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Effect of Cinnamon Powder Addition to a Portuguese Custard Tart (*Pastel de Nata*) on Healthy Adults' Postprandial Glycemia

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Abstract

Background and Objective: Cinnamon is a spice used over the years in cooking to impart aromatic, flavor and taste properties to food and beverages. Moreover, cinnamon has been used for its medicinal properties due to its potential phenolic content, which can protect against cardio-metabolic diseases. Previous studies reported an improvement of postprandial glycemia after addition of cinnamon powder to a high-sugar meal. The study aims at investigating the effect of adding cinnamon powder to a Portuguese custard tart (*Pastel de Nata*) on the postprandial glycemic response in healthy subjects.

Subjects and Methods: After review board and Ethic Committee of the State approval, thirty-two healthy human subjects were assigned in a controlled study and randomly allocated into 2 groups: 16 subjects ingested a custard tart with cinnamon powder (cinnamon group) and 16 subjects ingested a custard tart alone (control group). Blood glucose concentrations were measured before interventions and after 30, 60, 90 and 120 minutes. Chemical analysis was performed to quantify the total phenolic content and antioxidant activity.

Results: The postprandial blood glucose (PBG) area under the curve (AUC) was significantly lower ($p = 0.0005$) in the cinnamon group (599.2 ± 9.1) compared to the AUC of the control group (645.7 ± 7.7). The administration of cinnamon powder to the custard tarts slightly decreased PBG mean values compared to custard tart without cinnamon powder, although it did not reach statistical significance ($p = 0.273$). Cinnamon addition to custard tart improved the total phenolic content (1278.7 ± 0.7 compared to 253.7 ± 22.8 mg/L gallic acid) and antioxidant properties, increasing 4.4 times the capacity of free-radical scavenger compared with custard tart without cinnamon (IC_{50}).

Conclusion: The addition of cinnamon powder to custard tart could be beneficial to glycemic control.

Keywords: Cinnamon, Blood glucose, Postprandial, Antioxidant, Polyphenols.

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List of Abbreviations

AUC	area under the curve
$\Delta C_{\max}$	variation of maximal concentration
$C_{\max}$	maximal concentration
GAE	gallic acid equivalent
TAS	total antioxidant capacity
TEAC	total equivalent antioxidant capacity
BMI	body mass index
FM	fat mass
MM	muscular mass
WC	waist circumference
TEI	total energy intake
CD	carbohydrates
P	protein
L	lipid
PBG	postprandial blood glucose
GSK3 β	glycogen synthase kinase-3 β

Introduction

Westernized diets containing foods with high sugars and/or saturated fats and a low fiber content can lead to a number of chronic disorders, including cardiovascular diseases (CVDs), obesity, and diabetes [1,2]. Data from clinical trials suggest that low glycemic foods have small, but clinically useful effects on medium-term glycemic control in diabetic patients, which may be protective against cardio-metabolic diseases [3]. Cinnamon has been used for years as a spice, but just recently it has become increasingly popular for its benefits in glycemic control [4–6]. In short-term studies, the ingestion of either cinnamon powder or its equivalent in aqueous extract has been shown to significantly improve glycemia in healthy adults [7–11]. Moreover, the addition of cinnamon to a high-sugar meal seems to reduce postprandial glucose concentration and gastric emptying rate [7], and to reduce insulin concentrations [8]. Nevertheless, it was also reported that cinnamon powder added to a high-fat meal had little or no effect on postprandial lipidemic and glycemic responses or on the gastric emptying rate [12].

According to previous investigations involving several medicinal plants, cinnamon is among the most effective plants in the regulation of blood glucose *in*

vitro [13]. An *in vitro* study showed that this spice seems to exert a mimetic effect of insulin through increased glucose uptake in adipose tissue and skeletal muscles [14]. This effect is believed to be associated with its polyphenolic content, which is known to provide protection against cardio-metabolic diseases. Specifically, a water-soluble polyphenol type-A polymer compound isolated from *C. burmannii* has been shown to enhance insulin action by stimulating the insulin receptor *in vitro*, and it also shows antioxidant effects, which can protect against cardiovascular diseases [15]. Taking into account the literature, this study hypothesizes different postprandial blood glucose concentrations after ingestion of a custard tart without and with cinnamon powder. Thus, the aim of the study was to investigate the effect of adding cinnamon powder (3 g) to Portuguese custard tart (*Pastel de Nata*) on postprandial capillary blood glucose concentrations in healthy human subjects.

Subjects and methods

Subjects

Thirty-six healthy subjects between 18 and 50 years of age were recruited at Cooperativa de Ensino Superior Egas Moniz and the 32 subjects meeting the inclusion/exclusion criteria were enrolled. The flow diagram is depicted in Figure 1. The inclusion criteria were no symptoms and no history of gastrointestinal diseases, abdominal surgery, diabetes mellitus, lactose intolerance or cinnamon allergy. The exclusion criteria were impaired fasting glucose, pregnancy and lactating or menopausal conditions. Moreover, subjects should not take hyperlipidemic, hypoglycemic, anti-hypertensive, anti-coagulant or antibiotic medications. The participants were asked not to consume caffeine, alcohol, or tobacco, or practice physical exercise in the 8 hours prior to the intervention. A written informed consent was obtained from each participant after they were given written and oral information about the study. This study was approved by the Portuguese state-recognized Ethics Committee (Ethics Committee of Cooperativa de Ensino Superior Egas Moniz) and was carried out in accordance with the Helsinki Declaration of 1975 as revised in 2000.

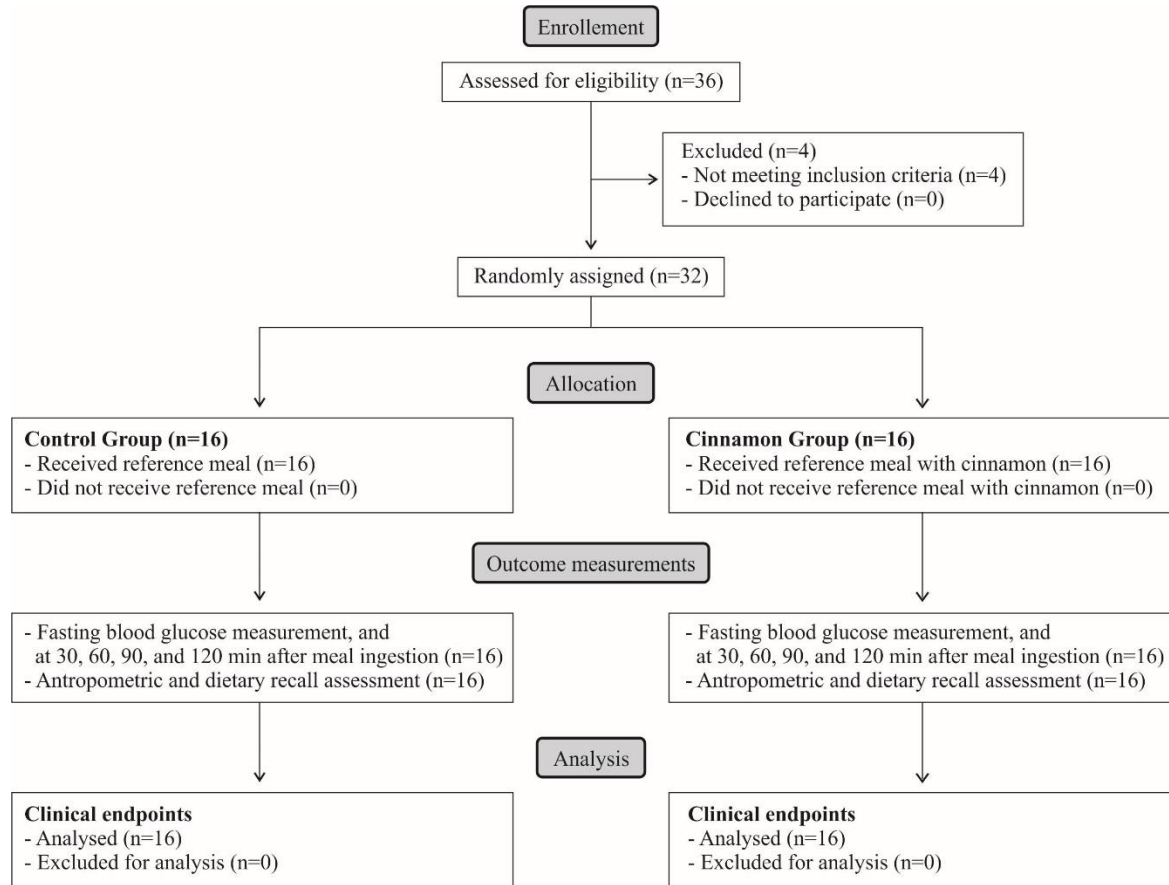


Figure 1. Flow diagram showing the experimental design and the participation of the subjects throughout the study. [RB: In bottom 2 boxes “Analyzed” should be used instead of “Analysed”, and “from” [not “for”] should be used in “Excluded from analysis”].

Study Design

Subjects were randomly assigned to 2 groups who randomly received a custard tart without (control) or with cinnamon added, Figure 1. Subjects were asked to fast the night before for at least 8 hours before the intervention. For both cinnamon and control groups, the intervention consisted of the ingestion of the reference meal either with or without cinnamon, respectively, in the morning (approximately 5-minute duration). The intervention in both groups was supervised by one of the study investigators.

The reference meal consisted of 60 g of custard tart (*Xandite* bakery, Caparica, Portugal), which contained 178.8 kcal: 65% of energy from carbohydrate (29.1 g), 31% of energy from fat (6.1 g) and 4% of energy from protein (1.7 g). Each subject from the control group had one reference meal. Each

subject from the cinnamon group had one reference meal containing 3 g of cinnamon (*Cinnamomum burmannii*; Sucrame, Produtos Alimentares, Lda; Nandi cafes, Portugal) powder.

Blood samples were taken by capillary finger stick before intervention (t_0) and at 30 (t_{30}), 60 (t_{60}), 90 (t_{90}) and 120 (t_{120}) minutes after ingestion of the meal. Sterilized lancets, glucometer equipment and strips for glucose meters (*FreeStyle_Abbott Diabetes Care*) were used to measure blood glucose concentrations.

An ADE stadiometer with a range of 85 to 210 cm (± 0.1 cm) was used for height assessment. Body Mass Index (BMI) was calculated based on the subjects' weight and height measurements. Weight, fat mass (FM) and muscular mass (MM) were measured using an *InBody 230* Body Composition

Analyzer. Waist circumference (WC) was measured with a metric tape (± 0.1 cm).

A last meal dietary recall was employed by the investigator during each intervention in order to compare food ingestion between groups following the last meal before the intervention. The *Portuguese Composition Food Table* was used to analyze the nutritional composition of the food. The blood glucose area under the curve (AUC) of each subject was calculated using GRAPH PAD PRISM software (version 5.01).

All data collection was registered in an Excel ® file where a coded number was assigned to each participant to blind the analysis.

Chemical Analysis

Ferric Chloride (III) hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), Folin-Ciocalteu, trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), TPTZ 2,4,6-tri(2-piridil)-s-triazine, methanol (CH_3OH), potassium persulfate (K_2SO_4), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt were from Sigma-Aldrich (Portugal), gallic acid-1-hydrate ($\text{C}_6\text{H}_2(\text{OH})_3\text{COOH} \cdot \text{H}_2\text{O}$) was from Acros Organics (Portugal) and sodium carbonate (Na_2CO_3) was from ICS Science group (France). All reagents were *pro analysis* grade.

All absorbance measurements were performed in a Perkin-Elmer Lambda 25. The reagents were weighed on an analytical balance (Sartorius, ± 0.0001 g).

Both custard tarts without and containing 3 g of cinnamon were subjected to a hydro-ethanolic (20:80) extraction. The mixture was filtered using the Whatman paper filter. Homogeneous samples were obtained and subjected to chemical analysis. Determinations were carried out in triplicate.

The total phenolic concentration of each extract was determined according to the Folin Ciocalteu method employing gallic acid as standard [16]. The results were expressed as mg of gallic acid equivalent (GAE)/L of extract. In order to obtain 500 μL , a sample extract water/ethanol (50:50), a volume of 312.5 μL of sample extract water/ethanol (20:80) was added to 187.5 μL of water. Folin Ciocalteu reagent (5 mL, 1:10 diluted with distilled water) and aqueous

Na_2CO_3 (4 mL, 1 M) were added to the sample extract. After 15 minutes, the absorbance was measured at 765 nm using a spectrophotometer.

The antioxidant effect (reducing ability) was evaluated by monitoring the formation of an intense blue color from the Fe^{2+} -TPTZ complex, according to the Ferric Reducing Antioxidant Power (FRAP) assay [17]. A fresh solution was prepared by mixing 25 mL of acetate buffer (300 mM, pH = 3.6), into 2.5 mL of TPTZ solution (10 mM TPTZ in 40 mM HCl) and 2.5 mL of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (20 mM). The solution was heated at 37°C. Samples (150 μL) were mixed with 2850 μL of the FRAP solution and was maintained in darkness for 30 minutes. The absorbance was measured at 593 nm using a spectrophotometer. Trolox was used as standard and the results were expressed in μmol Trolox/L.

The free radical scavenging capacity was also studied using the ABTS radical cation decolorization assay, where the antioxidant capacity was measured as the ability of test compounds to decrease the color reacting directly with the $\text{ABTS}^{+\bullet}$ radical (an intensely colored radical cation monitored in the range of 600–750 nm) [18]. This assay was performed by using two similar tests namely: TAS (total antioxidant status) and TEAC (total equivalent antioxidant capacity), both using Trolox as standard (mM Trolox/L). The TAS test was performed on a RANDOX RX Daytona model with RANDOX Kit-NX 2332. The TEAC test was performed by reacting 10 mL ABTS (7 mM) solution with 176 μL potassium persulfate (140 mM). The mixture stood in the dark at room temperature for 12 hours. This solution was diluted 70x with ethanol and then a volume of 2850 μL was added to 150 μL of each sample and absorbance was read at 734 nm using a spectrophotometer [19]. This test was performed for several extract concentrations in order to calculate IC_{50} (value defined as the total phenolic concentration required to achieve half maximal inhibition of $\text{ABTS}^{+\bullet}$ radical). All data collections were registered and analyzed in an Excel ® file.

Statistical Analysis

Statistical analysis was performed using the Excel[®] and SPSS[®] Statistics (Statistical Package for Social Sciences) version 20.0 software for Windows[®]. Data are presented as mean $\pm$ SD or SEM. Repeated measurement ANOVA of mixed type was used to assess the difference between the 2 groups for postprandial blood glucose at different times. The independent samples t-test was used to assess the difference between the 2 groups for total energy intake, carbohydrates, protein and lipid, $C_{\max}$, $\Delta C_{\max}$ and AUC values. All statistical tests were performed at the 5% level of significance.

Results

Characteristics of the Subjects

General characteristics of the subjects regarding age, body mass index (BMI), fat mass (FM), muscular mass (MM) and waist circumference (WC) are summarized in Table 1. Of the 32 subjects, 8 men and 24 women participated in the study. The data revealed no statistically significant differences between the control and cinnamon groups regarding the distribution of anthropometric variables ($P > 0.05$).

Table 1. Subjects characteristics for the control group (reference meal) and the cinnamon group (reference meal with cinnamon powder) at start of study¹

	Control Group	Cinnamon Group
Age ² (years)	22.06 $\pm$ 2.3	24.38 $\pm$ 9.4
BMI ² (kg/m ²)		
Men	21.3 $\pm$ 0.6	22.0 $\pm$ 1.0
Women	24.5 $\pm$ 1.3	23.9 $\pm$ 1.7
FM ² (%)		
Men	28.0 $\pm$ 1.2	30.0 $\pm$ 1.6
Women	21.0 $\pm$ 4.4	14.0 $\pm$ 3.6
MM ² (%)		
Men	37.8 $\pm$ 1.0	39.2 $\pm$ 0.7
Women	49.0 $\pm$ 2.2	45.0 $\pm$ 2.7
WC ² (cm)		
Men	71.2 $\pm$ 1.7	72.4 $\pm$ 2.6
Women	80.5 $\pm$ 3.8	83.4 $\pm$ 4.3

¹All values are expressed as mean $\pm$ SD, $n = 16$ for each group.

² No significant differences between the control and cinnamon groups were found, $P > 0.05$ (Independent sample *t* test).
FM, fat mass; MM, muscular mass; WC, waist circumference.

Table 2. Mean total energy intake (TEI), carbohydrates (CD), protein (P) and lipid (L) intake at last meal prior to the intervention¹

Dietary Parameters	Control Group	Cinnamon Group	<i>P</i> value ²
TEI (Kcal)	652.9 $\pm$ 187.8	581.5 $\pm$ 194.9	0.300
CD (g)	62.9 $\pm$ 26.4	52.8 $\pm$ 30.1	0.321
P (g)	26.8 $\pm$ 10.7	36.6 $\pm$ 22.7	0.213
L (g)	16.5 $\pm$ 8.2	20.2 $\pm$ 10.8	0.290

¹All values are expressed as mean $\pm$ SEM, $n = 16$ for each group.

² No significant differences between control and cinnamon groups were found, $P > 0.05$ (Independent sample *t* test).

Table 2 shows the total energy intake and macronutrient composition regarding carbohydrates, protein and lipid at the last meal before the interventions for each of the 2 groups. They can be considered to be homogeneous since they did not reveal any statistically significant differences in these dietary parameters ($P > 0.05$).

Postprandial Blood Glucose Concentrations

Table 3 shows the postprandial blood glucose (PBG) concentrations, which were measured for the 2 groups (control group and cinnamon group). Repeated measurement statistical analysis revealed that there was no interaction between the independent and repeated measures factors ($P = 0.530$), suggesting that it was not possible to infer about differences in PBG

at different times. The data further suggest that the administration of cinnamon powder to custard tart slightly decreased PBG mean values compared to custard tart without cinnamon powder, although that difference did not reach statistical significance ($P = 0.273$).

The results presented in Figure 2 show that the addition of cinnamon to custard tart resulted in a significant decrease in the blood glucose area under the curve (AUC) compared to the control group ($P = 0.0005$). However, no significant differences were observed in postprandial blood glucose in terms of maximal glucose concentration ($C_{\max}$) and variation of maximal concentration ($\Delta C_{\max}$) ($P > 0.05$) between the cinnamon and control groups (Table 4).

Table 3. Mean blood glucose concentrations¹ in healthy subjects obtained prior to the intervention (t_0) and after the ingestion of each meal consisting of custard tart (control group) and custard tart with cinnamon powder (cinnamon group) at 30 (t_{30}), 60 (t_{60}), 90 (t_{90}) and 120 (t_{120}) minutes after ingestion

Time	Control Group mmol/L	Cinnamon Group mmol/L
t_0	4.76 ± 0.14	4.70 ± 0.14
t_{30}	6.18 ± 0.24	5.71 ± 0.21
t_{60}	5.53 ± 0.24	5.41 ± 0.12
t_{90}	5.06 ± 0.17	4.84 ± 0.15
t_{120}	4.76 ± 0.14	4.72 ± 0.11

¹All values are expressed as mean $\pm$ SEM, $n = 16$ for each group. No interaction between the independent and repeated measures factors was found, $P = 0.530$, (Repeated Measures ANOVA of mixed type).

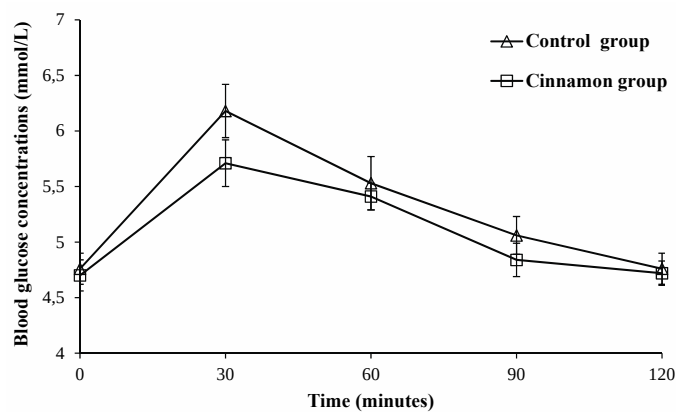


Figure 2. Time course curves showing mean ($\pm$ SEM) blood glucose concentrations (mmol/L) in healthy subjects after ingestion of custard tart alone (control group, $n = 16$) and custard tart with cinnamon (cinnamon group, $n = 16$). No interaction between the independent and repeated measures factors ($P = 0.530$) was found (Repeated Measures ANOVA of mixed type).

Table 4. Blood glucose area under the curve (AUC), maximal concentration ($C_{\max}$) and variation of maximal concentration ($\Delta C_{\max}$) in healthy subjects after ingestion of custard tart alone (control group) and custard tart with cinnamon powder (cinnamon group)¹

	Control Group	Cinnamon Group
AUC _{0-120 min}	645.7 $\pm$ 7.7	599.2 $\pm$ 9.1 ²
$C_{\max}$ (mmol/L)	6.2 $\pm$ 0.2	5.7 $\pm$ 0.2
$\Delta C_{\max}$ (mmol/L)	1.4 $\pm$ 0.2	1.0 $\pm$ 0.2

¹All values are expressed as mean $\pm$ SEM, $n = 16$ for each group.

²Significantly different from the control group, $P < 0.001$ (Independent sample t test).

Table 5. Total phenolic content, FRAP assay, TAS and TEAC (IC_{50}) tests of plain custard tart and custard tart containing 3 g of *C. burmannii*¹

	Custard tart	Custard tart with 3 g cinnamon
Total phenolic (mg/L gallic acid)	253.7 $\pm$ 22.8	1278.7 $\pm$ 0.7
FRAP assay (μ mol Trolox/L)	417.3 $\pm$ 12.7	963.0 $\pm$ 8.9
TAS test (mmol Trolox/L)	< 0.4	417.3 $\pm$ 12.7
TEAC (IC_{50}) (mg/L gallic acid)	257.5 $\pm$ 42.4	58.9 $\pm$ 0.1

¹All values are expressed as mean $\pm$ SEM.

Total Phenolic Content, FRAP Assay, TAS and TEAC (IC_{50}) Tests

Table 5 shows the results of the different tests for total phenolic content, FRAP assay, TAS and TEAC (IC_{50}) of plain custard tart and custard tart with 3 g of *C. burmannii*. The data revealed that custard tart, after addition of cinnamon powder, increased the total phenolic content. Moreover, custard tart with cinnamon addition showed high antioxidant activity and revealed 4.4 times more capacity of free radical scavenging compared with custard tart alone (IC_{50}).

Discussion

There are several different species of cinnamon that have been studied to determine their hypoglycemic effects [20] in both animal [14] and human [10] models. To date, not much work has been done using the *C. burmannii* species investigating both its effect in lowering blood glucose concentration and analyzing its antioxidant properties. The results from the present study have shown that the addition of *C. burmannii* powder to a Portuguese custard tart (*Pastel de Nata*) can significantly reduce blood glucose as expressed by the AUC in healthy adults. This result is

in agreement with previous studies, which demonstrated that the addition of cinnamon powder (6 g) from *C. cassia* to a high-sugar meal significantly lowered postprandial blood glucose, as expressed as the AUC [7], and blood glucose concentrations [10], compared with the reference meal. This result could be attributed to a delay in gastric emptying as a result of the cinnamon presence [7]. According to Horowitz et al., plasma glucose concentration in healthy subjects is inversely related to the rate of gastric emptying at 120 min [21]. Moreover, the gastric emptying itself does not seem to be the only mechanism accounting for the effect of cinnamon on glucose homeostasis. Cinnamon can markedly inhibit the enzymes involved in carbohydrate digestion, namely the intestinal α -glycosidase [22] and the pancreatic α -amylase, [23] which could prevent the absorption of carbohydrates into the small intestine.

In agreement with finding from others [8, 12], this study found that 3 g cinnamon powder did not significantly alter either $C_{\max}$, $\Delta C_{\max}$ or postprandial blood glucose concentrations. This result may be due to the fat content (31%) of custard tart, which seems to affect the glucose absorption, thereby interfering with postprandial glycemia. According to Normand et al., the carbohydrates-rich meal with medium doses of fat (33%) lowers the postprandial glucose peak

[24]. Moreover, in our previous work, cinnamon tea ingestion in a dose of 6 g significantly lowered postprandial $C_{\max}$, $\Delta C_{\max}$, and blood glucose in healthy subjects [11]. These observations suggest that cinnamon may act in a dose-dependent manner to improve glucose utilization through insulin receptor potentiation, as reported previously in an *in vitro* study [25].

In addition, chemical analyses performed in this study show that the addition *C. burmannii* to custard tart increased the total phenolic content. In addition, both the FRAP assay and the TAS test showed a high ability of cinnamon to reduce the ferric complex and to inhibit the free radical ABTS^{•+}, respectively. These results are in agreement with previous studies, which demonstrated strong antioxidant activity and high total phenolic content in different species of cinnamon extracts [26,27]. This high antioxidant property of cinnamon may be attributed to its high content in phenols. In a study by Dudonné et al., a significant relationship between antioxidant capacity and total phenolic content was found, suggesting that these compounds are the major contributors to the properties of this spice [27]. Specifically, polyphenol polymers of cinnamon with double-linked type-A procyanidin may potentiate insulin action by exerting an effect on glucose metabolism in fat cells [15]. Thus, cinnamon powder addition to custard tart could have a protective effect on diabetes, which is a risk factor of cardiovascular disease [28], due to its potential antioxidant activity.

Several mechanisms have been described for the action of polyphenols isolated from cinnamon. These compounds seem to modulate multiple steps of the insulin signal transduction pathway stimulating the glucose uptake and glycogen synthesis. These include: i) increased tyrosine phosphorylation activity of insulin receptors [29]; ii) increased amount of glucose transporter (GLUT4) protein [29]; and iii) increased glycogen synthase activity and decreased glycogen synthase kinase-3 β (GSK3 β) activity [30]. These bioactive compounds of cinnamon have also been shown to possess insulin-independent effects on the regulation of gene expression in adipocytes by inducing GLUT1 gene expression [31].

According to current evidence, the ingestion of cinnamon, either as whole cinnamon or as cinnamon extract, has been shown to enhance fasting blood

glucose [5,6]. However, it is particularly noteworthy that available studies in the literature are heterogeneous since they have employed different species and different compositions, study design, doses and formulations [32], which all limit the ability to apply cinnamon to nutritional care.

There are some limitations that should be acknowledged in the present study. It did not apply a 24-hour dietary recall, which could be an important measure to evaluate the food ingested the day before, affecting the fasting blood glucose concentration [3]. In future studies, it would be useful to apply the 24-hour dietary recall method for the dietary record. Moreover, the small number of participants in the present study compromised the external validation of these results and consequently the nutrition care process. To address this issue, a specific study focused on cinnamon powder used on large numbers of participants should be conducted.

As reported in other studies with cinnamon [8,10], the present study revealed a beneficial effect of adding cinnamon powder to a high-sugar meal, which could contribute to nutritional and alternative medicine practices.

Conclusion

The results from the present study revealed that 3 g of *C. burmannii* powder significantly decreased postprandial blood glucose as expressed by the AUC in healthy subjects. This result could be attributed to a delay in gastric emptying slowing the glucose absorption. Additionally, another possible mechanism of cinnamon action involves the inhibition of intestinal α -glycosidase and the pancreatic α -amylase, preventing the absorption of carbohydrates in the small intestine.

C. burmannii powder was also shown to increase the total phenolic content and to improve the antioxidant activity of Portuguese custard tart (*Pastel de Nata*). Further studies should investigate the long-term effect of cinnamon use in glucose homeostasis, especially in type 2 diabetic patients.

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The authors' responsibilities were: MMM and MAB conducted research (generation and collection of chemical data) and analysed chemical data; ARJ conducted research (generation and collection of clinical data); MAB and MLS analyzed clinical data and wrote the manuscript; MMM, MAB, JAB, JS, PMP and MFM revised the manuscript; JAB performed statistical analyses of the data; MFM provided the conception and design of the study and have primary responsibility for the final content; all authors read and approved the final manuscript. None of the authors declare a conflict of interest.

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