC indicates the effect, expressed in units of SD, in the response variable in relation to the variation of 1 SD in the explanatory variable. In SEM, because each variable is measured in a different unit, the use of standardized estimates facilitates the interpretation and comparison of results. The robust maximum likelihood method was used to estimate the parameters. This is a robust method that does not require the assumption of normal multivariate data distribution (Kline 2004).

In the evaluation of the variables associated with Hcys, additional adjustment was made for age, sex, and smoking, identified as predictors of its concentration (Ganji and Kafai 2003). To verify the fit of the model, some measures were analyzed. The root mean square error of approximation (RMSEA) and the standardized root mean square residual (SRMR) are based on model residuals, with values < 0.06 indicating that the theoretical model fits the data (Hu and Bentler 1999; Hooper et al. 2008). Moreover, the Tucker–Lewis index (TLI) and the comparative fit index (CFI), in which values above 0.90 indicate a good fit of the model (Kline 2004; Baltar et al. 2013), were also used.

Results

The median Hcys and CRP levels were 20.6 μmol/L (interquartile range (IQR), 15.9–27.0) and 4.2 mg/L (IQR, 1.8–13.5), respectively.

Fig. 1. Structural equation model for the relationship among food intake, clinical-nutritional status, inflammation, and plasma homocysteine (Hcys) in hemodialysis subjects. In structural equation modeling, all variables were considered continuous, with the exception of the presence of diabetes mellitus (DM). Associations with Hcys were further adjusted for age, sex, and smoking. BMI, body mass index; CRP, C-reactive protein; SFA/PUFA, relationship between intake of saturated and polyunsaturated fatty acids. Dashed lines indicate paths with statistical significance. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.



Among participants, 79.1% had hyperhomocysteinemia and 62.0% had high cardiovascular risk by CRP values.

Hcys median values were not statistically different ($p > 0.05$) according to sociodemographic, clinical-nutritional, and food consumption variables (Table 1). T1

The factor loadings of the indicators of the latent variable complex B vitamin intake were statistically significant, with the lowest load observed for folate intake (0.521) and the highest for vitamin B_2 intake (0.715) (Table 2). The SEM presented satisfactory T2 adjustment indices. The RMSEA was 0.014; SRMR, 0.050; CFI, 0.992; and TLI, 0.983, which suggest a good fit of the model (Table 2).

Figure 1 shows the SC for the direct effects of the SEM. CRP and Hcys were negatively associated (SC = −0.334; $p < 0.001$), indicating that the variation of 1 SD in CRP was associated with a 0.334 SD reduction in Hcys. The SFA/PUFA ratio, in turn, was positively associated with Hcys (SC = 0.126; $p = 0.046$). Positive association was also observed between the presence of DM and CRP (SC = 0.263; $p = 0.004$). Finally, an inverse relationship was observed between complex B vitamin intake and BMI (SC = −0.308; $p = 0.008$). Consumption of complex B vitamins, BMI, and the presence of DM did not have significant direct effects on Hcys (Fig. 1).

Table 3 presents, in addition to the direct effects, the indirect T3 and total effects for the relationships of the variables of food intake, clinical-nutritional status, and inflammation with Hcys. A significant indirect effect was observed only for a PCR-mediated relationship between the presence of DM and Hcys (SC = −0.088; $p = 0.022$). The SFA/PUFA ratio had only a direct effect on Hcys (SC = 0.009; $p = 0.046$). Clinical-nutritional status and complex B vitamin intake did not, in turn, have a significant direct, indirect, or total effect on Hcys.

Discussion

The study of the factors that trigger Hcys increase is a great challenge because this condition has several causes. Investigation of the mechanisms involved in Hcys' increment is relevant for the development of early intervention strategies and, consequently, decreased cardiovascular risk; this risk is highly prevalent in this population (Wald et al. 2012).

To our knowledge, this is the first study to investigate the role of inflammation in the relationship between clinical and nutritional factors and plasma Hcys. As a first result, CRP, a recognized inflammatory marker, was inversely associated with Hcys. This result can be attributed to a close and inverse relationship between malnutrition and inflammation (Zhang et al. 2016). Persistent low-grade systemic inflammation increases levels of circulating inflammatory markers such as CRP, interleukin-6, and tumor necro-

Table 1. Median values of plasma homocysteine according to sociodemographic, clinical-nutritional, and food intake variables of hemodialysis subjects (n = 129).

Variables	No. (%)	Plasma homocysteine (μmol/L) Median	Interquartile range	p
Sex				
Male	76 (58.9)	21.0	16.1–27.0	0.461
Female	53 (41.1)	19.9	15.0–26.1	
Age				
Elderly	78 (60.5)	20.6	16.4–24.9	0.704
Adult	51 (39.5)	20.7	15.4–28.2	
Smoker/former smoker				
No	100 (77.5)	20.7	16.4–27.6	0.133
Yes	29 (22.5)	19.9	14.5–23.3	
Presence of DM				
No	81 (62.8)	20.7	16.3–27.0	0.607
Yes	48 (37.2)	20.5	15.1–26.6	
BMI				
Low weight	28 (21.7)	18.8	15.7–22.9	0.201
Eutrophy	66 (51.2)	21.2	16.8–28.1	
Overweight	35 (27.1)	21.1	15.8–23.9	
CRP				
Low cardiovascular risk	17 (13.2)	20.5	15.5–23.1	0.068
Medium cardiovascular risk	33 (25.6)	23.2	19.2–28.8	
High cardiovascular risk	79 (61.2)	19.9	14.8–25.9	
SFA/PUFA*				
>Median (1.86)	51 (50.5)	21.0	17.2–26.1	0.761
≤Median (1.86)	50 (49.5)	20.9	15.1–27.4	
Vitamin B_2 intake*				
>Median (1.26 mg/d)	51 (50.5)	19.9	15.9–25.1	0.151
≤Median (1.26 mg/d)	50 (49.5)	21.7	16.6–31.0	
Vitamin B_6 intake*				
>Median (1.07 mg/d)	51 (50.5)	20.0	15.6–25.2	0.245
≤Median (1.07 mg/d)	50 (49.5)	21.6	17.4–28.4	
Folate intake*				
>Median (233.81 μg/d)	51 (50.5)	20.1	15.9–24.5	0.086
≤Median (233.81 μg/d)	50 (49.5)	22.1	17.0–29.3	

Note: BMI, body mass index; CRP, C-reactive protein; DM, diabetes mellitus; SFA/PUFA, relationship between intake of saturated and polyunsaturated fatty acids.
*Food intake variables: missing data for 28 subjects.

Table 2. Standardized coefficients for the latent variable and adjustment indices of the structural equation model.

Complex B vitamin intake			
	Standardized coefficient	SE	p
Folate	0.521	0.114	<0.001
Vitamin B_2	0.715	0.119	<0.001
Vitamin B_6	0.653	0.110	<0.001

Final model adjustment	
	Adjustment values
RMSEA	0.014
SRMR	0.050
CFI	0.992
TLI	0.983

Note: CFI, comparative fit index; RMSEA, root mean square error of approximation; SE, standard error; SRMR, standardized root mean square residual; TLI, Tucker–Lewis index.

sis factor-α (Dai et al. 2017), and subsequently, elevated CRP levels could inhibit albumin synthesis in HD patients (Kaysen 2009). In addition, > 70% of plasma Hcys bonds to protein, and albumin is the main Hcys-binding protein. Hence, the level of serum albumin is a strong determinant of the Hcys level in HD patients (Suliman et al. 2004). In fact, the CRP/albumin ratio showed a significant inverse association with Hcys (SC = −0.322; p = 0.002); that is, the higher the CRP, the lower the albumin and, consequently, the lower the Hcys.

This inverse association between Hcys and inflammation has also been observed in other studies in HD patients. Akgul and colleagues (2008) found that the CRP concentration was significantly higher in the group with the lowest Hcys level. Suliman and colleagues (2004) demonstrated that before the initiation of dialysis, subjects with end-stage renal disease with an inflammation diagnosis had lower Hcys and serum albumin levels than those in subjects without inflammation, and that CRP levels correlated negatively with Hcys level. Ducloux and colleagues (2006) reported that the effect of Hcys is dependent on a patient's nutritional and inflammatory status, and that those factors cannot be analyzed separately.

In fact, in our sample, the prevalence of hyperhomocysteinemia and high CRP, as in other studies, was high. In this sense, our results together suggest that inflammation and Hcys should be considered important cardiovascular risk factors in these individuals. In addition, the presence of Hcys should be interpreted with caution and cannot be considered in isolation without taking into account other factors such as inflammation.

In addition to the direct effect of inflammation on Hcys, our results show that the impact of the DM presence on plasma Hcys was mediated by inflammation. Controversial findings still exist about the impact of DM on Hcys levels (i.e., Hcys has been shown to be elevated or lower in diabetic subjects as compared with nondiabetics (Audelin and Genest 2001; Moraba et al. 2013)). Moreover, chronic hyperglycemia in DM causes oxidative damage and activates inflammatory signaling cascades (Brownlee 2001), in addition to increases in pro-inflammatory cytokines (Wellen and Hotamisligil 2005) and infiltrated macrophages (Derosa et al. 2013), thereby leading to local and systemic inflammation. Thus, we found that such controversial results regarding DM and Hcys may be related to better or worse glycemic control in these patients, affecting the inflammatory state, and consequently, Hcys.

Another important and unedited result of our study is the direct relation between dietary SFA/PUFA ratio and plasma Hcys. Although the mechanisms are not fully understood, a potential mechanism may be that n-3 PUFA, especially 22:6 n-3, modulates the gene expression of enzymes involved in the formation and metabolism of plasma total Hcys (Li et al. 2006). Huang and colleagues (2012) conducted a study of cell culture to examine the nutritional regulation of n-3 PUFA (22:6n-3, docosahexaenoic acid; 20:5n-3, eicosapentaenoic acid; 18:3n-3, α-alanine) on the messenger RNA (mRNA) expression of the genes encoding the key enzymes involved in Hcys metabolism. They observed that n-3 PUFA up-regulates cystathionine-γ-lyase and 5-methyltetrahydrofolate reductase mRNA expression and down-regulates the mRNA expression of the methionine adenosyltransferase involved in Hcys metabolism. The authors suggested that this regulatory effect on gene expression is associated with a decreased Hcys concentration. In addition, methionine synthase, the enzyme responsible for Hcys remethylation, is susceptible to indirect oxidative inactivation via inactivation of the cofactor cobalamin (Chen and Banerjee 1998). Studies evaluating the association between food intake and HD outcomes are scarce, but our results reinforce the importance of dietary assessment in this population for better clinical-metabolic and nutritional control.

Finally, the consumption of complex B vitamins was inversely associated with BMI. Studies in children (Gunanti et al. 2014) and adults (Pereira et al. 2019) also observed a negative association

Table 3. Direct, indirect, and total coefficients for the mediation relations of the structural equation model.

Relationship	Mediators	Effects	Standardized coefficient	SE	*p*
DM presence → plasma Hcys		Direct	–0.012	0.093	0.901
	BMI	Indirect	–0.004	0.012	0.716
	CPR	Indirect	–0.088	0.038	**0.022**
	BMI → CRP	Indirect	0.005	0.006	0.402
		Total	–0.099	0.091	0.278
Complex B vitamins intake → plasma Hcys		Direct	–0.213	0.134	0.113
	BMI	Indirect	0.011	0.028	0.702
	CRP	Indirect	0.005	0.061	0.934
	BMI → CRP	Indirect	–0.013	0.013	0.325
		Total	–0.210	0.142	0.139
SFA/PUFA → plasma Hcys		Direct	0.126	0.063	**0.046**
	BMI	Indirect	0.000	0.002	0.986
	CRP	Indirect	–0.078	0.048	0.102
	BMI → CRP	Indirect	0.000	0.003	0.986
		Total	0.048	0.050	0.340
BMI → plasma Hcys	CRP	Direct	–0.035	0.090	0.697
		Indirect	0.042	0.040	0.304
		Total	0.007	0.096	0.945

Note: *p* value in bold has statistical significance ($p < 0.05$). BMI, body mass index; CRP, C-reactive protein; DM, diabetes mellitus; Hcys, homocysteine; SE, standard error; SFA/PUFA, relationship between intake of saturated and polyunsaturated fatty acids.

between consumption of these vitamins and excess body mass/adiposity. Complex B vitamins play important roles in energy homeostasis, thermoregulation, and bioenergy metabolism (Huskisson et al. 2007; Depeint et al. 2006). Deficiencies in biotin, niacin, folate, and vitamins B_6 and B_{12} may affect energy metabolism, leading to increased production of reactive oxidants and increased inflammatory responses, and may also promote lipogenesis, leading to increased adiposity (Ullegaddi et al. 2004; Folsom et al. 2003). However, it is not known whether these associations result from alterations in the physiology and metabolism of complex B vitamins because of increased fat mass or from increases in adipogenesis among individuals with pre-existing vitamin B deficiencies or vitamin B-deficient diets (Gunanti et al. 2014). Thus, additional studies are needed to elucidate this issue, especially in HD individuals, who, as seen previously, may have low consumption, and in whom deficiency of these vitamins is common.

As a positive point, we can highlight that we did not find studies that used the SEM in this thematic; a differential of this study was the evaluation of the mediating effect of inflammation on the relationships among food intake, clinical-nutritional status, and Hcys plasma. However, this study has some limitations. The cross-sectional design did not allow us to assess the temporal direction of the investigated associations. Moreover, FFQ requires that individuals have the cognitive skill to remember the consumption of food items listed in the instrument and to distinguish the frequency of consumption over a previous period of time, thus ensuring that the response reflects the usual diet (Heber et al. 2006).

In conclusion, the SFA/PUFA ratio had a direct effect on plasma Hcys, whereas inflammation had a direct and mediating effect on the relationship of Hcys with DM in HD subjects. The direct relationship of SFA/PUFA with plasma Hcys indicates the importance of assessing food intake and adopting strategies for food and nutrition education to promote improvements in the quantity and quality of ingested nutrients and to reduce the high cardiovascular risk present in this population.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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References

Akgul, A., Bilgic, A., Sezer, S., Arat, Z., Ozdemir, F.N., and Haberal, M. 2008. Low total plasma homocysteine level in relation to malnutrition, inflammation, and outcome in hemodialysis patients. J. Ren. Nutr. **18**(4): 338–346. doi:10.1053/j.jrn.2007.11.007. PMID:18558298.

Amorim, L.D., Fiaccone, R.L., Santos, C.A., Santos, T.N., Moraes, L.T.L.P., Oliveira, N.F., et al. 2010. Structural equation modeling in epidemiology. Cad. Saúde Pública, **26**(12): 2251–2262. doi:10.1590/S0102-311X2010001200004. PMID:21243220.

Audelin, M.C., and Genest, J., Jr. 2001. Homocysteine and cardiovascular disease in diabetes mellitus. Atherosclerosis, **159**(2): 497–511. doi:10.1016/S0021-9150(01)00531-7. PMID:11730832.

Balbino, K.P., Epifânio, A.P.S., Ribeiro, S.M.R., da Silva, L.D.M., Gouvea, M.G., and Hermsdorff, H.H.M. 2017. Comparison between direct and indirect methods to diagnose malnutrition and cardiometabolic risk in haemodialysis patients. J. Hum. Nutr. Diet. **30**(5): 646–654. doi:10.1111/jhn.12468. PMID:28332268.

Baltar, V.T., Xun, W.W., Johansson, M., Ferrari, P., Chuang, S.C., Relton, C., et al. 2013. A structural equation modeling approach to explore the role of B vitamins and immune markers in lung cancer risk. Eur. J. Epidemiol. **28**(8): 677–688. doi:10.1007/s10654-013-9793-z. PMID:23532743.

Bazeley, J., Bieber, B., Li, Y., Morgenstern, H., de Sequera, P., Combe, C., et al. 2011. C-reactive protein and prediction of 1-year mortality in prevalent hemodialysis patients. Clin. J. Am. Soc. Nephrol. **6**(10): 2452–2461. doi:10.2215/CJN.00710111. PMID:21868617.

Brazilian Institute of Geography and Statistics. 2011. Research of Family Budgets. 2008–2009: Tables of nutritional composition of foods consumed in Brazil/IBGE, Coordination of Work and Income. Rio de Janeiro, Brazil.

Brazilian Table of Food Composition/Center for Studies and Research in Food. 2011. 4th ed., revised and expanded. NEPA- UNICAMP, Campinas, São Paulo, Brazil. 161pp.

Brownlee, M. 2001. Biochemistry and molecular cell biology of diabetic complications. Nature, **414**(6865): 813–820. doi:10.1038/414813a. PMID:11742414.

Chen, Y.F., Li, P.L., and Zou, A.P. 2001. Effect of hyperhomocysteinemia on plasma or tissue adenosine levels and renal function. Circulation, **106**(10): 1275–1281. doi:10.1161/01.cir.0000027586.64231.1b. PMID:12208805.

Chen, Z., and Banerjee, R. 1998. Purification of soluble cytochrome b_5 as a component of the reductive activation of porcine methionine synthase. J. Biol. Chem. **273**(40): 26248–26255. doi:10.1074/jbc.273.40.26248. PMID:9748308.

Dai, L., Golembiewska, E., Lindholm, B., and Stenvinkel, P. 2017. End-stage renal

disease, inflammation and cardiovascular outcomes. Contrib. Nephrol. **191**: 32–43. doi:10.1159/000479254. PMID:28910789.

Depeint, F., Bruce, W.R., Shangari, N., Mehta, R., and O'Brien, P.J. 2006. Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism. Chem. Biol. Interact. **163**(1–2): 94–112. doi:10.1016/j.cbi.2006.04.014. PMID:16765926.

Derosa, G., Fogari, E., D'Angelo, A., Bianchi, L., Bonaventura, A., Romano, D., et al. 2013. Adipocytokine levels in obese and non-obese subjects: an observational study. Inflammation, **36**(4): 914–920. doi:10.1007/s10753-013-9620-4. PMID:23467997.

Ducloux, D., Klein, A., Kazory, A., Devillard, N., and Chalopin, J.M. 2006. Impact of malnutrition-inflammation on the association between homocysteine and mortality. Kidney Int. **69**(2): 331–335. doi:10.1038/sj.ki.5000096. PMID:16408123.

Folsom, A.R., Desvarieux, M., Nieto, F.J., Boland, L.L., Ballantyne, C.M., and Chambless, L.E. 2003. B vitamin status and inflammatory markers. Atherosclerosis **169**(1): 169–174. doi:10.1016/s0021-9150(03)00161-8. PMID:12860264.

Ganji, V., and Kafai, M.R. 2003. Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988–1994. Am. J. Clin. Nutr. **77**(4): 826–833. doi:10.1093/ajcn/77.4.826. PMID:12663279.

Gunanti, I.R., Marks, G.C., Al-Mamun, A., and Long, K.Z. 2014. Low serum vitamin B-12 and folate concentrations and low thiamin and riboflavin intakes are inversely associated with greater adiposity in Mexican American children. J. Nutr. **144**(12): 2027–2033. doi:10.3945/jn.114.201202. PMID:25411037.

Hair, J.F., Black, W.C., Babin, B.J., Anderson, R.E., and Tatham, R.L. 2009. Multivariate Data Analysis. 6th ed. Bookman Publishing.

Heber, D., Blackburn, G.L., Go, V.L.W., and Milner, J. 2006. Nutritional Oncology. 2nd ed. Dietary Assessment. Elsevier, Calif., USA. pp. 367–375.

Hooper, D., Coughlan, J., and Mullen, M. 2008. Structural equation modeling: guidelines for determining model fit. EJBRM, **6**(1): 53–60. doi:10.21427/D7CF7R.

Hu, L., and Bentler, P.M. 1999. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. Struct. Equat. Model. **6**: 1–55. doi:10.1080/10705519909540118.

Huang, T., Wahlqvist, M.L., and Li, D. 2012. Effect of n-3 polyunsaturated fatty acid on gene expression of the critical enzymes involved in homocysteine metabolism. Nutr. J. **11**: 6. doi:10.1186/1475-2891-11-6. PMID:22260268.

Huskisson, E., Maggini, S., and Ruf, M. 2007. The role of vitamins and minerals in energy metabolism and well-being. J. Int. Med. Res. **35**(3): 277–289. doi:10.1177/147323000703500301. PMID:17593855.

Jamison, R.L., Hartigan, P., Kaufman, J.S., Goldfarb, D.S., Warren, S.R., Guarino, P.D., and Gaziano, J.M. 2007. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease. JAMA, **298**(10): 1163–1170. doi:10.1001/jama.298.10.1163. PMID:17848650.

Jankowska, M., Cobo, G., Lindholm, B., and Stenvinkel, P. 2017. Inflammation and protein-energy wasting in the uremic milieu. Contrib. Nephrol. **191**: 58–71. doi:10.1159/000479256. PMID:28910791.

Kalantar-Zadeh, K., Block, G., Humphreys, M.H., and Kopple, J.D. 2003. Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. Kidney Int. **63**(3): 793–808. doi:10.1046/j.1523-1755.2003.00803.x. PMID:12631061.

Kalantar-Zadeh, K., Kovesdy, C.P., Bross, R., Benner, D., Noori, N., Murali, S.B., et al. 2011. Design and development of a dialysis food frequency questionnaire. J. Ren. Nutr. **21**(3): 257–262. doi:10.1053/j.jrn.2010.05.013. PMID:20833073.

Kaysen, G.A. 2009. Biochemistry and biomarkers of inflamed patients: why look, what to assess. Clin. J. Am. Soc. Nephrol. **4**(Suppl. 1): S56–S63. doi:10.2215/CJN.03090509. PMID:19996007.

Kline, R.B. 2004. Principles and Practice of Structural Equation Modeling. 2nd ed. Guilford Press, New York, N.Y., USA.

Lai, S., Mecarelli, O., Pulitano, P., Romanello, R., Davi, L., Zarabla, A., et al. 2016. Neurological, psychological, and cognitive disorders in patients with chronic kidney disease on conservative and replacement therapy. Medicine, **95**(48): e5191. doi:10.1097/MD.0000000000005191. PMID:27902586.

Li, D., Mann, N.J., and Sinclair, A.J. 2006. A significant inverse relationship between concentrations of plasma homocysteine and phospholipid docosahexaenoic acid in healthy male subjects. Lipids, **41**(1): 85–89. doi:10.1007/s11745-006-5074-x. PMID:16555476.

Lipschitz, D.A. 1994. Screening for nutritional status in the elderly. Prim. Care, **21**(1): 55–67. PMID:8197257.

Manns, B., Hyndman, E., Burgwss, E., Parsons, H., Schaefer, J., Snyder, F., et al. 2001. Oral vitamin B12 and high-dose folic acid in hemodialysis patients with hyperhomocysteinemia. Kidney Int. **59**(3): 1103–1109. doi:10.1046/j.1523-1755.2001.00594.x. PMID:11231366.

Monteiro, J.P., Pfrimer, K., Tremeschin, M.H., Molina, M.C.C., and Chiarello, P. 2010. Food consumption viewing portions. Guanabara Koogan, São Paulo, Brazil.

Moraba, M., Sengwayo, D., and Motaung, S. 2013. Association of homocysteinaemia with hyperglycaemia, dyslipidaemia, hypertension and obesity. Cardiovasc. J. Afr. **24**(7): 265–269. doi:10.5830/CVJA-2013-059. PMID:24217303.

Myers, G.L., Rifai, N., Tracy, R.P., Roberts, W.L., Alexander, R.W., Biasucci, L.M., et al. 2004. CDC/AHA workshop on markers of inflammation and cardiovascular disease: application to clinical and public health practice: report from the laboratory science discussion group. Circulation, **110**(25): e545–e549. doi:10.1161/01.CIR.0000148980.87579.5E. PMID:15611379.

Panichi, V., Rizza, G.M., Paoletti, S., Bigazzi, R., Aloisi, M., Barsotti, G., et al. 2008. Chronic inflammation and mortality in haemodialysis: effect of different renal replacement therapies. results from the RISCAVID study. Nephrol. Dial. Transplant. **23**(7): 2337–2343. doi:10.1093/ndt/gfm951. PMID:18305316.

Pereira, G.A., Bressan, J., Oliveira, F.L.P., Sant'Ana, H.M.P., Pimenta, A.M., Lopes, L.L., et al. 2019. Dietary folate intake is negatively associated with excess body weight in Brazilian graduates and postgraduates (CUME Project). Nutrients, **11**(3) pii:E518. doi:10.3390/nu11030518. PMID:30823410.

Selhub, J., and Troen, A.M. 2016. Sulfur amino acids and atherosclerosis: a role for excess dietary methionine. Ann. N.Y. Acad. Sci. **1363**: 18–25. doi:10.1111/nyas.12962. PMID:26647293.

Stuhlinger, M.C., Tsao, P.S., Her, J.H., Kimoto, M., Balint, R.F., and Cooke, J.P. 2001. Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. Circulation, **104**(21): 2569–2575. doi:10.1161/hc4601.098514. PMID:11714652.

Suliman, M.E., Stenvinkel, P., Qureshi, A.R., Bárány, P., Heimburger, O., Anderstam, B., et al. 2004. Hyperhomocysteinemia in relation to plasma free amino acids, biomarkers of inflammation and mortality in patients with chronic kidney disease starting dialysis therapy. Am. J. Kidney Dis. **44**(3): 455–465. doi:10.1016/S0272-6386(04)00815-7. PMID:15332218.

Tinelli, C., Di Pino, A., Ficulle, E., Marcelli, S., and Feligioni, M. 2019. Hyperhomocysteinemia as a risk factor and potential nutraceutical target for certain pathologies. Front. Nutr. **6**: 49. doi:10.3389/fnut.2019.00049. PMID:31069230.

Ullegaddi, R., Powers, H.J., and Gariballa, S.E. 2004. B-group vitamin supplementation mitigates oxidative damage after acute ischaemic stroke. Clin. Sci. **107**(5): 477–484. doi:10.1042/CS20040134. PMID:15279619.

Wald, R., Yan, A.T., Perl, J., Jiang, D., Donnelly, M.S., Leong-Poi, H., et al. 2012. Regression of left ventricular mass following conversion from conventional hemodialysis to thrice weekly in-centre nocturnal hemodialysis. BMC Nephrol. **13**: 3. doi:10.1186/1471-2369-13-3. PMID:22260388.

Wellen, K.E., and Hotamisligil, G.S. 2005. Inflammation, stress, and diabetes. J. Clin. Investig. **115**(5): 1111–1119. doi:10.1172/JCI200525102. PMID:15864338.

Willett, W.C., and Stampfer, M. 1998. Implications of total energy intake for epidemiologic analyses. *In* Nutritional Epidemiology. *Edited by* W.C. Willett. Oxford University Press, New York, N.Y., USA. pp. 272–273.

World Health Organization. 2000. Obesity: preventing and managing the global epidemic: report of a WHO consultation. WHO Technical Report Series 894, World Health Organization. Geneva, Switzerland.

Zhang, K., Gao, J., Chen, J., Liu, X., Cai, Q., Liu, P., and Huang, H. 2016. MICS, an easily ignored contributor to arterial calcification in CKD patients. Am. J. Physiol. Renal Physiol. **311**(4): F663–F670. doi:10.1152/ajprenal.00189.2016. PMID:27335374.