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Original article

Dark chocolate (70% cocoa) attenuates the inflammatory marker TNF-α in patients on hemodialysis

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SUMMARY

Background: Inflammation and oxidative stress lead to a high risk of cardiovascular disease in patients with chronic kidney disease (CKD). Food rich in polyphenols such as dark chocolate may be an effective strategy to mitigate inflammation and delay CKD complications, outwith sensorial pleasure promotion. The aim of this study was to evaluate the effects of dark chocolate on inflammation and oxidative stress markers in patients with CKD on hemodialysis (HD).

Methods: A clinical trial was carried out with 59 patients who were allocated into the chocolate group [40g of dark chocolate (70% cocoa) offered during HD sessions, 3×/week] or the control group with any intervention for two months. Plasma levels of the inflammatory cytokines TNF-α and IL-6 were evaluated by the ELISA method. Thiobarbituric acid reactive substances such as malondialdehyde (MDA) and LDLox levels were evaluated as lipid peroxidation markers. Routine biochemical parameters were analysed using commercial BioClin® kits.

Results: Thirty-five patients completed the chocolate group (18 men, 53.0 (16) years and 31.0 (39) months on HD) and 11 in the control group (7 men, 48.0 (17.5) years and 44.0 (56.5) months on HD). Regarding the differences between the groups, the patients who received dark chocolate had reduced plasma levels of TNF-α compared to the control ($p = 0.008$). No significant changes were observed in the oxidative stress parameters evaluated in both groups. Routine biochemical (including phosphorus and potassium levels) and anthropometric parameters and food intake were not changed after the study period.

Conclusion: The intervention with dark chocolate (70% cocoa) for two months reduced the plasma levels of TNF-α in patients with CKD on HD. In addition, it is essential to emphasise that chocolate intake did not increase the plasma levels of phosphorus and potassium in these patients. This study was registered at clinicaltrials.gov as NCT04600258.

1. Introduction

Oxidative stress and inflammation present in chronic kidney disease (CKD) patients are described in the literature as the main contributors to the accelerated loss of renal function and also to a high rate of cardiovascular mortality in this population [1,2].

The inflammatory scenario in these patients can be explained by several factors, including uremia *per se*, immune system dysfunctions, accumulation of toxins in the blood, transcription factors activation, and dialysis procedures [3]. Inflammation is directly related to increased production of reactive oxygen species (ROS), causing oxidative stress [4], that in turn, also induces inflammation by activating the nuclear factor-kB (NF-kB) pathway, inducing inflammatory cytokines production such as tumour necrosis factor-alpha (TNF-α) and interleukins (IL) [5].

Therefore, in order to mitigate the inflammatory and oxidative process in these patients, much has been discussed about strategies to help delay CKD complications, especially concerning non-

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pharmacological treatments, encompassing the concept of "Food as Medicine" [6]. Bioactive compounds in several foods such as teas, wine, berries, fruit, vegetables and dark chocolate have immunoregulatory, antioxidant and anti-inflammatory properties [6,7,8,9].

Besides being a food of the gods and considered a sacred food for many chocolate lovers, chocolate is the source of several natural bioactive substances, such as polyphenols, flavonols, methylxanthines and proanthocyanidins [10]. It has been the focus of several studies, showing neuroprotective [11], anti-inflammatory [12], anti-oncogenic [13] and cardioprotective [14] effects, especially dark chocolate, with higher cocoa content and, consequently, more phenolic compounds and flavonoids than other chocolate types of chocolates.

According to current literature, the anti-inflammatory role of dark chocolate can be explained by the action of epicatechin, a flavonoid present in cocoa, capable of increasing the expression of erythroid nuclear factor 2 related to factor 2 (Nrf2), known as the primary factor transcription of endogenous defence, essential for the anti-inflammatory response and reduction of oxidative stress [9,15,16]. In addition, research elucidates that consumption of dark chocolate is associated with reduced blood pressure, improved endothelial function and dyslipidaemia [17–19]. However, studies are scarce concerning the effects of dark chocolate on patients with CKD, maybe due to the usual restriction on eating it since it may increase the potassium and phosphorus plasma levels. For a long time, the consumption of dark chocolate by patients with CKD was discontinued due to its phosphorus and potassium content [9]. Our perspective is to assess whether, in controlled doses, patients with CKD on HD could benefit from this food rich in bioactive compounds.

According to our literature search, there is only one study evaluating cocoa's effects on HD's endothelial function in CKD patients [20]. Thus, this study aims to assess whether dark chocolate consumption leads to positive effects in patients with CKD on HD, attenuating inflammation and oxidative stress without affecting plasma levels of potassium and phosphorus.

2. Methods

2.1. Patients

This longitudinal clinical trial was performed on 59 hemodialysis patients from the Associação Renal Vida clinic - Blumenau/SC, Brazil. According to the regular dialysis schedule, these patients undergo hemodialysis with a polysulfone membrane (three hemodialysis sessions per week, lasting 4 h each). The sample calculation of the research was performed using the G*Power software, with a test power of 80% [21], a significance level of 5% (two-tailed) and an effect size of 1,08. The sample obtained through the software was 30 patients, 15 in each group.

The Ethics Committee approved this study of the Faculty of Medicine of UFF-Niteroi, Rio de Janeiro-Brazil (CAAE 30116220.3.0000.5243), and it was registered in ClinicalTrials.gov under the number NCT04600258.

2.2. Inclusion and exclusion criteria

Patients with CKD on HD (both sexes, aged 20–65 years) with arteriovenous fistula (AVF) as vascular access (patients with catheter present high inflammatory status) who was on dialysis for more than six months (stable on dialysis) and following an individualised dietary prescription (25–35 kcal/kg/day and 1.0–1.2 g/kg/day of protein, as recommended by the NKF-KDOQI, 2020).

Patients with the habitual intake of any chocolate, antioxidant supplements and prebiotic, probiotic or symbiotic supplements were not included in the study; patients with allergy or intolerance to any of the ingredients present in chocolate were described in the section "Experimental Design"; smoking or pregnant patients; patients with infectious and autoimmune diseases, liver diseases, cancer, AIDS (acquired immunodeficiency syndrome), antibiotics and antithrombotic use were excluded.

2.3. Study design

Patients were allocated to the chocolate group, receiving standardised dark chocolate tablets (70% cocoa) at a dose of 40 g per serving on dialysis days (3×/week), totalling 30 days of intervention (two months). Patients received dark chocolate in the last hour of the dialysis session, and adherence to consumption was also verified. Patients in the control group went through the same period (2 months) without intervention. Blood samples were collected before and after the two months of the study.

The 70% dark chocolate ingredients used in this study were cocoa, cocoa butter, maltitol, polydextrose, sunflower lecithin emulsifier, natural sweetener thaumatin and natural vanilla extract, without lactose, gluten, sucrose, glucose and fructose. Table 1 shows the proximate composition of the chocolate used in the study (Nugali Chocolates®).

2.4. Food intake analysis

The assessment of food intake was performed at the beginning and end of the intervention using the three-day food record technique, covering two weekdays and one weekend day. The energy and macronutrient intake analysis was estimated using the NutWin® software.

2.5. Assessment of nutritional status

Due to the COVID-19 Pandemic and the recommendations of the Federal Council of Nutritionists in Brazil (CFN Resolution No. 646, March 18, 2020), the assessment of nutritional status was performed based on the collection of anthropometric data obtained from the patient's medical records. Information about body weight and height was collected. Nutritional status was evaluated according to the body mass index (BMI), obtained by the ratio between weight and height squared. Its classification followed that proposed by the World Health Organization (WHO, 1995; OMS, 1997).

2.6. Blood collection and biochemical analysis

Blood samples were obtained in the morning, before the dialysis procedure and immediately after the AVF puncture. Blood was collected in Vacutainer® tubes containing EDTA as an anticoagulant (1 mg/mL) or tubes without an anticoagulant. After collection, the blood was centrifuged at 3500 rpm for 15 min at 4 °C to obtain

Table 1
The centesimal composition of the 70% dark chocolate used in the study.

Parameters	Amount per serving (40g)
Energy (Kcal)	190,4
Carbohydrates (g)	14,88g
Proteins (g)	3,2g
Total fat (g)	15,52g
Saturated fat (g)	9,28g
Trans fats (g)	0,0g
Cholesterol (mg)	0,0 mg
Sodium (mg)	2,72 mg
Iron (mg)	7,68 mg
Calcium (mg)	17,6 mg

serum and plasma, distributed in 1.5 mL polypropylene eppendorfs® tubes and stored at −80 °C until further analysis.

2.7. Determination of the inflammatory cytokines

For the measurement of the inflammatory cytokines IL-6 and TNF-α, commercial ELISA kits (R&D Systems®) were used. TNF-α and IL-6 concentrations were measured within 31–2000 pg/mL using specific quantitative sandwich ELISA kits (PeproTech Inc.). A standard protocol was followed. Human capture TNF-α and IL-6 antibodies were diluted 1/100 (approximately 1 μg/mL) in PBS and used to coat a Maxisorp 96-well microplate overnight (Nunc, Fisher Scientific, Leicestershire) with 100 μl per well. The plates were washed with PBS +0.05% Tween and blocked with PBS +1% BSA before adding 100 μl samples and standards for 2 h at room temperature. Detection antibodies were used at a 1/660 dilution and incubated on the plates for 2 h. The streptavidin-HRP conjugate was diluted 1/2000 and incubated with 100 μl per well at room temperature for 30 min. Tetramethylbenzidine (Sigma, In) was used as a substrate for 20 min for colour development, and 1M HCl was used to stop the reaction before the optical density was read at 450 nm with correction at 650 nm. Standard curves were constructed, and samples were quantified only when the absorbance was in the linear portion of the standard curve.

2.8. Oxidative stress

Lipid peroxidation was used as a marker of oxidative stress, evaluated by thiobarbituric acid reactive substances (TBARS) using the modified Ohkawa method [22]. The samples were diluted with thiobarbituric acid (0.6% w/v), SDS (8.1% w/v) and phosphoric acid (1% w/v) and then heated at 95 °C for 60 min. The microtubules were centrifuged at 4000 rpm for 20 min at 20 °C, the supernatant was separated, and the absorbance was measured in a Synergy H1M microplate reader (Biotek) at 532 nm. Plasma levels of TBARS were expressed as nanomoles per millilitre.

Plasma levels of oxidized LDL (ELABSCIENCE) were also determined by a commercial ELISA kit (Elabscience ® - Catalog No: E-EL-H0124). Using the Sandwich-ELISA principle, the plate provided in this kit was pre-coated with an antibody specific for Human oxLDL. Samples were added to the plate wells and combined with the specific antibody. Afterwards, a detection antibody specific for Human oxLDL and the avidin-horseradish peroxidase (HRP) conjugate was added to each well of the microplate and incubated. Wells containing Human oxLDL, biotinylated detection antibody and Avidin-HRP conjugate reacted to form a blue colour. The enzyme–substrate reaction was stopped by adding a stop solution, changing the colour to yellow. Optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the Human OxLDL concentration. The concentration of Human oxLDL in the samples was calculated by comparing the OD of the samples with the standard curve.

2.9. Biochemical routine parameters

Plasma levels of biochemical parameters such as urea, creatinine, albumin, glucose, phosphorus, potassium, total cholesterol, high-density lipoprotein (HDL) and triglycerides were analysed using commercial kits from BioClin® according to the manufacturer.

2.10. Statistical analysis

The plasma levels of the variables studied were log-transformed when indicated. Various linear mixed-effects models were used to assess time–intervention interactions, and patients were considered as a random effect. Differences between final (2 months) and baseline times were assessed by multiple linear fixed-effects models and were adjusted for confounding variables (gender, age, and diabetes).

Results were presented graphically for the estimated average marginal effects and their 95% confidence intervals. All other variables in the mixed linear multiple models were held at their average values or equal proportions and by contrasts constructed from these average marginal effects estimated.

The Tukey Honest Significant Difference (HSD) method was used to correct p-values by the number of comparisons. p values < 0.05 were considered statistically significant. R software version 4.1.1, packages 'lme4', 'emmeans', and their dependencies were used to perform the statistical analyses.

3. Results

All 188 hemodialysis patients from Associação Renal Vida-Blumenau/SC were assessed for eligibility, and 59 patients met the inclusion criteria and were selected. Of these, 48 were allocated to the chocolate group, and 35 completed the intervention period in this group (18 men, 53.0 (16) years and 31.0 (39) months on HD). The other 11 selected patients (7 men, 48.0 (17.5) years and 44.0 (56.5) months on HD) were allocated to the control group, and all completed the study. Adherence to the intervention concerning absences was calculated at 85% in the chocolate group.

Regarding CKD aetiology, chronic glomerulonephritis was observed in 31.4% of the patients, with undetermined cause (17.1%), type I and II diabetic nephropathies (11.4%), hypertensive nephropathy and polycystic kidney disease (8.6%), other causes (5.7%), and chronic interstitial nephropathy and transplant rejection (2.9%).

Regarding the medications used, the most predominant were: Injectable Erythropoietin (r-HuEPO)/4000IU (77%), Iron Hydroxide (65.7%), 400 mg Sevelamer (57%) and Calcium Carbonate + Vitamin D3 (40%). There was no difference between groups, and they did not change during the study period.

The patients' baseline demographic and clinical parameters are described in Table 2. Table 3 shows the baseline values of biochemical parameters, and data on the food intake of the studied patients are shown in Table 4.

Figure 1 shows that plasma levels of phosphorus and potassium were not altered after ingestion of chocolate. The food intake, BMI and biochemical parameters of these patients during the study were not changed (data not shown).

Regarding the inflammation markers evaluated, there was no change in TNF-α and IL-6 plasma levels in both groups (Fig. 2). Likewise, no significant changes were observed in both groups regarding the oxidative stress markers evaluated, ox-LDL and MDA (Fig. 3).

Table 2
Baseline demographic and clinical parameters of patients with CKD on HD in chocolate supplementation and control groups.

Parameters	Chocolate group (n = 35)	Control group (n = 11)	p value
Demographics			
Age (years)	53.0 (16)	48.0 (17.5)	0.153
Gender % (n)			
Male	51.4 (18)	63.6 (7)	0.717
Female	48.6 (17)	36.4 (4)	0.717
Time in HD (months)	31.0 (39)	44.0 (56.5)	0.279
BMI (kg/m^2)	24.5 (5.25)	23.7 (5.55)	0.598

Data expressed as median and (interquartile range). **Abbreviations:** HD: Hemodialysis; BMI: Body mass index.

Table 3
Baseline biochemical parameters of patients with CKD on HD in chocolate supplementation and control groups.

Biochemical variables	Chocolate group (n = 35)	Control group (n = 11)	p-value
Glucose (mg/dL)	94.0 (34)	88.0 (89.5)	0.401
Total Cholesterol (mg/dL)	157.0 56.5)	174.0 (55)	0.857
Triglycerides (mg/dL)	140.0 (83)	93.0 (65)	0.094
HDL (mg/dL)	42.0 (12)	47.0 (14)	0.615
Calcium (mg/dL)	8.4 (1.2)	8.3 (0.75)	0.796
Phosphorus (mg/dL)	5.5 (1.95)	5.8 (1)	0.728
Potassium (mg/dL)	5.9 (0.75)	5.8 (1.05)	0.368
Sodium (mg/dL)	133.0 (3)	133.0 (3)	0.979
Urea (mg/dL)	136.0 (28.5)	136.0 (11)	0.554
Albumin (mg/dL)	4.0 (0.4)	4.0 (0.4)	0.383
C-Reactive Protein (mg/dL)	3.2 (5.75)	8.6 (7.6)	0.153
Hematocrit (%)	33.6 (9.5)	30.5 (8.55)	0.476
Hemoglobin (mg/dL)	10.8 (3.2)	9.7 (2.6)	0.390
Kt/V	1.5 (0.3)	1.5 (0.45)	0.123
TNF-alpha (pg/mL)	69.2 (125.3)	13.6 (8.7)	0.001
IL-6 (pg/mL)	93.3 (196.9)	57.3 (84.6)	0.080
oxLDL (pg/mL)	996.2 (270.1)	681.5 (205.9)	0.006
MDA (nmol/mL)	2.9 (3.65)	1.7 (2.1)	0.107

Data expressed as median and (interquartile range). **Abbreviations:** HDL: high-density lipoprotein; LDL: low-density lipoprotein; Kt/V: K – dialyzer clearance of urea, t – dialysis time, V – volume of distribution of urea; TNF-alpha: Tumor Necrosis Factors Alpha; IL-6: Interleukin-6; oxLDL: oxidized low-density lipoprotein; MDA: malondialdehyde.

Table 4
Food intake data of patients with CKD on HD in chocolate supplementation and control group.

Dietary variables	Chocolate group (n = 35)	Control group (n = 11)	p-value
Energy (Kcal/day)	1050.2 (298.3)	1323.1 (538.8)	0.393
Carbohydrates (g/day)	136.7 (51.1)	169.5 (84.9)	0.113
Protein (g/day)	49.5 (27.4)	56.8 (23.0)	0.501
Lipids (g/day)	34.5 (13.9)	36.6 (16.9)	0.262
Phosphorus (mg/day)	557.4 (426.4)	744.4 (388.5)	0.433
Potassium (mg/day)	1111.7 (560.5)	1092.7 (860.8)	0.604
Sodium (mg/day)	1312.4 (671.0)	1649.2 (691.5)	0.165
Calcium (mg/day)	286.8 (254.5)	324.3 (323.5)	0.510
Iron (mg/day)	7.3 (3.75)	9.4 (8.6)	0.101
Fibres (g/day)	12.6 (8.3)	14.4 (8.9)	0.911

Data expressed as median and (interquartile range).

However, when evaluating the differences between the control and chocolate groups after the two months of intervention, we observed that the chocolate group showed a reduction in plasma levels of TNF-α when compared to the control. Plasma levels of potassium, phosphorus, IL-6, ox-LDL and MDA between 2 months and baseline did not change after the interventions (Fig. 4).

4. Discussion

This study aimed to assess whether the consumption of dark chocolate by CKD patients on hemodialysis would be beneficial in mitigating inflammation and oxidative stress without affecting plasma phosphorus and potassium levels. After two months of intervention with dark chocolate (70% cocoa), we observed a significant reduction in the values of the inflammatory marker TNF-α in patients with CKD on hemodialysis. However, there were no changes in the inflammatory marker IL-6 and oxidative stress markers. The intervention did not affect biochemical parameters, food intake, and nutritional status, which makes this study interesting, showing that potassium and phosphorus did not change after chocolate consumption.

Dark chocolate has anti-inflammatory, anti-oncogenic, and cardioprotective effects [13,14], and the polyphenols stand out for the range of benefits found in chocolate. To the best of our knowledge, only one study evaluated cocoa intake in CKD patients on HD, and this study did not focus on the inflammatory state. [20]; in a randomised, double-blind, placebo-controlled study, observed that a 30-day intervention with a beverage rich in cocoa flavonols (900 mg/day) was effective in increasing basal flow-mediated dilation, reducing diastolic blood pressure and g endothelial dysfunction in 57 CKD patients on HD. According to the authors, such effects were possibly observed due to the ability of the flavonols present in cocoa to modulate nitric oxide levels by the plasmatic increase of nitrosothiol [23].

However, studies in non-CKD patients have shown that dark chocolate has effective anti-inflammatory properties. It was observed in diabetes patients that 30g of dark chocolate per day for eight weeks reduced the plasma levels of TNF-α [12,24]; associating the consumption of 30 g/d of dark chocolate (83% cocoa) with jumping rope exercises (3×/week, for six weeks) in obese patients, observed that dark chocolate intensified the reduction of IL-6 and TNF-α when compared with the groups that only performed the exercise.

Hypotheses can be raised to elucidate the action of dark chocolate in reducing TNF-α levels. The presence of phenolic compounds (especially procyanidins) gives dark chocolate a potent antioxidant character, making it capable of interfering with the enzymes involved in the synthesis of eicosanoids, leading to reduced levels of plasma leukotrienes and the

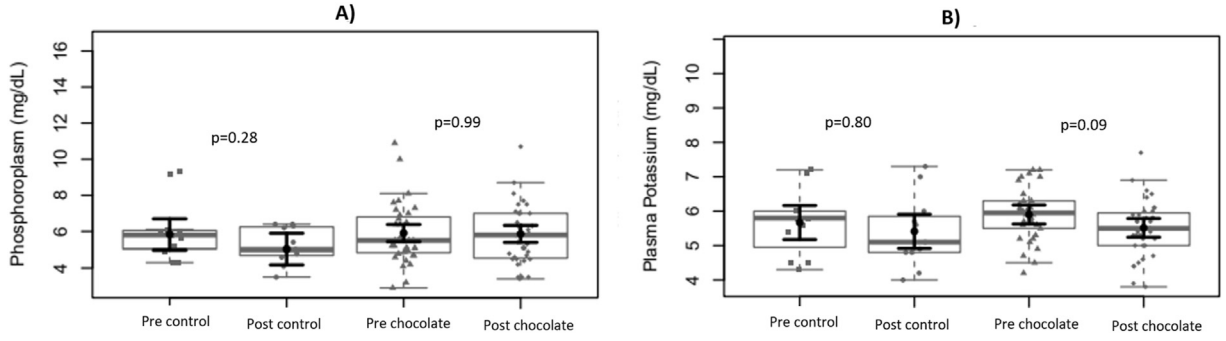

Fig. 1. Comparison of plasma levels of potassium and phosphorus before and after two months in the control and chocolate groups. We found no evidence of differences in plasma phosphorus (A) and potassium (B) levels after the intervention in the chocolate group nor after two months in the control group. The data sample distributions are represented in box plots and strip plots in grey. In black, the central circle represents the average marginal effect expected for each group estimated from linear mixed-effects models. The fixed effects in the models were the intervention group, the time, their interaction and the confounding variables Gender, Age, Hypertension and diabetes mellitus. Subjects were included as a random effect. The black horizontal bars represent the 95% confidence intervals of the expected mean marginal effects per group. The p-values were corrected for the number of contrasts/two-by-two comparisons by the Tukey Honest Significant Difference (HSD) method.

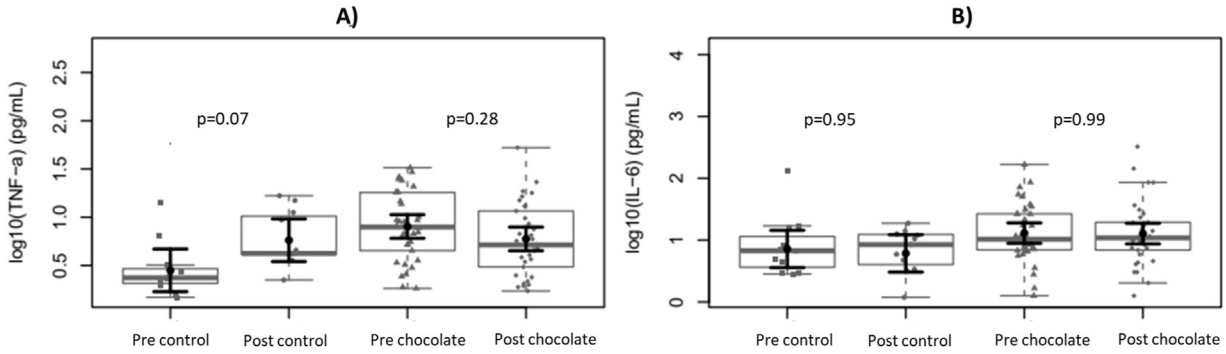

Fig. 2. Comparison of TNF-alpha and IL-6 plasma levels before and after two months in the control and chocolate groups. We found no evidence of differences in plasma levels of TNF-alpha (A) and IL-6 (B) after intervention in the chocolate group nor after two months in the control group. The data sample distributions are represented in box plots and strip plots in grey. In black, the central circle represents the average marginal effect expected for each group estimated from linear mixed-effects models. The fixed effects in the models were the intervention group, the time, their interaction and the confounding variables Gender, Age, Hypertension and diabetes mellitus. Subjects were included as a random effect. The black horizontal bars represent the 95% confidence intervals of the expected mean marginal effects per group. The p-values were corrected for the number of contrasts/two-by-two comparisons by the Tukey Honest Significant Difference (HSD) method.

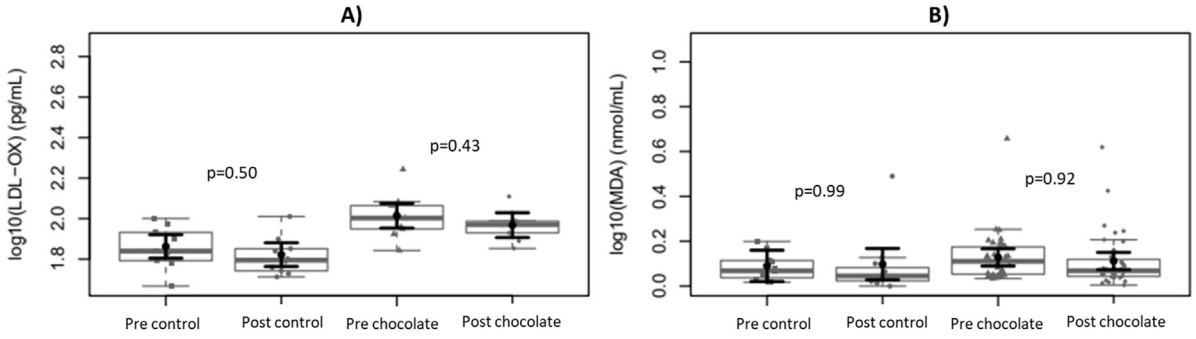

Fig. 3. Comparison of plasma levels of ox-LDL and MDA before and after two months in the control and chocolate groups. We found no evidence of differences in plasma levels of ox-LDL (A) and MDA (B) after the intervention in the chocolate group nor after two months in the control group. The data sample distributions are represented in box plots and strip plots in grey. In black, the central circle represents the average marginal effect expected for each group estimated from linear mixed-effects models. The fixed effects in the models were the intervention group, the time, their interaction and the confounding variables Gender, Age, Hypertension and diabetes mellitus. Subjects were included as a random effect. The black horizontal bars represent the 95% confidence intervals of the expected mean marginal effects per group. The p-values were corrected for the number of contrasts/two-by-two comparisons by the Tukey Honest Significant Difference (HSD) method.

leukotriene-prostacyclin ratio, factors that are involved in the inflammatory process [12,25]. In addition, another contributing factor is the ability of phenolic compounds to directly interfere with the NF-kB pathway, a key transcription factor involved in the inflammatory response [26,27]. The literature has already described that epicatechin (a phenolic compound present in chocolate) can inhibit IkB-kinases, resulting in the suppression of phosphorylation and degradation of IkB, and finally in the inactivation of NF-kB [28,29].

However, despite evidence supporting the anti-inflammatory role of dark chocolate in reducing inflammatory cytokines, our study did not observe significant changes in the serum levels of the inflammatory cytokine IL-6. Monagas et al. (2009) [30] also did not find any change in the levels of IL-6 and hs-CRP after supplementation with cocoa powder (40 g/day) in patients at high risk of cardiovascular disease. Such discrepancy can be explained due to the variability and quantification of circulating concentrations of cytokines present in these patients [31,32].

Although the hypothesis that flavonoid compounds present in dark chocolate can sequester and eliminate reactive oxygen species exerting direct antioxidant activity [33], we did not observe significant changes after the 2-month intervention on lipid peroxidation markers. A similar result was also seen by [34] when evaluating the effects of 100g of dark chocolate on arterial function in healthy individuals [35] also did not observe changes in MDA plasma levels in patients with type 2 diabetes who received 20 g/d of cocoa powder for six weeks. In patients with metabolic syndrome who received 40g and 20g of dark chocolate (76%) for eight weeks, the MDA plasma levels were unchanged [36].

In addition, it is worth highlighting a strong point observed in our study. It is known that chocolate is the source of phosphorus and potassium, approximately 220 mg of phosphorus and 432 mg of potassium for every 100g of chocolate [37], and it often becomes a prohibited food for patients with CKD to avoid hyperphosphatemia and hyperkalaemia [9]. However, during the two months of intervention with dark chocolate, no increase in serum levels of these minerals was observed in our study. In addition, during the entire study period, the patients reported no adverse effects from the consumption of dark chocolate. Such findings collaborate with the guidance that patients with CKD can benefit from consuming dark chocolate under an individualised nutritional prescription.

4.1. Limitations

When considering the study in question, some identified limitations are worth noting. First, the number of voluntary participants (mainly in the control group) may have limited our findings to more outstanding results. Likewise, a longer intervention time may also be promising to generate more significant results in these

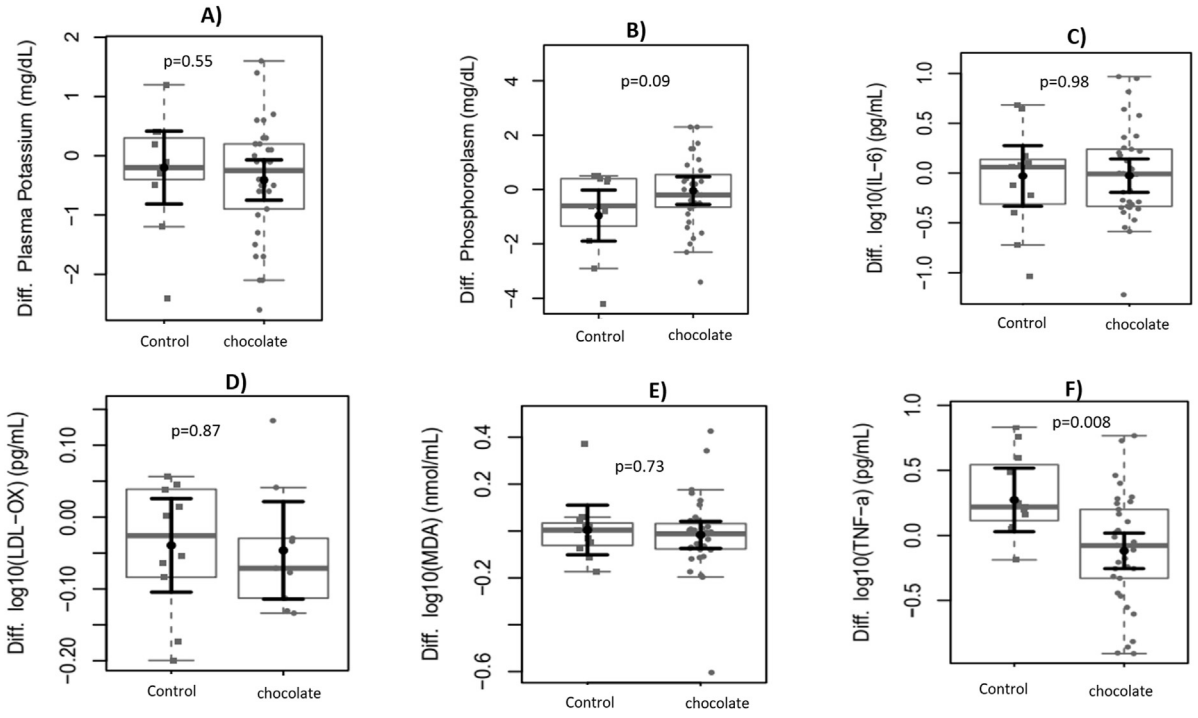

Fig. 4. Comparison of plasma levels of TNF-α, potassium, phosphorus, IL-6, LDL-ox and MDA between groups after two months of supplementation. We found no evidence of differences in potassium (A), phosphorus (B), Interleukin-6 (IL-6) (C), ox-LDL (D) and MDA levels (E). However, there was a difference in plasma levels of tumour necrosis factor-α (TNF-α) (F) after two months of intervention in the chocolate group compared to the control. The sample distributions of data differences are represented in box plots and strip plots in grey. In black, the central circle represents the average marginal effect expected for each group's differences estimated from linear fixed-effects models. Fixed effects in the models were the intervention group, time, their interaction and confounding variables Gender, Age, Systemic Arterial Hypertension (SAH) and diabetes mellitus (DM). Black horizontal bars represent the 95% confidence intervals of the marginal effects of the expected mean difference per group.

patients [38] elucidated in a recent systematic review of the literature on chocolate and cocoa for health that studies with a short intervention period (4–6 weeks) show more limited results. As performed in the study by Kord-Varkaneh et al. (2019), we identified that it would be interesting to carry out subgroups to evaluate other amounts of dark chocolate and its effects since the literature support those higher doses are related to positive effects of dark chocolate.

Although no significant differences were observed in the oxidative stress marker of these patients after the intervention with dark chocolate, we emphasise that this study contributes to the scientific literature regarding the effects of dark chocolate on inflammation in patients with CKD on HD.

Moreover, further studies analysing the mechanisms of action of dark chocolate on inflammation and oxidative stress in these patients are necessary since this group of patients can be significantly benefited from controlling the associated comorbidities and minimising the impact of CKD. Non-drug treatments should be prioritised, especially using food as medicine [6].

This is the first study on the intervention of dark chocolate in patients with CKD on hemodialysis and will pave the way for future studies to confirm and sustain the findings to date and determine whether the ingestion of dark chocolate in these patients is safe and healthy.

5. Conclusions

Given the above, it is possible to state that 40g of dark chocolate consumption for two months did not change the oxidative stress markers analysed. However, the plasma levels of TNF-α (an inflammation marker) in CKD patients on HD were reduced after the chocolate intervention. There was no significant difference in the nutritional status and food intake of these patients after the period of intervention with dark chocolate. It is worth noting that the intervention used in this study did not increase the plasma levels of phosphorus and potassium in these patients.

Our preliminary results open perspectives for new non-pharmacological strategies to reduce inflammation in patients with CKD. However, more studies using dark chocolate as an intervention in hemodialysis patients are needed, especially for chocolate lovers.

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Authors contributions

Marcia Ribeiro contributed to the data collection and analysis of samples and produced the figures, tables and writing. Susane Fanton and Beatriz Germer participated in data collection, randomisation and intervention. Bruna Paiva, Livia Alvarenga and Ludmila Cardozo actively contributed to analysing laboratory samples. Marcelo Ribeiro-Alves performed the statistical analysis; Denise Mafra coordinated the study and assisted in all stages of the research, restructured and revising the manuscript.

Declaration of competing interest

The authors declare they have no financial interests.

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