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Nasturtium (*Tropaeolum majus L.*) sub-chronic consumption on insulin resistance and lipid profile in prediabetic subjects. A pilot study

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ABSTRACT

Nasturtium (*Tropaeolum majus L.*) is a plant rich in bioactive compounds such as phenolic compounds, glucosinolates (GLS) and their hydrolyzed metabolites isothiocyanates (ITCs), which modulate signaling pathways related to lipid and carbohydrate metabolism. This randomized crossover trial explored the effect of intervention with a freeze-dried nasturtium drink on insulin response and lipid profile in prediabetic subjects. Ten (10) patients were randomly assigned to the following treatments: NT (nasturtium) and PLC (placebo) for 4 weeks and after this time the treatments were crossed for another 4 weeks. Biomarkers related to insulin resistance and lipid profile were measured at the beginning and the end of the intervention with each treatment. The consumption of 15 g NT (681 μmol of benzyl glucosinolate)/dose week for four (4) weeks resulted in a significant decrease in the concentration of LDL cholesterol (LDLc), Oxidized LDL (ox-LDL), Castelli's risk index I and II (TC/HDLc, and LDLc/HDLc) and Atherogenic Coefficient (non-HDLc)/HDLc). The results suggest that the nasturtium consumption might have a modulating effect on biomarkers related to cardiovascular disease, altered in subjects with chronic diseases, including type 2 diabetes. This study is the first *in vivo* study to explore the chronic effect of *T. majus* consumption in patients with prediabetes and shows the importance of continuing to explore this effect in studies with a larger number of participants.

1. Introduction

Tropaeolum majus is a flowering plant from the order Brassicales, family Tropaeolaceae native from Perú to Colombia. *T. majus*, known as garden nasturtium or Indian cress, occurs as a perennial plant and it is used mostly as an ornamental plant, although its leaves, flowers, and unripe green seeds are used in salads and sauces where they impart a spicy flavor (Wrolstad & Garzón, 2009). *T. majus* has been widely used in traditional medicine due to its antimicrobial and antiviral properties (Schiess et al., 2017); other reports have shown the anti-inflammatory

Abbreviations: GLS, glucosinolates; ITCs, isothiocyanates; NT, nasturtium; PLC, placebo; BITC, benzyl isothiocyanate; ox-LDL, oxidized LDL; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; TG, triglyceride concentration; TC, total cholesterol T2D, type 2 diabetes mellitus; WC, waist circumference; PPARγ, peroxisome proliferator activated receptor gamma; CEBPA, CCAAT/enhancer binding protein alpha; SREBF1, sterol regulatory element binding transcription factor 1; FAS, fatty acidy synthase; HUSI, Hospital Universitario San Ignacio; FINDRISC, finnish diabetes risk score; wk, week; HOMA-IR, Homeostasis model assessment of insulin resistance; FBG, fasting blood glucose; PPG, postprandial glucose AC, atherogenic coefficient; AIP, atherogenic index of plasma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; CBC, complete blood count; ELISA, enzyme linked immunosorbent assay; BMI, body mass index; SD, standard deviation; ANOVA, analysis of variance; BSP, broccoli sprout powder; CMIA, chemiluminescent microparticle immunoassay; HbA1c, glycated hemoglobin; CAD, coronary artery disease; NRF2, nuclear factor erythroid-derived 2-like 2; DNA, deoxyribonucleic acid.

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(Herz et al., 2016) and antioxidant effects related to its bioactive compounds (Wrolstad & Garzón, 2009). The leaves, petals, and seeds are a rich source of bioactive compounds, such as phenolic compounds and glucosinolates (GLS). The main GLS in narturtium is glucotropaeolin and its secondary metabolite, benzyl isothiocyanate (BITC), is formed by the enzymatic hydrolysis of the enzyme myrosinase present in the plant or the microbiota of humans (Barba et al., 2016). The biological effects after the GLS-rich sources consumption or cell stimulation are attributed to the presence of BITC.

Currently, around 537 million people in the world suffer from diabetes, with type 2 diabetes mellitus (T2D) representing 90% of cases (International Diabetes Federation. (2021), 2021). 1 in 10 people suffer from T2D, which is classified among the first five causes of mortality in Colombia (Fondo Colombiano de Enfermedades de Alto Costo Cuenta de Alto Costo (CAC), 2021).

T2D is characterized by a peripheral resistance to insulin which triggers a state of hyperglycemia. Altered blood glucose levels have wide-ranging metabolic consequences in the body with many deleterious downstream effects, such as chronic inflammation, loss of pancreatic β-cells, atherosclerosis, and heart disease (Sobczak, Blindauer, & Stewart, 2019). Epidemiologic studies have demonstrated that T2D is characterized by an altered lipoproteins metabolism with high plasma TG concentration, low HDLc concentration, and increased concentration of small dense LDLc particles (Mooradian, 2009). The phenotype of dyslipidemia in T2D led to the recognition of diabetes mellitus as a coronary heart disease-risk-equivalent condition (Mooradian, 2009).

The asymptomatic intermediate hyperglycemia condition which preceded T2D is called prediabetes, considered the main determining risk factor for the development of this disease. Prediabetes and T2D emerge due to unhealthy dietary patterns and low physical activity levels, therefore, improving diet and lifestyle are the best strategies for the prevention (American Diabetes Association, 2022; Barry et al., 2017).

In vitro and *in vivo* studies suggest that nasturtium might have a modulatory effect on biomarkers related to lipids and carbohydrate metabolism. Previous studies show that BITC extracted from *T. majus* leaves reduces the gene and protein expression of gluconeogenic enzymes and increases the expression of antioxidant proteins in hepatoma cells (Guzmán-Pérez et al., 2016). The modulation of cellular antioxidant response and glucose metabolism by BITC might represent advantages in the T2D condition (Guzmán-Pérez et al., 2016). A reduction in lipogenesis by nasturtium has been previously described. Kim, Kim, Kim, and Choi (2017) found an inhibition in the lipid accumulation and a reduction in the expression of peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT/enhancer-binding protein alpha (CEBPA), and sterol regulatory element-binding transcription factor 1 (SREBF1) in mouse preadipocyte cells. PPARγ, CEBPA and SREBF1 factors are considered responsible for adipocyte differentiation regulation in 3T3-L1 cells (Kim et al., 2017).

Other animal studies have reported comparable results where BITC reversed liver inflammation, lipid accumulation, weight gain (Chen, Yen, Kuo, Lo, Huang, Chen, & Lii, 2020), and insulin resistance (Alsanea & Liu, 2017) in mice fed with a high-fat diet.

Despite the *in vitro* and *in vivo* evidence, unfortunately, there is limited information about nasturtium's acute and sub-chronic consumption effect on the biomarkers related to insulin resistance and lipid profile in humans, to allow us to consider nasturtium as a useful ingredient for the development of nutritional or phyto-therapeutic supplements. Therefore, this study aims to explore the effect of sub-chronic consumption of nasturtium-based drinking on biomarkers for the evaluation of T2D and cardiovascular risk in subjects with prediabetes.

2. Materials and methods

2.1. Study design

This pilot study was designed as a double-blind, randomized, placebo-controlled, crossover clinical trial performed on patients with prediabetes. The study was conducted at the Pontificia Universidad Javeriana and Hospital Universitario San Ignacio (HUSI), Bogotá-Colombia. All procedures were approved by the Ethics Committee of Sciences and Medicine Faculties (FM-CIE-0162-19). The written informed consent forms were obtained from the patients before any clinical examinations were started at the beginning of the study.

2.2. Subjects

Subjects aged 25 to 70 y, who had 2 h glucose tolerance test between 140 and 199 mg/dL were recruited for the study. Patients were excluded from the trial if they had type 1 and T2D, severe impairment of cardiac, hepatic, or renal function, gestation, or lactation and if they used insulin injection, consumed vitamin K-antagonists, antioxidant supplements, drugs interfering with glucose tolerance (β-blockers, antidepressants, antibiotics, estrogens, etc.) and bile acid sequestrants or lipid-lowering drugs (statins, fibrates, and nicotinic acids).

For the participant's selection the FINDRISC (Finnish Diabetes Risk Score) screen test was used (Lindström & Tuomilehto, 2003). Those participants with a score >16 were scheduled by the research team for blood sampling and glucose intolerance confirmation. 287 subjects were screened with the FINDRISC questionnaire of which 68 patients were selected for blood sampling and glucose intolerance confirmation. A total of 10 patients with the confirmation of prediabetes were recruited and randomized by simple randomization using a random number table. Enrolled subjects started the study either within the placebo (PLC, n = 10) or nasturtium (NT, n = 10) treatment groups; after four (4) weeks (wks) intervention the treatments were exchanged, then the NT group received PLC and the PLC group received NT.

2.3. Intervention

Patients eligible for the study were asked to avoid GLSs-rich vegetables such as brassica family plants (broccoli, brussels sprouts, cabbage, cauliflower, or radish) and *Moringa oleifera* for the entire experimental period, beginning one week before the first intervention.

Each participant was supplemented with 15 g of freeze-dried nasturtium leaf powder (NT) or placebo (PLC) per wk for 4 wks. Each participant received 3 envelopes per wk with 5 g NT each or 3 g PLC. In total each participant was given 24 envelopes (12 NT and 12 PLC) packaged in the same way to be indistinguishable from each other. The participants were instructed not to make any other dietary changes or lifestyle changes for the length of the intervention.

Once the treatment began, the participants were instructed to take three days at wk one NT or PLC envelope diluted in 300 ml (about 10.14 oz) in chilly water in a bottle provided for the study. After pouring the NT into the water it was necessary to wait 10 min with the bottle closed, to stir, and consume. The exact process was recommended for PLC. Within the 10 min, the myrosinase in the freeze-dried plant material converted the benzyl glucosinolate into the bioactive BITC (Hanschen, Lamy, Schreiner, & Rohn, 2014). It was recommended to consume the drink between meals in the morning to reduce gastrointestinal symptoms. Participants were instructed to keep the envelopes frozen to avoid the degradation of the bioactive compounds (Platz et al., 2016). After the first period of intervention, the participants had 1 wk of washout and the treatments were exchanged, those participants who started with PLC received NT and those who began with NT received PLC for 4wks (Fig. 1).

The participants were appointed for three visits to the HUSI clinical laboratory (certified by norm 9001:2008) to measure primary and

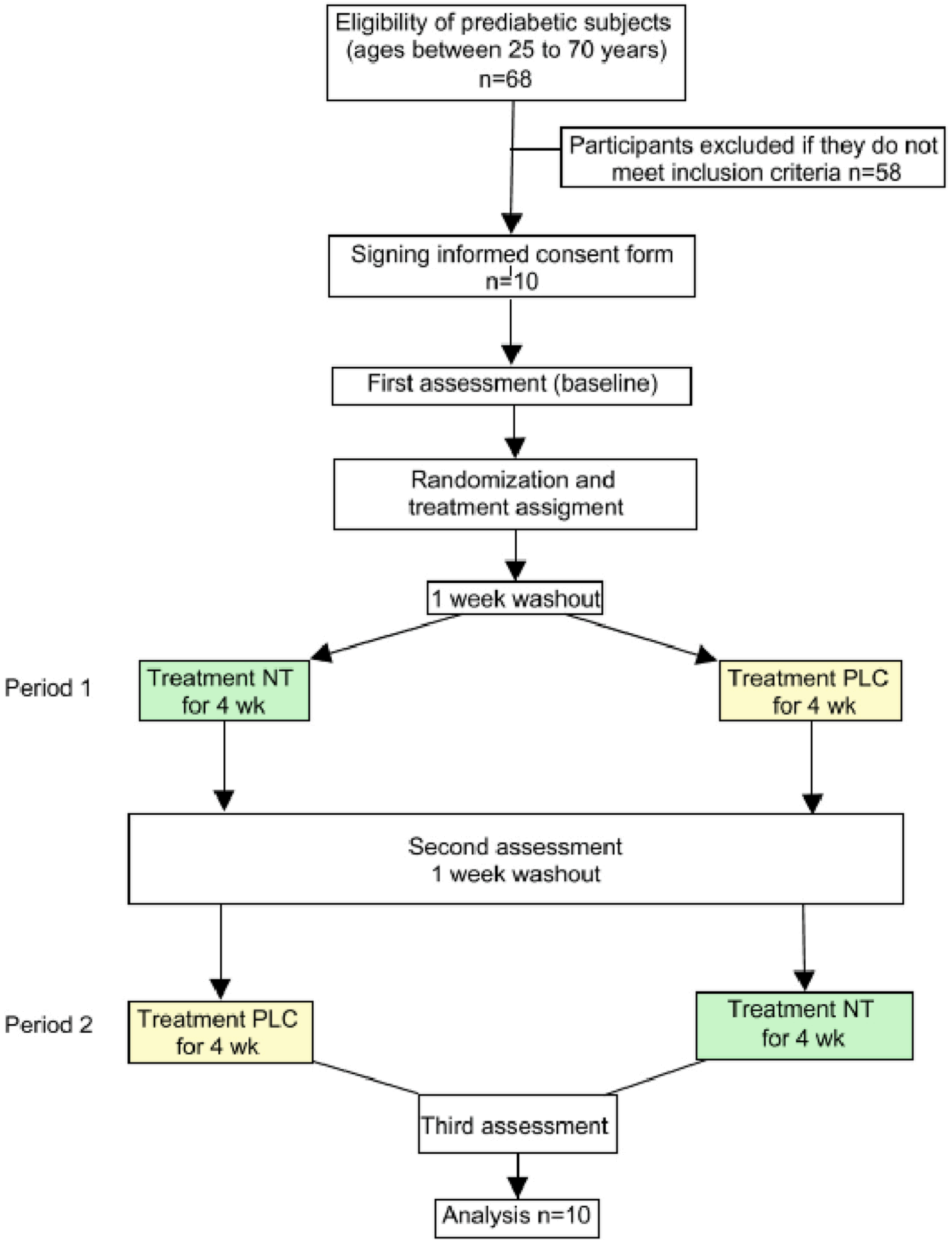

Fig. 1. Flow chart of study subjects. PLC, Placebo; NT, Nasturtium; wk, week.

secondary outcomes at baseline (0 wk.) and at the end of the intervention with NT or PLC (4 wks.). The compliance, gastrointestinal symptoms, GLS-rich foods consumption, and physical activity were recorded every week throughout the study. For the food consumption information, a GLS-rich food consumption frequency questionnaire was used. A short international physical activity questionnaire was used to record information related to the weekly physical activity. During the intervention, a photographic recording was performed with the empty envelope and bottle photos sent by the participants. The compliance was 100% and no participant withdrew from the study.

2.4. *Nasturtium and placebo drinking development*

The NT used for the intervention was provided by the Leibniz Institute of Vegetable and Ornamental Crops, Grossbeeren, Germany. Nasturtium (*Tropaeolum majus L.*) plants were grown in a greenhouse at the Leibniz Institute of Vegetable and Ornamental Crops e.V., Großbeeren, with a daily mean temperature between 19 and 20 °C and a light intensity of 300–350 μmol $m^{-2}s^{-1}$. At the time of harvest, only the leaves of the plants were flash-frozen in liquid nitrogen, freeze-dried, ground to a fine powder, and mixed homogenously. The GLS content was determined as desulfoglucosinolate according to Wiesner, Zrenner, Krumbein, Glatt, and Schreiner (2013). The conversion to BITC was measured according to Hanschen, Klopsch, Oliviero, Schreiner, Verkerk, and Dekker (2017). For 1 g freeze-dried plant material, 45.4 $\mu molg^{-1}$ BITC was determined. Each NT envelope contained 5 g freeze-dried nasturtium leaf powder with a total content of 227 μmol BITC. The PLC contained 3 g collagen (Solugel® collagen peptide) colored with 0.05 g green pigment used in the food industry. The NT and PLC nutrient composition and BITC content are presented in Table S1 and S2. The proximate composition of raw NT was carried out in the food chemistry laboratory in the Sciences Faculty of Pontificia Universidad Javeriana. Moisture content was quantified by freeze-drying. The total fat content was determined by solvent extraction (AOAC 920.39). Crude protein determination was performed by using the Kjeldahl procedure (AOAC 978.04). Crude ash was determined by incineration in a muffle furnace

at 500 °C (AOAC 930.05) and crude fiber was obtained as the organic residue remaining after digesting with H_2SO_4 and NaOH (AOAC 930.10). The carbohydrate content was calculated by difference. All experiments were conducted for five independent replicates. NT micronutrient composition was carried out in Lepton S.A.S analytical laboratory (ISO 9001:2015), located in Bogotá DC, Colombia.

2.4.1. Primary and second outcomes

Following 12-h fasting, blood was withdrawn from the antecubital vein, collected in different vacutainer tubes for specific biomarker analysis. All biochemical biomarkers were determined at the HUSI clinical laboratory (certified by norm 9001:2008). The primary outcomes were the glycemic markers, fasting (FBG) and postprandial blood glucose (PPG), insulin, HOMA-IR index, and lipid profile (Total cholesterol, HDL-cholesterol, LDL-cholesterol, and triacylglycerol). Secondary outcomes were oxidized-low density lipoprotein (ox-LDL), Castelli's risk index I and II (TC/HDLc and LDLc/HDLc), Atherogenic Coefficient (AC, non-HDLc)/HDLc), Atherogenic Index of Plasma (AIP, TG/HDLc), liver function tests aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), creatinine and complete blood count (CBC).

2.4.2. Glycemic assessments

Glucose concentration was measured by enzymatic colorimetric analysis according to the standard procedures (Beckman Coulter, CA, USA). FBG was measured following 12-h fasting and PPG at 2 h was performed in all subjects by taking 75 g oral glucose solution after overnight fasting and then 2 h later, blood glucose level was measured. Insulin was measured by chemiluminescent microparticle immunoassay (CMIA) (Abbott, IL, USA). HOMA-IR index was calculated with the formula (Basal Insulin (μU/L) × fasting blood glucose (mmol/L)/22.5) (Matthews et al., 1985).

2.4.3. Lipid profile assessments

Serum TC and TG levels were measured by enzymatic colorimetric analysis (Beckman Coulter, CA, USA). HDLc was measured by enzymatic colorimetric analysis (Beckman Coulter, CA, USA) and LDLc was measured by an enzymatic method (Beckman Coulter, CA, USA). To quantify the concentration of serum ox-LDL used an ELISA kit (Novus Biologicals, USA). Castelli's risk index I and II, AC, and AIP were calculated as cardiovascular risk factors parameters, at baseline and 4 weeks after treatment.

2.4.4. Assessment of secondary outcome measures

Different safety parameters were evaluated for each patient, such as renal function through serum creatinine; liver function by evaluating AST, ALT, and GGT; and hematologic function by CBC.

2.4.5. Anthropometry assessments

Anthropometrical measurements taken on the participants included weight, height, waist circumference, and bioimpedance analysis (Seca 515/514; TANITA). All measurements were taken barefoot and with standard methods. Height was measured only at basal visit with a stadiometer (0.5 cm precision) and weight at each visit with the TANITA scale at the nearest 0.1 kg precision. BMI was calculated as Weight (kg)/Height $(m)^2$. Waist circumference was measured with an elastic tape (Seca, precision 0.5 cm) by standard procedures.

2.5. Statistical methods

Statistical analysis was performed with SPSS (version 21; SPSS, Inc., Chicago, IL, USA). The data are expressed as mean ± standard deviation (SD). Before data analyses, the distribution of the variables was checked through Shapiro-Wilk. To analyze the effectiveness of nasturtium vs. placebo, the difference in glycemic and lipid values before and after the intake was calculated using Student's *t*-test for related samples or Wilcoxon's signed-rank test based on the compliance of the assumption of normality of the dependent variables.

The effectiveness of the intervention was verified by comparing the intakes difference of NT and PLC using the student's *t*-test for related samples or the Wilcoxon signed-rank test depending on the fulfillment of the assumption of normality for glycemic and lipid variables. To study whether the effect of NT or PLC intake interacts with gender and BMI, the F test for the interaction of the ANOVA of a partially repeated measures design with the factor age and BMI was used. The percent change for each variable was also calculated by the formula [(4-weeks values - baseline values)/baseline values × 100]. $P < 0.05$ was considered significant.

3. Results

The flow chart of participants is presented in Fig. 1. The blood samples of sixty-eighty (68) volunteers were evaluated and ten (10) met the requirements for the intervention, which were treated with NT and PLC. None of the participants withdrew from the study. The mean age was 51 ± 11 years and 8 of 2 participants were female. The anthropometrical characteristics of the participants are presented in Table 1. No differences were observed in the sex proportions and age in the NT and PLC groups. The intervention with NT or PLC did not modify the anthropometrical measures and body composition (Table 1). The analysis of renal, hepatic, and hematological function in NT and PLC groups showed no differences or adverse effects, these results are shown in Table S3. After the NT intervention, the participants reported nausea 60% (n = 6), bloating 30% (n = 3), and discomfort due to the spicy taste of the drinking 20% (n = 2).

The intervention effects on glycemic and insulin resistance parameters by the treatment with NT and PLC are shown in Table 2. No difference was found in FBG and PPG, insulin, and HOMA-IR index where NT and PLC groups were compared.

Table 3 shows the effects of intervention with NT and PLC on lipid profile. The intervention with NT led to a significant reduction ($P < 0.05$) of LDLc and ox-LDL compared to PLC. A similar effect was observed for Castelli's risk index I and II and the AC. Fig. 2 presents the mean percentage of changes in the groups after 4 weeks of intervention. Ox-LDL, TC/HDLc, LDLc/HDLc, and AC significantly decreased after NT intervention.

4. Discussion

In the current study, supplementation with NT in prediabetic patients for 4 wks had favorable effects on lipid-related parameters, as risk factors for cardiovascular disease in these patients. No effect was found

Table 1
Baseline characteristics of participants.

	Baseline	Placebo (TB) n = 10	Nasturtium (TA) n = 10	*P* for treatment effect
BMI (kg/m^2)	31.7 ± 8	31.7 ± 8	31.7 ± 8	NS
WC (cm)	93.1 ± 13	92.7 ± 12	93.2 ± 11	NS
Lean mass index (kg/m^2)	17.6 ± 3	17.7 ± 2	17.8 ± 3[†]	NS
Fat mass index (kg/m^2)	13.8 ± 5	14.0 ± 6	14.0 ± 6	NS
Visceral fat (L)	2.8 ± 1	2.9 ± 1	3.0 ± 1	NS

BMI: body index mass; L: liter; WC, waist circumference. All values are mean ± SD. NS: no significant differences between the two groups (student paired *t*-test); [†] Significantly different from baseline (NT vs baseline).

Table 2
Glycemic control biomarkers in patients with prediabetes in NT and PLC groups.

	Baseline	Placebo (PLC) n = 10	Nasturtium (NT) n = 10	*P* for treatment effect
FBG (mmol/L)	6.5 ± 1	6.2 ± 1‡	6.0 ± 1	NS
PPG (mmol/L)	8.7 ± 1	7.4 ± 2‡	7.9 ± 2†	NS
Insulin (μU/mL)	15.2 ± 8	14.8 ± 10	16.7 ± 14	NS
HOMA-IR	4.5 ± 3	4.3 ± 3	4.5 ± 4	NS

FBG, fasting blood glucose; PPG, Postprandial glucose; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance. All values are mean ± SD; NS: no significant differences between two groups NT vs PLC (student paired *t*-test); ‡ Significantly different from baseline (PLC vs Baseline); † Significantly different from baseline (NT vs baseline).

in glycemic parameters and HOMA-IR index, but a reduction was found in ox-LDL and the cardiovascular indexes TC/HDLc, LDLc/HDLc, and non-HDLc/HDLc which provide evidence of the NT functional effect.

The elevation of LDLc together with the use of cardiovascular indexes, such as the Castelli's index I and II, AIP, and AC are considered strong predictors of cardiovascular events in T2D (Baral & Hamal, 2019). This study showed that 15 g of NT/ wk for 4 wks induced a reduction of 6.5% in TC/HDLc, 8% in AC, and 13% in ox-LDL. So far, no study had assessed the effect of sub-chronic NT intervention in humans on the glycemic indexes and lipid profile parameters, which suggests that administration of NT as a supplementary treatment for chronic diseases such as metabolic syndrome and T2D might have favorable effects on lipid profiles, as risk factors for cardiovascular disease.

The effect of polyphenols and GLS-rich foods consumption, different from *T. majus*, on glucose and lipid biomarkers in T2D patients, has been evaluated previously in *in vivo* studies. Bahadoran et al. (2012) showed a significant reduction in FBG, TC, TG, LDLc, ox-LDL, and AIP after the intervention with 10 g of broccoli sprout powder (BSP) daily for 4wks. Recently, Gómez-Martínez et al. (2022) showed a reduction in FBG and glycated hemoglobin (HbA1c) after the intervention with 2400 mg/day of *Moringa oleifera* dry leaf powder over 12 wks in prediabetic subjects. NT consumption in our study did not lead to relevant changes in the glucose and HOMA-IR index, due to the small amount of NT supplemented and the small size of the sample (10 subjects). Nevertheless, the 15 g/wk NT (681 μmol BITC) supplemented in our study was defined considering the freeze-dried NT that would allow the development of an acceptable drinking for consumption. A wide standard deviation in insulin concentration was observed in the present study, which might have had an impact on the non-significant HOMA-IR index. The high inter-variability observed in insulin concentration might be related to the prediabetes condition evolution, genetic factors, waist circumference, and body composition documented in previous studies (Hansen et al., 2020).

Table 3
Effect on lipid profile in patients with prediabetes of the NT and PLC groups.

	Baseline	Placebo (PLC) n = 10	Nasturtium (NT) n = 10	Mean difference (%)	*P* for treatment effect
TG (mmol/L)	2.3 ± 1	2.3 ± 1	2.4 ± 1	5.0	NS
TC (mmol/L)	55.9 ± 11	52.7 ± 14	50.0 ± 11†	−3.7	NS
LDLc (mmol/L)	33.7 ± 9	32.7 ± 11	28.9 ± 8	−9.1	0.04
HDLc (mmol/L)	10.7 ± 2	10.0 ± 2‡	10.3 ± 2	2.9	NS
Ox-LDL (pg/mL)	1726.3 ± 398	1746,6 ± 383	1472.4 ± 401†	−12.8	0.00
TC/HDLc	5.3 ± 1	5.4 ± 2	5.0 ± 1	−6.5	0.03
LDLc/ HDLc	3.2 ± 1	3.3 ± 1	2.9 ± 1	−11.8	0.01
AIP	0.2 ± 0	0.2 ± 0	0.2 ± 0	2.2	NS
AC	4.3 ± 1	4.4 ± 2	4.0 ± 2	−7.7	0.03

TG, triglyceride concentration; TC, total cholesterol; LDLc, low-density lipoprotein; HDLc, high-density lipoprotein; Ox-LDL, oxidized-low density lipoprotein; AIP, atherogenic index of plasma (TG/HDLc); AC, Atherogenic coefficient (non-HDLc/HDLc). NS: no significant differences between two groups NT vs PLC (student paired *t*-test); The $P < 0.05$ significantly different between two groups NT vs PLC (student paired *t*-test); † Significantly different from baseline (NT vs baseline), ‡Significantly different from baseline (PLC vs baseline); Mean difference was calculated for each patient according to the formula [(nasturtium values - placebo values)/ placebo values) x100].

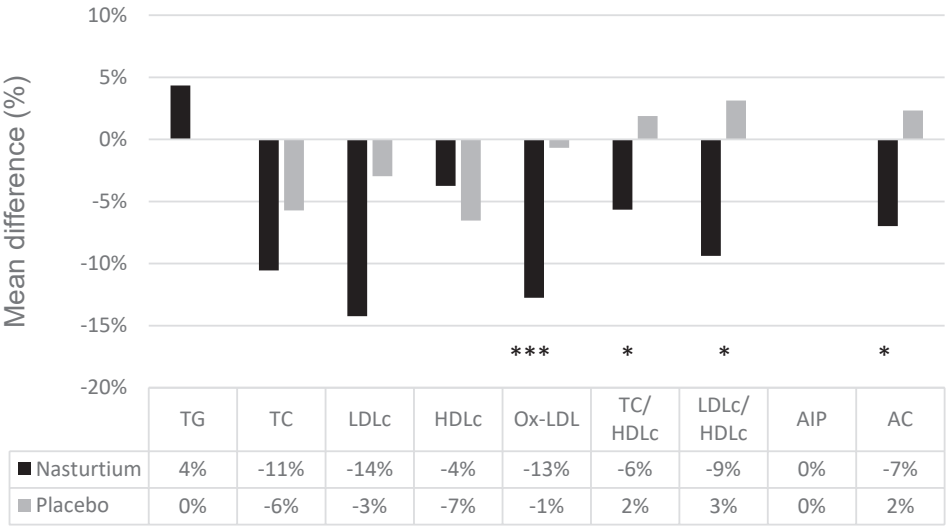

Fig. 2. Mean percentage of change in the groups compared to baseline values after 4 weeks of intervention. TG, triglyceride concentration; TC, total cholesterol; LDLc, low-density lipoprotein; HDLc, high-density lipoprotein; ox-LDL, oxidized-low density lipoprotein; AIP, atherogenic index of plasma, (TG/HDLc); AC, Atherogenic coefficient (non-HDLc/HDLc). *Significantly different compared with baseline values according to the formula [(4-weeks values - baseline values)/baseline values × 100] ($P < 0.05$); *** significant difference compared with baseline values ($P < 0.001$).

The reduction found in our study in the cardiovascular indexes TC/HDLc, LDLc/HDLc, and non-HDLc/HDLc might be explained by the effect of phenolic compounds and ITCs. Although phenolic compounds were not determined in the present study, previous reports have documented the presence of anthocyanins and flavonoids in nasturtium (Garzón, Manns, Riedl, Schwartz, & Padilla, 2015). Those compounds have been associated with a reduction in the lipoprotein's oxidation and a reduction in cardiovascular molecular biomarkers (Reis et al., 2016). There is evidence showing that ITCs modulate protein expression of lipogenic factors such as peroxisome proliferator-activated receptors (PPAR), sterol regulatory element binding protein-1c (SREBP-1c) and fatty acidy synthase (FAS) (Chuang, Liu, Huang, Lo, Yao, Chen, & Lii, 2019), which regulates the expression of a family of genes involved in glucose utilization and fatty acid synthesis (Wang, Viscarra, Kim, & Sul, 2015). A previous study in mice fed with a very high-fat diet supplemented with *Moringa oleifera* showed a reduction in hepatic lipid accumulation, TG and TC decrease, and an inhibition in the gene expression of SREBP-1c and FAS (Nova et al., 2020).

The elevation in ox-LDL has been reported in T2D patients (Bonilha et al., 2021) and is a significant and independent predictor for future cardiac events in T2D patients with coronary artery disease (CAD) (Shimada, 2004). In the present study, the supplementation with NT led to a 13 % reduction of ox-LDL which might be associated with a decreased risk of atherogenic complication development in the prediabetic patients. Furthermore, the effect of NT on ox-LDL in our study might be related to the regulation of antioxidant factors such as nuclear factor erythroid-derived 2-like 2 (NRF2) by BITC, which was previously reported by us in a previous study (Guzmán-Pérez et al., 2016). NRF2 is a transcription factor that regulates the gene expression of antioxidant enzymes (Tonelli, Chio, & Tuveson, 2018). NRF2 plays a vital role in cellular defense. The binding of NRF2 to the antioxidant response elements in the DNA induces the transcription of antioxidant and detoxification enzymes (Soundararajan & Kim, 2018).

The consumption of NT did not alter hepatic, renal, or hematological function (Table S3. supplementary material), indicating no damaging side effects after the supplementation. At the amounts of NT used in this study, it is not expected that long-term consumption might induce accumulative toxic effects. As reported in other studies, GLS-rich foods have been used as the source of supplementation without any adverse effects (Bahadoran et al., 2012; Schiess et al., 2017). At the end of the intervention, participants referred to mild gastrointestinal events such as nausea, bloating, and flatulence, respectively.

The authors are aware that this study has limitations: first, although in the crossover study each subject is his own control and that allows a smaller number of patients, the low sample size used in the study might have an impact on the nonsignificant results found with FBG and HOMA-IR index. Second, the small sample size did not allow controlling the effect of sex and age on the variables studied. Third, given that an initial clinical approach to prediabetic patients is carried out with lifestyle modifications (Galaviz, Narayan, Lobelo, & Weber, 2015), the present study aimed to include those patients with glucose intolerance but without glucose medication. However, it was a challenge to find prediabetic participants without oral hypoglycemic medication. Fourth, the number of blood samples collected during the intervention (basal, post NT, and PLC intervention) represented a limitation for the analysis, since the blood sample taken before the first intervention might not reflect the basal condition for NT or PLC after the washout at the end of the study. The length of the washout period was established according to the time required to eliminate the bioactive compounds of nasturtium from the body, estimated in previous studies as 48 h (Platz et al., 2016). Therefore, the definition of seven (7) days for washout in this study was considered appropriate. Fifth, although there were no dropouts in the present study and the participants fully adhered to the intervention, the NT-based drinking gastrointestinal symptoms were an issue that should be considered in future studies.

Despite these limitations, the current study provided evidence of NT effects on lipid profile parameters and ox-LDL reduction compared to PLC intervention, while being the first study to show the effect of chronic intervention with *Tropaeolum majus* on glucose and lipid metabolism biomarkers in humans with prediabetes, therefore the data should be regarded as a suggestion for further studies with bigger sample size and with an NT higher concentration.

5. Conclusion

In conclusion, administration of 15 g/wk of NT as supplementary treatment in prediabetic patients could have favorable effects on lipid profile and ox-LDL, as risk factors for cardiovascular disease, suggesting that the functional and therapeutic effect of nasturtium must be evaluated in future studies with bigger sample size for the prevention of chronic diseases including T2D and cardiovascular disease.

6. Data availability Statement

The data presented in this study are available on request from the corresponding author, due to privacy restrictions. The study has been registered at https://www.ClinicalTrials.gov (Identifier: NCT05346978).

CRediT authorship contribution statement

Yudy V. Barrantes-Martínez: Investigation, Writing – original draft. **Martha Liévano:** Formal analysis, Investigation, Writing – review & editing. **Álvaro J. Ruiz:** Conceptualization, Formal analysis, Writing – review & editing. **Isabel Cuéllar- Rios:** Investigation, Writing – review & editing. **Diana Paola Valencia:** Investigation, Writing – review & editing. **Diego Ballesteros-Vivas:** Methodology, Writing – review & editing. **Valentina Guzmán-Pérez:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2022.105189.

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