CÓDIGO:

UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM NUTRIÇÃO

SELEÇÃO DE MESTRADO ACADÊMICO EM NUTRIÇÃO – 2019

CAPA DA PROVA ESCRITA

INSTRUÇÕES	
1	Para o mascaramento da prova escrita, sortear o CÓDIGO, preencher o nome completo e assinar o formulário. Esse formulário será depositado em envelope lacrado e assinado pelos Fiscais. A identificação do candidato em qualquer outro local da prova resultará na eliminação do Processo Seletivo.
2	Preencher os campos CÓDIGO nos seguintes locais: **capa da Prova Escrita e páginas destinadas para responder as questões dissertativas.** A falta deste procedimento impedirá a correção da prova, culminando com a eliminação do candidato.
3	Essa Prova Escrita contém 02 (duas) questões dissertativas.
4	Quando o Fiscal autorizar, verifique se a prova está completa e sem imperfeições gráficas que impeçam a leitura. Detectado algum problema, comunique-o imediatamente ao Fiscal. Não destaque nenhuma folha da prova.
5	As questões dissertativas serão avaliadas considerando apenas o que estiver escrito nas páginas pautadas, utilizando caneta esferográfica azul ou preta. Se necessário, solicite folhas adicionais ao fiscal. Escreva de modo legível.
6	Os rascunhos em qualquer outro espaço, fora das páginas pautadas, não serão considerados para efeito de correção.
7	Os candidatos dispõem de, no máximo, **4 (quatro) horas** para responder toda prova escrita.
8	Antes de retirar-se definitivamente da sala, devolva ao Fiscal a prova, juntamente com as páginas destinadas para responder as questões dissertativas.

CÓDIGO:

QUESTÕES DISSERTATIVAS

Leia atentamente as informações extraídas do artigo "*White grape juice increases high-density lipoprotein cholesterol levels and reduces body mass index and abdominal and waist circumference in women"* publicado, no periódico *Nutrition (2019)*, de autoria de Zuanazzi C. et al. (Nutrition. 2019 Jan;57:109-114. doi: 10.1016/j.nut.2018.05.026), e responda os itens que se seguem:

(1) Interprete os resultados apresentados nas Tabelas 2, 3 e 4.

(2) Elabore um resumo estruturado sobre o artigo, contendo os seguintes itens: Introdução, Objetivos, Métodos, Resultados e Conclusão.

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Applied nutritional investigation

White grape juice increases high-density lipoprotein cholesterol levels and reduces body mass index and abdominal and waist circumference in women

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Materials and methods

White grape juice

For this study, concentrated WGJ (100% grape) was used. The juice did not contain added sugar or preservatives, was produced from *Vitis labrusca* grapes, and was provided by a company located in Vale dos Vinhedos, Bento Gonçalves, Rio Grande do Sul, Brazil.

Physicochemical assay

The physicochemical parameters of WGJ were evaluated in accordance with the methodologies described by the Instituto Adolfo Lutz [9]. The relative density, pH, total titratable acidity, volatile acidity, and solvable solids content were determined.

Phenolic compounds

The concentration of total phenolic compounds was determined with the colorimetric method as described by Singleton and Rossi [10]. For the chemical characterization of WGJ, the levels of caffeic acid, ferulic acid, coumaric acid, (+) catechin, (-) epicatechin, and trans-resveratrol were determined by high-performance liquid chromatography, using a precolumn Zorbax SB C18 (250 mm × 4.6 mm; 5 μm) and column Zorbax SB 300 C18 (12 mm × 4.6 mm; 5 μm). The wavelengths were 204, 280, and 320 nm for the diode array detector with excitation at 280 nm and emission at 320 nm for the fluorescence detector.

Antioxidant activity

The in vitro antioxidant activity of WGJ was evaluated using the 2.2-diphenyl-1-picrilhidrazil (DPPH) and 2.2-azino-bis (3-ethylbenz−6-sulphonic acid; $ABTS^{+}$) methods as described by Yamaguchi et al. [11] and Re et al. [12], respectively. The results were expressed as μL for the amount of juice necessary to reduce the radicals by 50%.

Volunteers and experimental design

A total of 25 women were included in this study. The sample size was calculated using Programs for Epidemiologists version 4.0 and based on the study by O'Byrne et al. [4] to detect differences among the volunteers before and after WGJ supplementation. The significance level was set at 5%, power of 90%, and a size of standardized effect of at least 0.8 standard deviations in the parameters of oxidative markers.

The participants agreed to consume WGJ and were instructed to maintain their habitual diet. To standardize the intake of calories, we included the energy (kcal) of WGJ to adapt the intake to the participants' diets. The volunteers were from the city of Caxias do Sul in Rio Grande do Sul, Brazil, and ages 50 to 67 y. Women who smoked, were taking dietary supplements (especially vitamins), were diabetic, and currently undergoing chemotherapy were excluded from the study.

The volunteers were instructed to consume 7 mL/Kg/d of WGJ during 30 d without other changes in diet energy consumption or lifestyle. They were advised to avoid consuming grape derivate products throughout the intervention period (30 d). The juice intake was administered into two to three portions, and the volunteers were instructed to consume WGJ with morning and afternoon snacks and at dinner time in accordance with their daily intake. The first blood collection was performed before the start of the supplementation, and the last collection after supplementation for 30 d.

A nursing technician collected 10 ml of blood in dry tubes of Vacutainer (BD Diagnostics, São Paulo, Brazil) from volunteers' antecubital veins after 12 h of fasting. The tubes were centrifuged for 15 min at 4°C to obtain the serum, which was immediately pipetted to Eppendorf tubes and stored at −80°C until the analysis. All experimental procedures conducted in accordance with the Declaration of Helsinki. Moreover, the Ethics Committee in Human Beings from the Universidade de Caxias do Sul approved the study (authorization number 1.093.796; 06/02/15). The participants signed an informed consent form to authorize participation in this project.

Questionnaires and interviews

The participants answered three questionnaires, always under the supervision of a dietitian. The first questionnaire was about sociodemographic and anthropometric characteristics. Physical activity was defined as the practice of any type of exercise for at least 30 min. Individuals were classified into three categories according to the amount of time spent and the frequency of the physical activity (i.e., no activity, 1–2 times/wk, and ≥3 times/wk) [13].

Body mass index (BMI) was calculated from the relationship between weight (kg) and the square of height (m). For women ages 50 to 60 y, the classification per the World Health Organization guidelines [14] was <18.5 kg/m^2 low weight, >18.5 kg/m^2 and <24.9 kg/m^2 eutrophy, >25.0 to 29.9 kg/m^2 overweight, and >30 kg/m^2 obese. For women ages 60 y, the classification per the Pan-American Health Organization [15] was <23 kg/m^2 low weight, >23 kg/m^2 to <28 kg/m2 eutrophy, >28 kg/m^2 to <30 kg/m^2 overweight, and >30 kg/m^2 obese.

The second questionnaire evaluated participants' dietary intake before starting the supplementation through a 24-h dietary recall. Individuals were prompted to remember all foods including snacks and beverages that were consumed during the preceding day. Interviews were conducted from Tuesday to Thursday because the weekend could be an atypical feeding day [13]. Dietwin Software (Software Program for Nutritional Assessment professional version 2008, Brazil) was used for the data analysis to quantify the content of the total calories and macro- and micronutrients in food and beverages on the basis of regional tables of food chemical composition.

The third questionnaire was developed to determine the total diet antioxidant capacity (DTAC) in 48 h as described by Flogel et al. [16]. Volunteers were asked to recall their intake of antioxidant food and beverages during the 2 d before the questionnaire. DTAC was calculated in accordance with the antioxidant capacity of each food or drink, multiplied by the amount consumed per day. The results were expressed as mg vitamin C equivalents per day (mg VCE/d).

Lipid profile

Serum lipid measurements were determined by evaluating the total cholesterol, high-density lipoprotein (HDL) cholesterol, and triacylglycerols using a detection kit (Labtest Diagnostic S/A, Minas Gerais, Brazil) in accordance with the manufacturer's instructions. Estimates of LDL cholesterol were calculated using the Friedewald formula as follows: [LDL cholesterol] = (total cholesterol − HDL cholesterol) − (triacylglycerols/5). The results were expressed in mg/dL.

Serum glucose and insulin levels

Glucose serum concentration was determined through an enzymatic-colorimetric test using a detection kit (Labtest Diagnostic S/A, Minas Gerais, Brazil) in accordance with the manufacturer's instructions. The results were expressed in mg/dL. Insulin levels were quantified with a commercial kit (Beckman Coulter) and the results were expressed as μIU/mL.

Oxidative damage to lipids

Lipid peroxidation in serum was evaluated through the determination of thiobarbituric-acid reactive substances (TBARS) as described by Wills [17]. Malondialdehyde (MDA) was used as the standard, and the results were expressed in nmol MDA/mL.

Nitric oxide levels

Nitric oxide (NO) levels were assessed from the spontaneous decomposition of sodium nitroprusside. Once generated, NO interacts with oxygen to produce nitrite, which was measured by Griess reaction [18]. For NO quantification, a standard curve with sodium nitroprusside was used, and the results were expressed in mmol/L.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined in accordance with the method by Bannister and Calabrese [19]. The results were expressed in units of SOD (USOD) per mg of protein. One USOD was defined as the amount of enzyme necessary to decrease adrenochrome formation by 50%.

Statistical analysis

The statistical analyses were performed with STATA software version 12.0 (Stata Corp., College Station, TX) and SPSS version 20.0 (SPSS Inc., Chicago, IL). The sample characteristics were described through absolute and relative frequency. The paired *t* test was used to compare the averages of anthropometric and laboratory measurements before and after WGJ supplementation. To check the association between the independent variables and DTAC, a Spearman correlation analysis was used. The results were considered statistically significant if $P \leq 0.05$.

Table 1
Chemical physical analysis, phenolic profile, and antioxidant activity of white grape juice

Chemical physical analysis	Mean	SD
Density (g cm^{-3})	1.06	0.00
pH	3.39	0.10
Total acidity (g L^{-1})	0.50	0.10
Volatile acidity (g L^{-1})	0.01	0.10
Solvable solids (° Brix)	14.4	0.10
Phenolic profile		
Total phenolic (GAE mg L^{-1})	267.90	0.07
Caffeic acid (mg L^{-1})	13.94	0.09
P-Coumaric acid (mg L^{-1})	3.07	0.24
Ferulic acid (mg L^{-1})	1.10	0.01
(+) Catechin (mg L^{-1})	11.29	0.17
(-) Epicatechin (mg L^{-1})	5.95	0.48
Trans-resveratrol (mg L^{-1})	0.54	0.03
Antioxidant activity IC$_{50}$ (μL)		
DPPH$^{\bullet}$	12.95	0.01
ABTS^{+}	45.41	0.03

ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, equivalent of gallic acid; IC$_{50}$, amount of juice (μL) that inhibits 50% of DPPH$^{\bullet}$ and ABTS^{+}; SD, standard deviation.
Data were expressed in mean ± standard deviation.

Table 2
Description of women's sociodemographic characteristics, nutritional status, and lifestyle (n = 25)

Variable	Frequency	%
Age (y)		
50–55	10	40
56–60	7	28
61–67	8	32
Income (minimum wage)*		
No income	4	16
1–2	4	16
3–4	5	20
≥4	12	48
Education level		
Incomplete primary education	4	16
Complete primary education	1	4
Complete secondary education	6	24
Incomplete college/university	4	16
Complete college/university	7	28
Complete postgraduate course	3	12
Use of medication		
Yes	22	88
No	3	12
Weekly physical activity practice		
Yes	21	84
No	4	16
Minutes of weekly physical activity		
<150	17	81
>150	4	19
Nutritional status		
Eutrophic	11	44
Overweight	10	40
Obese	4	16

* Ranges of minimum wage: 1–2: R$ 937.00 (US$ 299.25)–R$ 1.874 (US$ 598.49); 3–4: R$ 1.875 (U$ 599.00)–R$ 3.748 (U$ 1.196.99); ≥5: R$ ≥3.750 (U$ 1.198.20).

Table 3
Anthropometric measurements and biochemical assays before and after supplementation with white grape juice

	Before supplementation			After supplementation			
Variable	Mean	SD	Median	Mean	SD	Median	*P*-Value
Body mass index (kg/m²)	25.6	3.8	25.7	25.4	3.7	25.4	<0.001*
Waist circumference (cm)	85.5	9.5	85.0	83.6	8.9	85.0	<0.001*
Abdominal circumference (cm)	90.5	10.0	92.0	88.7	9.4	91.0	<0.001*
Systolic blood pressure (mmHg)	123.6	16.4	118.0	121.8	11.7	124.0	0.5
Diastolic blood pressure (mmHg)	77.7	11.6	79.0	74.5	10.5	75.0	0.07
Total cholesterol(mg/dL)	182.6	30.0	181.5	182.1	23.9	179.8	0.9
HDL cholesterol (mg/dL)	55.9	15.2	52.0	64.2	18.4	62.5	<0.05†
LDL cholesterol (mg/dL)	102.0	36.0	102.4	91.8	28.9	95.1	0.2
Triacylglycerols (mg/dL)	128.6	31.2	135.6	130.8	38.6	127.3	0.7
Glycemia (mg/dL)	88.3	20.6	88.5	79.0	13.1	76.0	0.06
Insulin (μUI/mL)	5.2	2.9	4.9	5.3	3.4	4.4	0.9
TBARS (nmol MDA/mL)	4.6	0.6	4.6	4.5	0.7	4.5	0.4
Nitric oxide (mmol/L)	27.5	30.2	18.5	33.7	43.0	17.9	0.5
SOD (USOD/mg of protein)‡	89.5	33.9	86.6	93.9	30.9	89.8	0.6

HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase activity; TBARS, thiobarbituric acid reactive substances; USOD: one unit of superoxide dismutase activity.
Data were expressed in mean ± SD.
* Statistical significance $p < 0.001$.
† Statistical significance $p < 0.05$.
‡ One unit of SOD is defined as the amount of enzyme that is needed to decrease to half the spontaneous adrenochrome formation velocity.

Table 4
Levels of nutrient and energy intake and correlations with DTAC

				DTAC	
Nutrient	Mean	SD	Median	r*	*P*-value
Total energy intake (Kcal)	1645.00	338.8	593.25	0.05	0.82
Carbohydrate (% TEI)	55.70	6.4	54.70	0.48	0.02†
Protein (% TEI)	17.92	4.1	17.50	0.07	0.74
Fat (% TEI)	26.18	5.1	26.00	−0.62	0.001‡
Total fiber (g)	21.21	6.4	18.50	0.06	0.77
Calcium (mg)	771.58	406.04	722.73	0.06	0.78
Iodine (μg)	122.56	113.72	95.06	−0.03	0.89
Iron (mg)	9.20	3.20	8.40	0.11	0.61
Magnesium (mg)	260.70	120.03	248.06	−0.06	0.78
Potassium (mg)	2377.76	783.21	2391.85	0.10	0.61
Selenium (μg)	27.10	28.76	21.05	−0.23	0.53
Sodium (mg)	1920.93	1394.10	1377.12	−0.18	0.38
Zinc (mg)	8.10	3.70	7.10	0.07	0.72
Folate (μg)	149.62	76.74	137.9	0.45	0.03†
Vitamin A (μg)	4419.09	5636.71	2669.82	−0.31	0.14
Vitamin B1 (mg)	1.30	1.44	0.89	−0.16	0.44
Vitamin B6 (mg)	0.89	0.26	0.83	0.23	0.27
Vitamin B12 (μg)	2.16	1.90	2.00	0.25	0.23
Vitamin C (mg)	120.00	50.80	118.10	0.21	0.32
Vitamin D (μg)	3.77	4.33	2.67	0.06	0.78
Vitamin E (mg)	5.13	4.73	3.79	−0.27	0.19
Polyphenols (mg GAE)	1806.60	923.20	2069.60	0.74	<0.001‡
β−Carotene (μg)	137.44	131.33	87.38	0.14	0.52
DTAC (mg VCE/d)	1194.30	563.60	1101.70	—	—

DTAC, dietary total antioxidant capacity; GAE, gallic Acid equivalent; SD, standard deviation; TEI, total energy intake.
Data expressed with mean ± SD mean.

* Spearman correlation with; Spearman correlation with statistical significance.

† $P < 0.05$.

‡ $P < 0.001$.

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Utilize o espaço pautado a seguir para responder a questão 2. Não será considerada para correção nenhuma letra ou palavra escrita fora deste espaço.

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