Different postprandial acute response in healthy subjects to three strawberry jams varying in carbohydrate and antioxidant content: a randomized, crossover trial

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Abstract

Purpose: Dietary food composition influences postprandial glucose homeostasis. Thus, the objective was to investigate the effects of an acute intake of three different types of strawberry jam, differing in carbohydrate and antioxidants content, on postprandial glucose metabolism, lipid profile, antioxidant status and satiety.

Methods: Sixteen healthy adults participated in a randomized, crossover, double-blind study with three arms, receiving 60g of three different strawberry jams. Blood samples were collected at fasting and at 30, 60, 90 and 120 min after its intake. Blood analyses were performed with validated procedures and satiety was estimated with visual analogue scale (VAS).

Results: Blood glucose concentrations were maintained at normal values and without peaks within the 2 h after consumption of low-sugar jams. However, blood glucose and insulin were significantly higher at 30 and 60 min after high-sugar (HS) jam intake versus both low-sugar jams. Furthermore, HS jam produced more satisfaction at short time, but decreased as soon as blood glucose concentration began to decrease. Moreover, HS ingestion produced lower free fatty acid levels ($p<0.05$) throughout the trial with respect both the low-sugar jams However, no additional benefits on oxidative status (malondialdehyde, glutathione peroxidase, total antioxidant capacity, and uric acid), glucose, lipid and satiety variables were observed due to the inclusion of an antioxidant to low-sugar jam.

Conclusions: This study reinforces the idea that products without added sugars are appropriate for the management of glycaemic alterations and provides further insight into the effect of natural antioxidants as a functional ingredient on oxidative status and related metabolic disturbances. Registered at www.clinicaltrials.gov as NCT01684332.

Keywords: Antioxidant, Glucose metabolism, Jam, Polyphenols, Postprandial, Strawberry

Abbreviations

FFA: Free fatty acids

GI: Glycaemic index

GPx; Glutathione peroxidase

HDL-c: High-density lipoprotein-cholesterol

HOMA-IR: Homeostasis model assessment-estimated insulin resistance

HS: High-sugar jam

LDL-c: Low-density lipoprotein-cholesterol

LS: Low-sugar jam

LSA: Low-sugar jam including antioxidant extract

MDA: Malondialdehyde

TAC: Total antioxidant capacity

TG: Triglycerides

VAS: Visual analogue scale

Introduction

Westernized diets, which contain foods high in simple sugars and/or saturated fats, together with the increasing consumption of processed foods, refined grains and a lower fiber intake, have been associated with the risk of suffering from chronic diseases [1]. The postprandial oxidative stress caused by the intake of this type of diet may be a key factor in the development of conditions such as diabetes, dyslipidemia, hypertension, inflammation, etc. [2,3]. The postprandial state is a dynamic period, where the absorbed substrates are metabolized [4], but also pollutant molecules known as reactive oxygen species are generated, which may influence long-term health status [5].

Antioxidant intake has been often inversely linked with the onset of metabolic syndrome and cardiovascular disease [6,7]. Fruits, which are sources of fiber, vitamins, polyphenols, and other bioactive compounds, have a recognized antioxidant capacity [7]. In turn, data from clinical trials suggest that low glycaemic response diets based on fruits, favourably modulate oxidative stress, inflammation, lipid, and glucose metabolism as well as appetite, while high-sugar consumption is negatively associated with the development of obesity and associated disorders, mainly type 2 diabetes and metabolic syndrome [7-9]. For these reasons, studies have been designed to assess the health effects of fruits and fruit-derived products such as juices, jams, or syrup in humans [10,11]. Thus, it has been hypothesized that, like fresh fruits, these products could induce healthy properties due to the antioxidant and fiber content, occurring naturally or added [12], as well as being involved in carbohydrate metabolism regulation [13,14]. Strawberries are a rich source of antioxidants, polyphenols, and vitamin C, which gives it an important antioxidant activity. Strawberries can be eaten fresh, but it is also widely consumed as jam, especially as part of breakfast. Taking into account the positive aspects of strawberries (antioxidant content), we may consider that it is probably a food that confers protection against pathologies related to oxidative stress as the underlying mechanism [15]. To our knowledge, only one previous study has investigated the effect of strawberry jam. In that study, five strawberry jams with different carbohydrate composition were assessed to observe the effect on GI (glycemic index) and postprandial blood glucose [16]. In that study, Kurotobi et al. [16] concluded that the jam containing the highest level of glucose showed a high GI, while those containing a high concentration of fructose showed a low GI. However, it has not been evaluated the effects of a low-sugar jam with added antioxidants on whole carbohydrate metabolism, lipid metabolism, oxidative status, and hunger scores in humans.

Therefore, the aim of this study was to investigate the acute consumption effect of three different types of strawberry jams, high-sugar (HS), low-sugar (LS) and low-sugar including antioxidant (LSA), with different carbohydrate and antioxidant content, on postprandial glucose metabolism, lipid profile, antioxidant profile, and satiety in healthy adult men and women.

Materials and Methods

Subjects

Six men and ten women (body mass index 23.99±3.05 kg/m^2; Age: 25.94±3.02 years old) were enrolled in the study (baseline characteristics are shown in Table 1). Conjointly a physician, a nurse, and a dietician performed the screening of volunteers by means of medical history, blood biochemical analysis, physical examination, anthropometric, and body composition measurements. Subjects with diabetes, a low body weight (body mass index <18.5 kg/m^2), or obese (body mass index >29.9 kg/m^2) status were excluded. Other exclusion criteria included any chronic disease status related to metabolism, following concomitant medications, use of birth control pills, slimming or hormone replacement treatments, inability to perform the follow-up, smoking, known food allergies, and pregnant, lactating, or menopausal women. After having read the study information and asking all the questions they had, all subjects gave written informed consent to participate in the trial, which had been previously approved by the Research Ethics Committee of the University of Navarra (ref.no 078/2010) and followed the Helsinki Declaration guidelines. This trial was registered at www.clinicaltrials.gov as NCT01684332.

Strawberry jam composition

In the study, three different types of strawberry jam: HS, LS and LSA jam were investigated (Table 2). The HS jam contained naturally occurring sugars and added sugars (41.8±1.6 g/100 g). The other two types of jams contained only naturally occurring sugars (LS 2.6±0.1 g/100 g and LSA 2.7±0.1 g/100 g), without added sugar. The most important differences between LS and LSA jams lie in free polyphenols (369.03±64.79 vs. 839.81±43.30 μmol catechin/100 g) and fiber (1.12±0.2 vs. 1.64 g/100 g) contents, respectively. The antioxidant extract added to the LSA jam is a natural mixture, obtained from the strawberry pulp. The three types of jams were analyzed by the National Centre for Food Safety and Technology (CNTA, Spain).

Study design and procedures

The trial was a randomized, crossover, double-blind study with three arms. Each volunteer attended the Metabolic Unit (Department of Nutrition, Food Science and Physiology of the University of Navarra) once a week during three consecutive weeks, at the fasting state. The participants subsequently received 60 g of jams HS, LS or LSA in a randomized order in days 0, 7 and 14 (with a wash-out period of 7 days, among assays). The randomization code was performed using the "random between 1 and 3" function in the Microsoft Office Excel 2003 software (Microsoft Ibérica, Spain).

The volunteers were instructed not to change their physical activity patterns, or performing extreme physical activities prior to the test days. Also, the volunteers were asked, during the 3 nights previous to the intervention, to consume a standardized dinner (100 g lettuce, 150 g tomato, 50 g onion, 150 g chicken breast, 15 g oil and 200 g apple) at least 10 h before visiting the facilities (around 10:00 p.m), as well as to avoid antioxidant-rich food products the week before to the test meals. At visit 1, volunteers attended the Metabolic Unit, where anthropometric measures were taken and a blood sample was extracted between 8:00 and 8:30 a.m (time 0). Afterwards, each volunteer was provided with the visual analogue scale (VAS) questionnaire to assess the appetite feelings [17]. Once the volunteer had correctly filled the questionnaire, he/she was provided with the portion (60g) of the randomly assigned jam to be consumed in less than 5 min. At 30, 60, 90 and 120 min after the jam intake, new blood samples were extracted, and a new VAS questionnaires were completed. At visits 2 and 3, the same procedure as in visit 1 was conducted, consuming the remaining types of jams (Fig. 1).

Blood pressure, anthropometry and body composition

Blood pressure was measured following World Health Organization criteria [18]. Body weight was assessed using Tanita bioelectrical impedance (SC-330, Tanita, Tokyo, Japan), and height was measured using a wall-mounted stadiometer (Seca 220, Vogel & Halke, Germany). Both measurements were taken in underwear after an overnight fast. The waist circumference was measured at the narrowest point between the rib cage and the iliac crest and the hip circumference at the widest point over the buttocks.

Blood glucose metabolism, lipid metabolism and oxidative stress profiles

On intervention days, volunteers arrived to the Metabolic Unit where a cannula was inserted into the antecubital vein. Blood samples were taken every 30 min and were collected using EDTA and CLOT tubes. Then, blood samples were left at room temperature for 10 min. Afterwards, samples were

centrifuged for 30 min at 2,013 × g (3,500 rpm) and 4ºC in a standard centrifuge (Eppendorf 5804R, Hamburg, Germany) to obtain plasma and serum aliquots, which were stored at -80 ºC for subsequent analyses. Plasma concentrations of glucose, total cholesterol, high-density lipoprotein-cholesterol (HDL-c), triglycerides (TG), free fatty acids (FFA), and uric acid were measured by specific colorimetric assays in an autoanalyzer Pentra C200 (Horiba ABX Diagnostics, Montpellier, France) and insulin was measured by an Enzyme Linked ImmunoSorbent Assay technique (Mercodia, Upssala, Sweden) in a Triturus autoanalyzer (Grifols, Barcelona, Spain). Low-density lipoprotein-cholesterol (LDL-c) concentration was calculated following the Friedewald equation. Insulin resistance was calculated by the homeostasis model assessment-estimated insulin resistance (HOMA-IR) index [19]. Serum concentrations of malondialdehyde (MDA), glutathione peroxidase (GPx), and total antioxidant capacity (TAC) were assessed by validated colorimetric assays (Thermo Scientific, Vantaa, Finland). MDA by Bioxytech LPO-586 kit (OxisResearch Inc, Portland, EE.UU), GPx by Glutathione Peroxidase Assay (Cayman Chemical, Ann Arbor, USA) and TAC by Antioxidant Assay (Cayman Chemical Company, Canada /EE.UU). The coefficients of variation were lower than 8% in all the assays.

Satiety score (VAS)

Visual analogue questionnaire is based on 5 lines of 10 cm in length with words anchored at each end, reporting the most positive and the most negative rating of each question as described elsewhere [17]. The lines measured hunger, satiety, satisfaction, desire to eat, and thirst feelings asking the questions below: How hungry do you feel? How full do you feel? How satisfied do you feel? How much do you think you can eat? and How thirsty do you feel? Quantification was done by measuring the distance from the left end of the line to the mark.

Statistical analysis

According to the screened literature, MDA concentration was considered as the main variable. Assuming as significant, a difference ($p<0.05$) between jams on MDA levels of 0.5 ± 0.4 mg/dl [9,20] and 90 % statistical power ($\beta = 0.90$), it was estimated that 13 volunteers would be needed as sample size. Expecting a dropout rate of 25%, the total number of participants needed for the study was established in 16 subjects. Statistical analysis of data consisted on a descriptive presentation of the parameters analyzed in the nutritional intervention. Data are shown as means ± SD. Differences between groups in continuous variables were assessed using Student's *t* test. To study the postprandial evolution of the variables' repeated measures ANOVA and Post hoc tests (Bonferroni) were performed. p values <0.05 were

considered statistically significant. Analysis were carried out using SPSS 15.0 software for Windows (SPSS Inc, Chicago, USA)

Results

Adherence to the study

As shown in Fig. 1, adherence to the study was 100%, and the 16 participants who went under randomisation completed the three visits. In order to choose the participants, 21 subjects were screened and 5 subjects were excluded.

Blood pressure, anthropometry and body composition measurements

The age of female and male participants was not statistically different. As expected, some phenotypical differences were found at baseline due to the sex concerning body mass index, body composition etc. (Table 1). No differences were found in blood pressure, anthropometry and body composition after following the experimental protocols (data not shown).

Glucose and lipid metabolism determinations and oxidative profile

The postprandial glycemic response following consumption of jams without added sugar (LS and LSA) was significantly different ($p<0.001$) compared to the increase obtained after the intake of HS jam at the first 30 min. After the consumption of LSA and LS, blood glucose concentrations were maintained within normal blood glucose values (<100 mg/dl), without peaks in the 2 h after consumption. The insulin and HOMA-IR index response after the ingestion of jam behaved in the same way as blood glucose. Thus, insulin and HOMA-IR index significantly increased after HS jam intake at 30 min ($p<0.001$) compared with both LS and LSA jams. However, insulin and HOMA-IR values decreased at 30 min after HS intake, showing significant higher levels ($p<0.001$) at 30 and 60 min when it was contrasted with the concentrations obtained after LS and LSA jams ingestion (Fig. 2).

After consumption of the three different types of jams, the levels of total cholesterol, HDL-c, and TG decreased ($p<0.05$) between 0 and 30 min, showed no differences between jams (Table 3). After LSA and HS jam consumption, no differences were found in LDL-c concentrations during different times, and only a decrease was observed between 0 and 30 min after LS jam consumption. In the case of FFA, an interaction between jam and time ($p=0.013$) was identified. The time curve of FFA obtained after the consumption of both LS and LSA jams was similar and significantly different ($p<0.05$) to the one that was obtained after the consumption of HS jam, which maintained lower FFA levels ($p<0.001$) at 60, 90,

and at 120 min (p<0.001 and p=0.001) in relation to LS and LSA jams (Fig. 2). At 30 min after consumption, only differences were between LSA and HS (p<0.01) jams.

Regarding the antioxidant profile, no conclusive changes concerning GPx, uric acid, TAC and MDA measurements were found, all showed similar outcomes (Table 4). Curiously, the only difference observed was in time of the uric acid (p=0.032) and MDA (p=0.003) variables. However, after a post hoc analysis (Bonferroni), differences between times in every jam were not maintained.

Satiety score

Concerning the satiety assessment, only differences in fullness and satisfaction were evidenced. The analyzed jams produced a different full feeling sensation (p=0.035). However, after pos hoc screening, no significant differences between jams at different time points were observed. HS jam produced higher full feeling than LSA jam at 30 min after consumption, but without statistical significance. In terms of satisfaction, differences were observed within the time (p=0.038). increasing the level of satisfaction (p=0.018) after consumption of HS jam during the first 30 min, which did not occur after the intake of both LS and LSA jams (Fig. 3).

Discussion

Despite some debate, consuming foods containing antioxidants, either occurring naturally or added, might be useful for preventing or reversing oxidative stress [2,5,21]. Moreover, products with low amounts of simple sugars, low glycemic index or rich in fiber can be of interest in the management of blood glucose alterations [22-24]. In that context, fruits and fruit-derived products have shown some healthy benefits, improving carbohydrate, lipid profile and, oxidative status [25,26]. However, to date little is known about the effect of the addition of natural antioxidants to fruit-derived products, such as jams. For this reason, the present study was conducted to verify whether a low-sugar jam with natural added antioxidant (LSA) is more effective to control postprandial glucose, lipid, and antioxidant status comparing with low-sugar jam without antioxidant extract (LS). Simultaneously, the acute effect of those jams compared to a traditional strawberry jam with added sugar (HS) and without added antioxidants was taken into account.

At 30 min after HS jam intake, a peak in blood glucose concentration was observed, which is in agreement with other studies [27]. Accordingly, after the jam ingestion, insulin also increased, in response to the elevation of blood glucose concentration [22,28,29]. The intake of simple sugars, such as

In this study, the results confirm that total cholesterol, HDL-c, LDL-c (only with LS jam), and TG concentrations decreased during the first 30 min after consumption of the three different types of jams, which might be partly attributed to the fact that insulin promotes the transfer of LDL-c from peripheral blood to liver, reducing the circulating LDL-c concentrations [35,36]. In addition, the FFA value obtained after the consumption of LS and LSA jams was similar, but significantly different from those obtained after the consumption of HS jam, which maintained lower FFA levels during and at the end of the trial. The mechanism by which this reduction seems to occur could be mediated by insulin, which stimulates lipoprotein lipase and inhibits hepatic lipase. Consequently, chylomicrons and very low-density lipoproteins (rich in TG), that are synthesized after jam consumption, are hydrolyzed to release FFA, while intracellular lipolysis is reduced. These findings are important, because the result is a lower availability of FFA in plasma, but more FFA to be accumulated into the adipocyte. Additionally, insulin induced stimulation of glucose metabolism in fat cells increasing the availability of glycerol to re-esterify FFA and to accumulate into the adipocytes as TG [29]. Moreover, fructose may play an important effect on hepatic de novo lipogenesis, hereby also insulin resistance, increasing TG concentration. The average amount of fructose in the dinner previous to the intervention day was 14 g in all groups. Although the quantity of fructose contained in the jams was different and may have influenced some biomarkers, which

must be considered in result interpretation. However, the experimental meal, consisting only in 60g of jam, would not have been enough to induce changes in serum cholesterol after 2 h.

In relation to oxidative status, the natural antioxidant extract derived from strawberry pulp and added to jam produced neither additional benefits nor differences on measured oxidative stress parameters (MDA, GPx, uric acid, and TAC) that were obtained after LSA consumption. Regarding these outcomes, there are controversial opinions. Some authors have reported that after the consumption of antioxidant products by subjects with an optimal antioxidant status, no differences or improvements have been observed [37-39]. However, in subjects with low antioxidant status, beneficial effects have been shown [40,9]. In this trial, the antioxidant profile of the volunteers was probably good, because all participants were healthy, young, and non-smokers. Furthermore, there are also some studies which have reported an antioxidant effect in vitro, but not in vivo [41]. Moreover, the strawberry variant and temperature conditions are also important factors in the loss of antioxidants content [33,34].

Concerning the VAS, the literature is inconsistent. Glucostatic hypothesis, which was proposed in 1953 by Mayer, for the regulation of food intake, established that a high glucose concentration in blood produces satiety [42]. After the intake of highly digestible carbohydrates, hunger decreases rapidly and satisfaction increases in a short period of time. Moreover, when blood glucose concentration starts to decrease, hunger appears again and the feeling of satisfaction decrease [43]. In our trial, significant differences were observed in satisfaction during the first 30 min after HS jam consumption, which contained rapid digestible carbohydrates. Thus, it produced higher glucose and insulin responses and higher satisfaction than both low-sugar jams at first 30 min after intake. However, once the peak of blood glucose occurred, satisfaction started to decrease again. In contrast, LSA and LS jams, which contained sweeteners (sucralose) did not increase blood glucose or insulin levels, thus did not increase satiety during the first 30 min after it consumption. This fact is probably due to the different carbohydrate content of the jams (HS 46.0 ± 2.1; LS 4.2 ± 0.8; LSA 5.1 ± 0.9 g per 100g). Furthermore, all of these results can be related to the GI of foods [44]. High GI carbohydrates, such as glucose and sucrose (≥ 55 units), suppress food intake at short term (1-1.5h) after consumption [45], while the satiating effect of low GI carbohydrates appears later after ingestion (2-3h) and result in lower postprandial blood glucose and insulin, thereby reducing appetite [46]. Moreover, the taste or sweetness of the jams is an important factor which could probably influence the satiety response [47].

Conclusions

Our results indicate that an acute intake of low-sugar jam provided a more favourable postprandial blood glucose concentration than a jam with added sugar, due to the maintenance of blood glucose levels after its consumption. However, the addition of a natural antioxidant extract to a low-sugar jam did not involve any additional benefit. Even so, this study contributes to elucidate the effect of natural antioxidants as functional ingredients.

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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FIGURE TITLES AND CAPTIONS

Fig. 1 Flow-chart of the intervention

After 21 subjects recruitment, medical history, blood biochemical analysis, physical examination, anthropometry, and body composition measurements were taken. Finally, 16 subjects were randomized

and consumed the three types of jam in three different days with one week wash-out period. At baseline and the end of the study anthropometry, body composition and blood pressure measurements were obtained. At baseline, 30, 60, 90 and 120 min after the jam intake, a blood sample was extracted and a VAS questionnaire was completed.

Fig. 2 Postprandial serum glucose metabolism during 2 h after jam's consumption (*n*=16)

(a) Postprandial blood glucose concentration, (b) Postprandial blood insulin concentration, (c) Postprandial HOMA-IR index, and (d) Postprandial Free fatty acids

p values concerning the effect of jam, the effect of time, and the effect of Jam × time interaction have been shown. $p<0.05$ has been considered statistically significant. Values are mean ± SD, n=16. Differences between jams identified as *asterisk*. Data were analyzed by repeated measures ANOVA followed by Bonferroni post hoc analysis.

HOMA-IR Homeostasis model assessment-estimated insulin resistance, *HS* high-sugar jam, *LS* low-sugar jam, *LSA* low-sugar jam including antioxidant extract

Fig. 3 Postprandial satiety score during 2 h after jam's consumption (*n*=16)

(a) How hungry do you feel? (b) How satisfied do you feel ? (c)How full do you feel? and (d) How much do you think you can eat?

Repeated measures ANOVA analyzed for Jam × time interaction was significant ($p<0.05$). Values are mean ± SD, n=16. Differences between jams identified as *asterisk*. Differences between times identified as *ash symbol*. Data were analyzed by repeated measures ANOVA followed by Bonferroni post hoc analysis

HOMA-IR Homeostasis model assessment-estimated insulin resistance, *HS* high-sugar jam, *LS* low-sugar jam, *LSA* low-sugar jam including antioxidant extract