Effect of Sugary Beverages Ingestion on Thirst Sensation

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Porto, 2014
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Acknowledgments

This thesis was made possibly thanks to the contribution of a lot of people and institutions to whom I am grateful:

My beloved parents and sister
My supervisor, example and friend Prof. Dr. Vitor Hugo Teixeira
Arq. Luzia Gama
Prof. Dr. Pedro Moreira
Dra. Mónica Sousa
Prof. Dr.ª. Patrícia Padrão
Mestre Renata Barros
Prof. Dr. José Soares
Prof. Dr. Nuno Borges
Eng. Rui Chilro
Engª. Maria de Jesus
Prof. Dr. Paulo Marques
Prof. Dr. Franklim Marques and his team
Prof. Dr. João Tiago Guimarães and his team
Dr. Gustavo Costa
Dr. Ricardo Pinto
Dr. Filipe Barbosa
Sr. João Noronha, Sra. Mónica Lamas and Sra. Fernanda Cardoso
All my PhD colleagues
All the participants of the trials
Sumol+Compal
SASUP
Instituto de Hidratação e Saúde
European Hydration Institute
List of Abbreviations

BIA - Bioelectrical Impedance Analysis
BMI – Body Mass Index
CCK – Cholecystokinin
ECW – Extracellular Body Water
GIP – Gastric Inhibitory Polypeptide
GLM – General Linear Model
GLP-1 – Glucagon-like Peptide-1
GLUT 5 – Glucose Transporter 5
HFCS – High Fructose Corn Syrup
HS – “High” Sucralose
ICW – Intracellular Body Water
IT – Iced Tea
LS – “Low” Sucralose
NFM – Non-fat Milk
NRS – Numeric Rating Scale
NST – Nucleus of the Solitary Tract
OJ – Orange Juice
PDS – Pinapple Diet Soda
PS – Pineapple Soda
SGLT-1 – Sodium-glucose Cotransporter 1
SPSS – Statistical Package for Social Sciences
TBW – Total Body Water
VAS – Visual Analog Scale
W – Water
Sugary beverages are believed to increase thirst sensation due to their hyperosmolality face to plasma but the few studies that analyzed this relationship show contradictory results. In addition to this, non-energetic sweetened beverages and beverages with different sweetness levels and sugars could be hypo-osmolar in comparison to blood and their impact on thirst is still unknown.

This work aimed to compare the effect on thirst sensation of: beverages sweetened with sugar and non-caloric sweeteners; beverages with different sweetness levels and beverages with distinct dietary sugars.

In three distinct clinical trial series with a crossover design, participants consumed a standardized breakfast followed by three different chilled preloads: 1) 500ml of water, regular and diet pineapple soda; 2) 500ml of water, high-sweetened diet pineapple soda and low-sweetened pineapple soda; 3) 330ml of water, non-fat milk, orange juice and iced tea. They rated thirst, desire to drink, mouth dryness, nausea, hunger and desire to eat at baseline and at 30-min intervals until a standardized lunch with ad libitum water intake being served 2h30 after preload. Hydration status, glycaemia, plasma osmolality and sodium (in third trial) were evaluated at baseline and before lunch. Until the end of the day, participants recorded all food and fluid intake.

Thirst sensation does not differ greatly between all beverages studied and only a tendency to higher thirst ratings after non-fat milk intake face to water and iced tea was observed in men. A tendency to higher water intake at subsequent meal was detected in high-sweetened, low-sweetened and regular pineapple soda face to water, and in non-fat milk face to water and iced tea. Sugary beverages led to a more pronouncedly decrease in glycaemia face to water but hydration status, plasma osmolality and sodium does not exhibited major differences between beverages. Energy, sugars, caloric beverages and total fluid intake after lunch and until the end of the day does not differ significantly regardless of the morning preload.

Thus, despite different sugar, sweetness and osmolality levels, our work suggests that sugary and sweetened beverages, when presented chilled, are as thirst-quenching as water, with a potential exception of non-fat milk. These beverages does not elicit major differences in hydration status and other physiological parameters involved in thirst regulation and in short-term nutritional intake.
Resumo

Presume-se que as bebidas açucaradas aumentem a sensação de sede pelo facto de, na sua maioria, serem hiperosmolares em relação ao plasma. Ainda assim, os estudos que analisaram esta relação mostraram resultados bastante contradiatórios. Para além disso, as bebidas com edulcorantes e com diferentes graus de doçura e açúcares distintos, podem ser hipoosmolares relativamente ao plasma e o seu impacto na sensação de sede é ainda desconhecido. Este trabalho teve assim como objectivo comparar o efeito na sensação de sede de: bebidas açucaradas e com edulcorantes; bebidas com diferentes níveis de doçura e bebidas com diferentes fontes de açúcar.

Em três series distintas de ensaios clínicos com design crossover, os participantes ingeriram um pequeno-almoço estandardizado seguido da ingestão de 3 conjuntos de bebidas-teste frescas: 1) 500ml de água, refrigerante de ananás e refrigerante light de ananás; 2) 500ml de água, refrigerante de ananás muito doce e refrigerante de ananás pouco doce; 3) 330 ml de água, leite magro, sumo 100% laranja e chá gelado. Foram classificadas as sensações de sede, desejo de beber, secura da boca, náusea, fome e desejo de comer no início do ensaio clínico e a cada 30 minutos até a um almoço com ingestão de água ad libitum ser servido, 2h30 após a ingestão das bebidas-teste. O estado de hidratação e glicemia, osmolaridade e sódio plasmático (no último ensaio) foram avaliados no início no protocolo e antes do almoço, sendo que após este e até ao final desse dia, os participantes registaram em diários alimentares todos os alimentos ingeridos.

A sensação de sede não diferiu de modo significativo entre todas as bebidas estudadas, sendo que apenas uma tendência para maiores níveis de sede após a ingestão de leite magro comparativamente a água e chá gelado foi observada nos homens. Uma tendência para uma maior ingestão de água no almoço subsequente foi igualmente detectada após a ingestão de todos os refrigerantes de ananás em comparação com a água e após a ingestão de leite magro em comparação com a água e chá gelado. As bebidas açucaradas levaram a uma descida mais pronunciada dos níveis de glicemia em comparação com a água mas os níveis do estado de hidratação, osmolaridade e sódio plasmático não mostraram diferenças significativas entre bebidas. A ingestão energética, de açúcares, bebidas calóricas e fluidos ao longo do dia do ensaio clínico não foi substancialmente diferente independentemente da bebida-teste ingerida nessa manhã.

Assim, apesar dos diferentes níveis de açúcar, doçura e osmolaridade, o nosso trabalho sugere que bebidas com açúcar e edulcorantes, quando ingeridas frescas, são tão eficazes quanto a água na saciação da sede, com uma potencial excepção do leite magro. Nenhuma das bebidas promoveu diferenças significativas no estado de hidratação e nos outros parâmetros fisiológicos envolvidos na regulação da sede bem como na ingestão nutricional a curto prazo.
Health, and particularly nutrition, are perhaps the matters more susceptible to myths and fads. The classical recommendation to avoid sugary beverages when thirsty, due to their hypothetic inefficacy in thirst-quenching capacity is a great example of a nutrition myth with no scientific evidence. The mechanism behind this recommendation is the fact that most of sugary beverages are hyperosmolar face to plasma and an increase in blood osmolality could possibly trigger thirst sensation. However, until now, no studies have analyzed specifically the impact of commercially available sugary beverages on thirst sensation. The few ones that partially investigate the impact of sugar and sugary beverages on thirst does not show a coherent pattern that allow us to draw any conclusion. Firstly, in a study that infused various hypertonic solutions in healthy humans, revealed that saline and mannitol infusions led to increases in plasma osmolality and vasopressin concentration, but hypertonic glucose, although had significantly increase plasma osmolality, decreased plasma vasopressin and had no detectable effect on thirst [1]. Other studies [2-6] have analyzed the impact of different beverages such as milk, fruit juices and cola beverages on satiety and subsequent energy intake but with thirst assessment only as a secondary outcome. Even so, the results were contradicting. In a study when participants ingested 360g of water, diet cola, regular cola, orange juice, 1% milk or no beverage during an *ad libitum* lunch reveal that thirst ratings were lower after consumption of diet cola than after consumption of juice or milk. Subjects’ ratings of fullness after lunch did not differ among beverages, but were lower for the non-beverage condition, and energy intake from lunch (including beverage) did not differ among the caloric beverage conditions [2]. Another study compared the effects of 590ml of orange juice, low-fat milk (1%), regular cola and sparkling on hunger, thirst, satiety and energy intake at next meal. Although four beverages have satisfied thirst equally well, a significant beverage by gender interaction occurred with water and orange juice satisfying thirst better than did the cola beverage in women. The three energy-containing beverages were associated with higher fullness and reduced hunger compared to water, but energy intake at subsequent lunch were the same across all four beverage conditions [3]. A study of Monsivais et al, compared the effects of 475ml diet cola, 495ml 1%milk, 525ml sucore-sweetened cola, 475ml of high-fructose corn syrup-sweetened cola with 42% of fructose (HFCS 42) and 525ml of high-fructose corn syrup-sweetened cola with 55% of fructose (HFCS 55) on hunger, satiety and energy intake at next meal. Here, the 5 beverages did not differ significantly from each other in thirst ratings, hunger and satiety profiles with milk being the only beverage to partially suppress energy intake at next lunch [4]. Another study that aimed to measure the effects of oro-sensory stimulation and energy content on taste-related brain activations of 450ml of sucrose-sweetened orangeade and non-caloric sweetened orangeade, reported that thirst decrease equally in the two beverages [6]. The only study that reported an effective increase in thirst after the intake of sugary beverages is from 1990 and showed that thirst sensation decreased less after the consumption of 235ml of sucrose sweetened lemonade than of the same amount of water and aspartame-sweetened
lemonade. However, this was observed when the beverages were ingested in a context of an *ad libitum* self-selected lunch, and when the beverage volume doubled to 470ml this effect faded [7]. Since the main objective of almost all of these studies was to evaluate the effect of beverages on satiety, hunger and energy intake at subsequent meal, physiological parameters involved in thirst regulation were not evaluated nor as *ad libitum* water intake in subsequent meal. Even with this methodological issues, in this short review, we can thus conclude that does not exist a linear relationship between the amount or even the presence of sugar in a beverages and a positive impact on thirst sensation.

Regarding the ingestion of sugary beverages and their metabolic effect, Kristek *et al* [8], proposed an interesting theoretical model. In this model, it is postulated that sucrose drinks, with an osmolality near 400mOsm/kg, require small amounts of water to reach isotonicity (0.5 liters only needs 0.16 liters of water to become 300mOsm). Then, 50% of the total intestine volume (near 0.33 liters) will be quickly absorbed with glucose, and the rest will slowly follow fructose absorption with fluid volume load being optimized within 3 or 4h. A different process would occur with HFCS-containing soft drinks, because, when 500ml of a soft drink with HFCS (800mOsm/Kg) is ingested, it needs to be diluted to a total volume of more than a 1 liter to become isotonic. Nearly 45% of this volume is expected to be absorbed within 2 hours, along with glucose absorption, but the reestablishment of hydration levels, affected by the need to dilute the hyperosmotic HFCS beverage, takes the next 2 hours along with slow fructose absorption, not being thus a surprise (according with the author) that thirst can be sooner and better satisfied by fresh water than by hyperosmolar soft drinks.

Nevertheless, this mechanism and expected results in thirst sensation was not confirmed in clinical trials cited above.
References


2. Hypothesis and Aims

Hypothesis

Sugary beverages are less effective than water in thirst satisfaction due to their hyperosmolality face to plasma

Aims

The specific aims of this thesis were:

1. Compare the effect of beverages sweetened with sugar and non-caloric sweeteners on thirst sensation

2. Analyze the impact of the beverage sweetness level on thirst

3. Compare the effect of energetic beverages with distinct dietary sugars on thirst
Experimental protocol of the trials

- Fasting + 500 ml water before bedtime and after waking

- Clinical Trial Eve
  - Restriction of:
    - Alcohol Ingestion
    - Caffeine Ingestion
    - Physical Activity

- Initial Outcomes:
  - Height
  - Weight
  - Total Body Water (TBW)
  - Extracellular Body Water (ECW)
  - Intracellular Body Water (ICW)

- Preload Ingestion
  - (Plasmatic Sodium)
  - Blood Glucose
  - Osmolality

- Motivational Rating (MR) 0 + Breakfast

- MR 1

- MR 2

- MR 3

- MR 4

- MR 5

- MR 6

- MR 7

- MR 8

- Completion of Food Records

- Rest of the day

- Restriction of:
  - Physical Activity

- Final Outcomes:
  - TBW
  - ECW
  - ICW

- Lunch

- Ad libitum water ingestion

- Beverage Acceptability/Sweetness Rating

- (Plasmatic Sodium)

- Blood Glucose

- Osmolality
Abstract
The correct physiological function of all systems in the body and body fluid homeostasis requires a constant supply of water and sodium. Fluid balance is maintained via thirst, a feedback-controlled process regulated acutely by central and peripheral mechanisms. Increases in blood osmolality draws water from cells into the blood, dehydrating specific brain receptors that stimulate drinking and the release of vasopressin. Water losses are lowered by increases in water reabsorption in kidneys and reduction of urine volume. Dehydration caused by losses of water from extracellular compartment, stimulate vascular receptors that signal brain centers to initiate drinking and vasopressin release. In kidneys, baroreceptors release renin that starts a cascade of events to produce angiotensin II that in addition to initiate drinking and vasopressin release, also stimulates aldosterone to reduce the sodium losses in urine. At same time, vasopressin and angiotensin II exerts a vasoactive effect to reduce blood vessel diameter around the remaining blood. This integrated response to dehydration allow cardiovascular system to maintain a constant perfusion pressure particularly to the brain, however, other factors beyond osmolality and plasma volume seem to affect thirst and drinking since pre-absorptive mechanisms such as oropharyngeal receptor stimulation are capable to decrease thirst sensation before body fluid restoration is achieved. Sensory characteristics of beverages are also a major determinant of their thirst-quenching ability with attributes intimately related with mouth-wetting effect such as cold, sour and acid having a thirst supressing capacity, with the opposite being observed for beverage sweetness, thickness and after taste.
Introduction

Thirst is a sensation that is difficult to describe and a physiological state often difficult to diagnose, despite being a process very finely controlled [1]. It has been described as “the perception of one’s need for drink” [2], as “the consequence of the need to moisten the mouth” [3] or as “a physiological state linked to fluid deficit” [4, 5]. Thus, despite being a subjective perception, thirst plays a key role in the regulation of body fluid homeostasis since correct physiological function of all the systems in the body requires a constant supply of water and sodium [6]. Fluid balance is then maintained via thirst, a feedback-controlled variable, regulated acutely by central and peripheral mechanisms.

Physiological Regulation of Thirst

Under ideal conditions, where all physiological systems function correctly, the osmolality of all fluid compartments is the same and water is distributed proportionally (2/3 intracellular and 1/3 extracellular). Sodium is the principal cation of the extracellular fluids with potassium being that of the intracellular compartment. The balance between these two cations plays an essential role in the regulation of plasma osmolality, the gradient that regulates the movement of water within the body [1]. Small increases of 1-2% in the effective osmotic pressure of plasma result in stimulation of thirst. This can be achieved by an increase in sodium concentration like a meal or solutes like sodium chloride or sucrose [7]. In intracellular or osmotic thirst, when an increase in osmolality of extracellular space occurs, water draws from the intracellular space to reestablish the condition of equiosmolality. This increase in osmolality triggers a response by neurons existing in central nervous system and in the periphery (osmoreceptors) transmitting a neural input to the brain to initiate the search for water and reduce osmolality to a physiological set point. This search for water is then mediated by osmoreceptors existing in the brain and in the periphery (kidneys) and baroreceptors present in cardiopulmonary system and arterial walls which connect with the paraventricular and supraoptic nuclei of hypothalamus to liberate vasopressin from their axon terminals in the posterior neurohypophysis into blood stream (reviewed by [1, 8]). The osmotic threshold for both thirst and vasopressin release is generally considered to be very similar and once defined as 281mOsm/Kg for thirst and 285mOsm/Kg for vasopressin [9]. This antidiuretic hormone is also responsible for increase the membrane permeability in distal tubules and collecting ducts of nephrons and thereby increases water reabsorption and the concentration of excreted urine [10]. Any water drunk at this time would be rapidly absorbed into the blood supply reducing the concentration gradient and reestablishing the normal concentrations and volumes in both compartments [6].
In extracellular or volemic thirst, a fluid loss from the vasculature in the order of 5-8% (e.g. hemorrhage, vomiting, sodium loss or edema) leads to a decrease in blood volume and, consequently, fluid from the interstitial compartment move into the blood flow to reestablish the homeostasis. Detectors in the arch of aorta, carotid sinus and great veins sense this decrease in plasma volume and increase the production of vasopressin with consequent reduction in urine production. Simultaneously, pressure detectors in juxtaglomerular apparatus of the kidney sense the decrease in perfusion pressure and promote the release of renin. Renin acts on circulating angiotensinogen released from liver producing angiotensin I. This decapeptide is then converted in the lung to angiotensin II, by angiotensin-converting enzyme and this octapeptide exerts several actions having a vasoactive effect in the reduction of the diameter of the blood vessels and also stimulate the release of vasopressin and another hormone – aldosterone - from the adrenals. Aldosterone is the hormone responsible for sodium regulation, decreasing the excretion of this cation in urine via stimulation of sodium potassium ATPase pump in kidney distal tubule and collecting duct. Aldosterone also sensitizes hypothalamus to the circulating levels of angiotensin II which stimulates the ingestion of sodium, the “sodium appetite”. This concerted actions promote the ingestion of water and sodium and ensure that blood volume does not decrease to a volume that is dangerous to health. As blood volume increases, the stimuli for thirst disappear, and vasopressin and renin levels goes down allowing the vasculature to accommodate the increase in volume without the consequent increase in blood pressure that is inherent to the vasoconstrictor character of vasopressin (reviewed by [1, 6]).

Besides hypothalamic control of thirst, other neural mechanisms are involved in homeostatic regulation of fluid intake. Since angiotensin and vasopressin cannot cross the blood-brain barrier, the translation of the endocrine signal into conscious awareness of thirst occurs by activation of receptors for these hormones in two circumventricular organs (subfornical organ and the organum vasculosum of the lamina terminalis) situated in the anterior wall of third ventricle, outside the hypothalamus [11]. The destruction by lesion of these organs cause an inhibition of thirst in rodents [12]. Even knowing that data on this kind of lesions in humans is obviously limited, disease states affecting hypothalamus, such as Huntington's disease, are associated with altered vasopressin levels, increased thirst levels and dry-mouth sensations [5, 13]. So, although hypothalamus was classically considered the “thirst center”, these interactions with extra-hypothalamic regions create a complex neural circuitry regulating fluid intake [14, 15].

Nevertheless, other factors beyond osmolality and plasma volume seem to affect thirst and drinking [16]. The fact that humans stop drinking following dehydration-induce thirst well before body fluid restoration is achieved [17] and osmolality only starts to decrease about 10 minutes after cessation of drinking [4] shows that pre-absorptive mechanisms such as oropharyngeal receptor stimulation need to be involved. At this regard, a study where the water previously ingested was extracted from the stomach via nasal-gastric tube found that subjects’ thirst returned to baseline values even though no water as absorbed [17]. This same oropharyngeal receptors are responsible for the ergogenic effect of carbohydrate mouth rinses in athletic performance, since it has become clear that the underlying mechanisms for the ergogenic effect during exercises with rel-
atively short duration (~1h) may reside in the central nervous system and are not confined to its conventional metabolic advantage [18].

Thus, it is clear that hyperosmolality and/or hypovolemia stimulate thirst. However, it is not clear whether drinking is a response of changes in osmolality or plasma volume during normal daily conditions since fluid consumption can be made well before any water deficit occur [19] and approximately 75% of fluid intake occurs peri-prandially [20] which reinforces the importance of the non-homeostatic influences on drinking [21].

How can we measure thirst?

Thirst assessment relies upon and individual’s recognition, perception, and explanation of the sensation [15]. The methods more commonly used to measure thirst were visual analog scales (VAS) and categorical scales, such as numeric rating scales (NRS). A recent review [22] of clinical trials that investigated the relationship of thirst to specific physiologic thirst-related correlates and associated thirst mediators showed that all studies used VAS or NRS to quantify thirst sensation. To our knowledge, a comparison of the sensitivity or applicability of these instruments in thirst assessment has never been made. Although the majority of the trials included in the review cited above used VAS as a method to assess thirst, the opposite succeed in studies that investigated the impact of sugary and sweetened beverages on thirst. Here, most of the studies used unipolar adjective scales anchored at each end with labels 1=not thirsty at all and 9=extremely thirsty [23-26], notwithstanding that some of them used a 100-mm VAS with the same labels [27, 28]. In addition to this, correlations between thirst and biomarkers of the physiological systems that regulate fluid balance (e.g. short-term body mass (water) loss, serum osmolality, plasma volume) are weak and there is no single index that is a reliable predictor [29, 30].

Regardless of the method used, a very pertinent question remains: Is perceived thirst translated into higher ad libitum fluid intake? Most of the studies does not include a procedure that evaluate this relationship and it is known that thirst ratings, in particular, does not show an unequivocal correspondence with drinking raising the concept of hedonic thirst, where drinking may be driven more by pleasure and reward than by fluid balance [31].
Sensory and environmental influences in thirst

The environmental changes that promoted a constant availability of foods and beverages and the multiple social contexts that encourage eating and drinking in the absence of energy and fluid needs have partially degraded the functional relationship between appetitive sensations and food and beverages intake [31]. On the other hand, when we try to find some reasons to the lack of response to physiological signals of thirst, we see that the same environmental issues can also be responsible for this situation, as showed by the main barriers to fluid consumption by kidney stone formers: “not knowing the benefits of fluid”, “not remembering to drink”, “disliking the taste of water”, “lack of thirst and lack of availability”, and finally “the need to void frequently and related workplace disruptions” [32]. Particularly in these individuals with chronic lower fluid ingestion, the high palatability of many energy-yielding or non-energetic sweetened beverages may facilitate their consumption through the enhancement of rewarding sensory associations. Beverage properties such as flavor [33], color [34], sweetness [35] and temperature [36, 37] can all be manipulated to enhance non-homeostatic ingestive behaviors such as drinking highly palatable energy-yielding beverages to satisfy thirst in the absence of energy need. In the study of McKiernan et al [20] where relationship between human thirst, hunger, drinking and feeding was evaluated, individuals responded “appropriately” by consuming water in response to thirst, in the absence of hunger, only 2% of the time. They responded “inappropriately” (i.e., thirsty and hungry but did not drink or eat; not thirsty and not hungry but drank and/or ate; not thirsty but hungry and drank but did not eat; thirsty but not hungry and did not drink but ate) 62% of the time.

Regarding the influence of beverage sensory characteristics on thirst sensation, the review from Labbe et al [5] present a very interesting relationship between specific beverage attributes and their thirst-quenching capacity (Fig. 1). In this context, beverages that promote a higher mouth-wetting effect due to their cold temperature or due to their acid flavor and subsequent increase in saliva secretion are perceived as more effective in thirst satisfaction [38]. Sensory trained panels [38-40] are also in agreement regarding the attributes positively (coldness, sourness, clear appearance) and negatively (thickness, sweetness, after-taste) correlated with the refreshing perception and thirst-quenching ability of beverages. Beverage carbonation play also a role in thirst perception. Levels of carbonation equal or in excess of 2.3 volumes of CO₂ can negatively impact drink acceptability, voluntary fluid intake and thirst quenching capacity [41], although a more recent study does not report any difference in thirst ratings between sugar-sweetened beverages with low (1.7), medium (2.5) and high (3.7) volumes of CO₂ [42]. Therefore, sensory characteristics of the beverages have a crucial role in thirst satisfaction and in drinking behavior.
Thus, regarding the physiological, sensory and environmental factors comprising thirst sensation, the conclusions of the review of Richard Mattes [21] provide a great comprehension about the integration of all these dimensions on drinking behavior: A) thirst sensations are high and stable over the day; B) the health consequences of drinking in moderate excess of need are minimal; C) there are strong non-homeostatic influences on drinking; D) beverages are highly palatable, inexpensive and convenient; E) it is socially acceptable to drink in many social and professional settings and F) beverages elicit weak compensatory dietary responses.

Figure 1. Schematic representation of the construction of refreshing perception
Image from D. Labbe et al. (2009)
References


3.2
Effects of Energetic and Non-energetic Sweetened Beverages Ingestion on Thirst Sensation

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Submitted for publication

Abstract

Sugary and sweet beverages are assumed to be less effective in satisfaction of thirst than water, but a direct link between sugar and thirst has never been described. In this study, we compared the effects of water (W), regular (PS) and diet (PDS) pineapple soda intake on thirst, mouth dryness, hunger and water ingestion at subsequent meal as well as their impact on hydration status, glycaemia and blood osmolality. In a crossover design, twenty-four participants (13 male), aged 19-28 years consumed in three consecutive weeks a standardized breakfast followed by a 500ml preload of water, regular or diet pineapple soda. They rated thirst, desire to drink, mouth dryness, nausea, hunger and desire to eat at baseline and at 30-min intervals until a standardized lunch with ad libitum water intake being served 2h30 after preload. Hydration status, glycaemia and plasma osmolality were evaluated at baseline and before lunch. Until the end of the day, participants recorded all food and fluid intake. Thirst sensation does not differ between beverages along the trial but PS preload revealed a tendency to higher water ingestion at lunch face to W (P=0.087). No differences on hydration status and plasma osmolality were observed between beverages but PS led to a more accentuated glycaemia decrease compared to the other preloads (P=0.004). Energy, sugars, fluid and caloric beverages intake throughout day showed no differences between beverages. These results reveal an absence of a link between the ingestion of sugary and sweet beverages and thirst sensation.

Keywords: sugar, osmolality, water, sweetener, soft-drinks
Introduction

The positive relationship between sugary beverages and thirst is classic. The biological explanation for this common sense belief includes the elevation of blood glucose levels and subsequent rise in blood osmolality, which would be responsible for triggering the thirst sensation. Due to the efficiency of water excretion, there are less consequences to humans of drinking in excess – except in extreme cases – than eating in excess [1]. Furthermore, thirst sensations are relatively stable over the day, with 75% of total fluid intake occurring peri-prandially revealing a more consistent pattern than the episodic fluctuations generally observed for hunger [2]. This condition has been contributing to the scarce investigation about the impact of nutritional composition of beverages on thirst so far. Moreover, it is more difficult to record changes in thirst sensation than in hunger feeling, which can also contribute to this gap in thirst research. The few studies [3-8] that analyzed the impact of different sugary beverages on thirst sensation were inconclusive and do not allow to draw any association between the amount - or even the presence - of sugar in a beverage, and an impact on thirst. Also, the effect of beverage sweetness has never been isolated from the effect of sugar on thirst. In fact, all these studies adopted a classical ingestive model (i.e., preload) to evaluate the impact of different beverages (water, milk, yogurt, cola, orange juice, etc.) on satiety and subsequent energy intake, and thirst sensations were only measured as a secondary outcome. Furthermore, no biochemical parameters such as glycaemia, blood osmolality or plasmatic sodium were measured, being the impact on thirst sensation extrapolated only by individuals' subjective ratings.

So, with our study we aimed to determine the impact of sweetened beverages intake on thirst sensation, on physiological parameters involved in their regulation, and also distinguish the effects of sugar than that of artificial sweeteners in this regard.

Material and methods

Participants

Thirty participants were recruited at University of Porto through e-mail advertisings. During the study, 6 dropouts occurred so the final sample included 24 subjects (13 male). The eligibility criteria included: age between 18-35 years; body mass index (BMI) between 18.5 – 25 kg/m²; non-smokers; non-athletes; weight stable on the previous 6 months; not dieting to gain or lose weight; not using any kind of medication (except oral contraceptives in women) and not being pregnant and nursing. To minimize variability, each participant was asked to be fast since 22h00 of the previous day and to abstain from alcohol, caffeine and strenuous physical activity on the day of the trial and the day before. To ensure euhydration status and avoid mouth dryness, subjects were
instructed to drink 500 ml of water the evening before the trial and 500 ml water on the morning of the trial immediately after wake up [9]. Participants who met these requirements and accepted to participate in this study were informed verbally and in writing regarding the experimental procedures before giving their written informed consent. Participants received a financial compensation upon submission of meal and transportation expenses resulting from travel to our lab.

**Study design**

The trials followed a single blind, randomized cross-over mode with treatment order in counterbalanced design. Each participant came for three sessions, separated by a week, and lasting from 08h00 to 14h00. On all testing occasions was offered to each participant a standardized breakfast followed by a preload stimuli 1h30 after. A standardized lunch was served 2h30 after preload. The time interval set between preload and lunch was longer than the previous studies in this area [3, 5, 8].

**Preload stimuli**

The 3 beverages studied were Water (W), Pineapple Soda (PS) and Pineapple Diet Soda (PDS). Energy, nutritional and chemical composition of the beverages are presented in Table 1. Beverages were presented chilled (8 – 10º C), but without ice, in 500 ml portions in opaque plastic containers. Participants were asked to consume the entire amount within 15 min. The sodas were previously decarbonized by the manufacturer to avoid any kind of interference of beverage carbonation [6, 10]. All beverages were analyzed previously in laboratory in order to obtain their pH, osmolality and sodium characteristics.

<table>
<thead>
<tr>
<th>Preload</th>
<th>Energy</th>
<th>Carbohydrates</th>
<th>Sugars</th>
<th>Protein</th>
<th>Fat</th>
<th>Energy Density</th>
<th>pH</th>
<th>Osmolality</th>
<th>Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kcal</td>
<td>g</td>
<td>g</td>
<td>g</td>
<td>g</td>
<td>kcal/g</td>
<td></td>
<td>mOsm/Kg</td>
<td>mg</td>
</tr>
<tr>
<td>Water</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5.8</td>
<td>3</td>
<td>2.05</td>
<td></td>
</tr>
<tr>
<td>Pineapple Soda</td>
<td>220</td>
<td>55</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>0.44</td>
<td>3.5</td>
<td>409</td>
<td>13.5</td>
</tr>
<tr>
<td>Pineapple Diet Soda</td>
<td>22</td>
<td>5.5</td>
<td>5.5</td>
<td>0</td>
<td>0</td>
<td>0.044</td>
<td>3.8</td>
<td>81</td>
<td>19.5</td>
</tr>
</tbody>
</table>

a Fastio ™, Portugal  
b Sumol Ananás™, Portugal  
c Sumol Zero Ananás™, Portugal

*Table 1.* Energy, nutritional and chemical composition of the 500ml preloads
Motivational Ratings

Participants rated their thirst, mouth dryness, desire to drink, hunger, nausea and desire to eat using a nine point Likert scale. The unipolar adjective scales were anchored at each end with labels $1 = \text{not at all}$ and $9 = \text{extremely}$. Participants also rated their liking for the beverage in a nine point hedonic preference scale, where $1 = \text{dislike extremely}$ and $9 = \text{like extremely}$. All scales were marked on paper.

Meals Provided

Breakfast was served at 9h30 and consisted in 5 cookies and 200 ml of orange juice. Participants had to consume the entire breakfast within 15 min. A lunch meal prepared without added salt was served at 13h00 and consisted in, approximately, 180 g of vegetable soup, 200 g of boiled rice, 150 g of grilled pork chop and 100 g of apple. Participants were allowed to drink ad libitum the water provided and the amount was measured afterwards. Identical meals were provided on each occasion and their energy and nutrient composition are represented in Table 2. Information was obtained from the label or from software Food Processor SQL Edition, version 10.0 (ESHA Research Inc., Salem, OR, USA).

<table>
<thead>
<tr>
<th>Food</th>
<th>Portion</th>
<th>Energy</th>
<th>Carbohydrates</th>
<th>Sugar</th>
<th>Protein</th>
<th>Fat</th>
<th>Energy Density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>kcal</td>
<td>g</td>
<td>g</td>
<td>g</td>
<td>g</td>
<td>kcal/g</td>
</tr>
<tr>
<td>BREAKFAST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cookies</td>
<td>25</td>
<td>107</td>
<td>19.8</td>
<td>6</td>
<td>1.75</td>
<td>2.25</td>
<td>4.28</td>
</tr>
<tr>
<td>Orange Juice</td>
<td>200 ml</td>
<td>88</td>
<td>21</td>
<td>21</td>
<td>1.2</td>
<td>0</td>
<td>0.44</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
<td>40.8</td>
<td>27</td>
<td>2.95</td>
<td>2.25</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>LUNCH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable Soup</td>
<td>180</td>
<td>39.6</td>
<td>8.7</td>
<td>2.9</td>
<td>1.4</td>
<td>0.54</td>
<td>0.22</td>
</tr>
<tr>
<td>White Rice</td>
<td>200</td>
<td>260</td>
<td>57.2</td>
<td>1</td>
<td>4.8</td>
<td>0.42</td>
<td>1.3</td>
</tr>
<tr>
<td>Pork Chop</td>
<td>150</td>
<td>266.4</td>
<td>0</td>
<td>31.9</td>
<td>14.5</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td>100</td>
<td>47.8</td>
<td>12.7</td>
<td>9.6</td>
<td>0.24</td>
<td>0.16</td>
<td>0.48</td>
</tr>
<tr>
<td>Total</td>
<td>613.8</td>
<td>78.5</td>
<td>13.5</td>
<td>38.3</td>
<td>15.6</td>
<td>0.97</td>
<td></td>
</tr>
</tbody>
</table>

* Makro™, Portugal

Table 2. Energy and nutrient composition of foods provided at breakfast and lunch
Procedures

Participants arrived at the laboratory at 8h00 to assess height (stadiometer SECA 220) and weight, total body water (TBW), intracellular water (ICW) and extracellular water (ECW) with a Segmental Multi Frequency Body Composition Monitor (TANITA MC 180 MA®). Then, a blood sample (8 ml) was collected from the antecubital vein by a trained phlebologist for analysis of blood glucose (ABX Pentra 400; ABX Diagnostics, Montpellier, France) and osmolality (Löser Micro-Osmometer Type 15; Löser Messtechnik, Germany). During the session participants were allowed to read, listen to music with earphones, or use their portable computers with the exception of internet access to minimize visual cues (e.g., unwanted publicity for beverages or visiting Web sites showing pictures of food and drinks), which may have had effect on thirst. Breakfast was served at 9h30 on every occasion, the preload beverage was offered exactly 60 minutes after breakfast (10h30) and lunch was provided at 13h00. Motivational ratings were firstly obtained at the end of blood sampling (baseline or time 0) and every 30 minutes thereafter until lunch time (times 1 through 7). Before lunch, another blood sampling was collected and TBW, ICW and ECW were evaluated once again. Immediately after lunch (time 8), participants completed the last set of ratings and a food record was given to record all food and fluid intake from that moment until 00h00 of that day. Energy and nutritional intake were estimated by Food Processor SQL Edition, version 10.0 (ESHA Research Inc., Salem, OR, USA) added with nutritional information of Portuguese recipes and the following parameters were extracted: Energy, sugars, caloric beverages intake and total fluid intake.

The procedures were approved by Ethics Committee of the Universidade do Porto (Nº30/CEUP/2011), and were registered in ClinicalTrials.gov with (NCT ID: NCT01502722).

Data Analysis

All statistical analyses were conducted using IBM Statistical Package for Social Sciences (SPSS) 20.0 for Windows. Normality was determined by the Kolmogorov-Smirnov test (normal if P > 0.05). When the assumption of normality was violated, it was assured that variables skewness was < 3 and kurtosis < 10 [11]. Sphericity was determined by Mauchly’s test (sphericity assumed when P > 0.05). When the assumption of sphericity was violated, univariate tests of within-subjects effects were subject to Greenhouse Geisser correction (when epsilon < 0.75) and to Huynh-Feldt correction (when epsilon > 0.75). General linear models (GLM) with repeated measures were used to analyze motivational ratings with beverage and time postingestion (times 3-7) as within-subjects factors and sex as the between-subjects factor. Analyses of glycaemia, plasma osmolality, extracellular, intracellular and total body water used a GLM with repeated measures with beverage and time as within-subjects factors and sex as between-subjects factor. When a main effect of time was observed in these variables, a t-student test was performed to find differences between moments for each beverage condition. Analyses of water intake at lunch and energy and nutritional intake after lunch used a GLM with repeated measures with beverage as within-subjects factors and sex as between-subjects factor. Bonferroni-adjusted pairwise comparisons were made when differences in GLM’s were found. Only when there was a gender interaction, data were analyzed...
separately for each group. Data was expressed as mean ± standard deviation. The level of significance was set at P < .05.

Results

The mean age (± SD) of participants was 21.1 ± 1.75 years for men, 22.1 ± 2.79 years for women, and 21.5 ± 2.33 years for the whole group. Mean body mass index (BMI; in kg/m²) was 21.8 ± 1.77 for men, 20.3 ± 1.55 for women, and 21.1 ± 1.75 for the whole group.

Motivational Ratings

Thirst sensation ratings (Fig. 1) suffered a rough decrease after the preload consumption (P<0.001). They gradually increased until lunch time, but it was not observed a main effect of beverage [F (2, 42) = 0.54; P=0.59]. Only a main effect of time was detected [F (1.4, 28.4) = 12.6; P<0.01] and no beverage×sex [F (2, 42) = 2.13; P=0.13], or beverage×time interaction [F (8, 168) = 1.5; P=0.16] were observed.

Figure 1. Temporal profile of thirst sensation by beverage
In mouth dryness ratings (Fig. 2) no differences in beverage type were observed \([F (2, 42) = 0.59; \ P=0.56]\). Only a main effect of time was detected \([F (2.3, 49.1) = 6.32; \ P=0.002]\) and no sex \([F (2, 42) = 0.55; \ P=0.58]\), or beverage*time interaction \([F (4.3, 89.2) = 1.2; \ P=0.32]\) were observed.

![Mouth Dryness (9-point scale)](image)

**Figure 2.** Temporal profile of mouth dryness by beverage

For desire to drink ratings (Fig. 3), only a main effect of time was observed \([F (1.8, 39.3) = 14.7; \ P<0.001]\). There was no effect of beverage \([F (1.7, 36.5) = 1.22; \ P=0.3]\), and no beverage*sex \([F (1.7, 36.5) = 2.28; \ P=0.13]\) or beverage*time interaction \([F (4.3, 94.8) = 1.5; \ P=0.27]\) were noticed.
Hunger ratings (Fig.4), showed a significant effect of beverage \([F (1.6, 35.2) = 83.2; P<0.001]\), with the pairwise comparisons revealing higher hunger scores with Water than with the other two beverages \((P<0.001)\). There was also a significant effect of time \([F (2.2, 47.2) = 35.5; P<0.001]\), and an interaction beverage*time \([F (8, 176) = 8.35; P<0.001]\), revealing that hunger scores increased more in W condition than in PS and PDS. No influence of sex was observed \([F (1.6, 35.2) = 0.87; P=0.41]\).

Desire to eat ratings corroborate the trend of hunger ratings, although the beverage influence did not reach statistical significance \([F (2, 44) = 2.87; P = 0.07]\). Similar to other ratings, it was registered a significant effect of time \([F (2.7, 58.5) = 74.0; P<0.001]\), and no interaction of beverage*sex \([F (2, 44) = 0.74; P=0.482]\) or beverage*time \([F (7.4, 162.6) = 1.55; P=0.15]\).

Ratings of nausea were not affected by time or beverage, and no sex interaction was observed (data not shown).
Hydration Status and Biochemical Parameters

Table 3 show, for each preload condition, the TBW, ICW and ECW at the beginning of the trial and at the pre-lunch evaluation. No sex interaction was observed for any of these parameters. Similarly, there was not observed a significant effect of beverage in TBW: \[F (2, 44) = 0.77; P=0.47\], ICW: \[F (1.2, 26.1) = 0.33; P=0.61\] and ECW: \[F (2, 44) = 0.40; P=0.67\]. Only a main effect of time was observed in the rise of TBW values \[F (1, 22) = 22.9; P<0.001\] indicating that all three beverages had an equal capacity to increase total body water values during the trial, as revealed by T-test (W, P<0.001; PS, P=0.005; PDS, P=0.009).

<table>
<thead>
<tr>
<th>Preload Condition</th>
<th>Initial TBW (Kg)</th>
<th>Final TBW (Kg)</th>
<th>Initial ICW (Kg)</th>
<th>Final ICW (Kg)</th>
<th>Initial ECW (Kg)</th>
<th>Final ECW (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>37.3 ± 7.38</td>
<td>37.7 ± 7.61*</td>
<td>15.0 ± 2.67</td>
<td>15.1 ± 2.74</td>
<td>22.3 ± 4.72</td>
<td>22.6 ± 4.89</td>
</tr>
<tr>
<td>Pineapple Soda</td>
<td>37.4 ± 7.21</td>
<td>37.7 ± 7.37*</td>
<td>15.1 ± 2.64</td>
<td>15.1 ± 2.68</td>
<td>22.3 ± 4.59</td>
<td>22.6 ± 4.77</td>
</tr>
<tr>
<td>Pineapple Diet Soda</td>
<td>37.3 ± 7.03</td>
<td>37.5 ± 7.22*</td>
<td>15.0 ± 2.60</td>
<td>15.1 ± 2.63</td>
<td>22.2 ± 4.45</td>
<td>22.5 ± 4.61</td>
</tr>
</tbody>
</table>

* - P < 0.001 vs. Initial TBW
Table 4 expressed the glycaemia and plasma osmolality at the beginning of the trial and at the pre-lunch evaluation. There was a clear decrease in osmolality values over time \( [F (1, 22) = 25.7; P<0.001] \), with T-test revealing differences between this two moments for all beverages (W, \( P=0.001 \); PS, \( P=0.002 \); PDS, \( P=0.039 \)). No difference between beverages was noticed \( [F (2, 44) = 0.19; P=0.83] \) nor interaction between beverage*time \( [F (2, 44) = 0.79; P=0.46] \) or beverage*sex \( [F (2, 44) = 113; P=0.33] \). Thus, despite the different osmolality values between beverages, in this trial, their impact on plasma osmolality was similar.

Regarding glycaemia, it was observed a significant effect of time on its decrease \( [F (1, 22) = 18.1; P<0.001] \). A significant interaction beverage*time \( [F (2, 44) = 6.28; P=0.004] \) was found, indicating that in PS condition, the decrease in glycaemia was more pronounced than in the other two beverages as we can see by “P” values of T-Test: (W, \( P=0.03 \); PS, \( P<0.001 \); PDS, \( P=0.03 \)).

<table>
<thead>
<tr>
<th>Preload Condition</th>
<th>Osmolality (mOsm/Kg)</th>
<th>Glycaemia (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final*</td>
</tr>
<tr>
<td>Water</td>
<td>313 ± 14.3</td>
<td>299 ± 8.86</td>
</tr>
<tr>
<td>Pineapple Soda</td>
<td>311 ± 10.8</td>
<td>303 ± 8.25</td>
</tr>
<tr>
<td>Pineapple Diet Soda</td>
<td>310 ± 13.9</td>
<td>301 ± 16.1</td>
</tr>
</tbody>
</table>

Table 4. Glycaemia and plasma osmolality values for each preload condition.
* - \( P < 0.001 \) vs. Initial Osmolality
*+ - \( P < 0.001 \) vs. Initial Glycaemia

Beverage Acceptability and Water Intake

Table 5 shows hedonic ratings of the three beverages with a main effect of beverage type \( [F (1.34, 29.4) = 52.2; P<0.001] \). Bonferroni comparisons showed a difference between water and the other beverages \( (P<0.001) \).

<table>
<thead>
<tr>
<th>Preload</th>
<th>Beverage Acceptability</th>
<th>Water Ingestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1-9)</td>
<td>mL</td>
</tr>
<tr>
<td>Water</td>
<td>4.4 ± 1.7*</td>
<td>192 ± 122</td>
</tr>
<tr>
<td>Pineapple Soda</td>
<td>7.0 ± 1.1</td>
<td>256 ± 173</td>
</tr>
<tr>
<td>Pineapple Diet Soda</td>
<td>6.8 ± 1.1</td>
<td>225 ± 133</td>
</tr>
</tbody>
</table>

Table 5. Beverage Acceptability and Water ingestion at Lunch
* - \( P<0.001 \) vs. PS
*+ - \( P<0.001 \) vs. PDS

Regarding the amount of *ad libitum* water intake at lunch for each preload condition, there was a main effect of beverage type \( [F (2, 44) = 3.62; P=0.04] \), although the pairwise comparison revealed a non-significant difference between W and PS \( (P=0.087) \). Even so, a tendency to a higher ingestion of water at lunch was observed in PS condition face to W condition.
Energy, sugar, caloric beverages and total fluid intake throughout the day

Table 6 shows the energy, sugar, caloric beverages and total fluid intake after lunch and until the end of the day. The three beverages led to similar energy intake [F (2, 44) = 0.45; P=0.64]. Regarding sugar intake, the higher values seen after the ingestion of sugary preload does not have statistical significance [F (2, 44) = 1.57; P=0.22]. The caloric beverages intake revealed a significant beverage*sex interaction [F (2, 44) = 3.39; P=0.043]. Analyzing the data separately by sex, a main-effect of beverage was observed in men [F (2, 24) = 3.42; P=0.049], but pairwise comparisons failed to detect a difference between PS and W (P=0.305) and PS and PDS (P=0.111). The volume of all fluids ingested was also higher in PS condition, although no main effect of beverage was observed [F (1.76, 38.6) = 1.52; P=0.232].

<table>
<thead>
<tr>
<th>Preload</th>
<th>Energy kcal</th>
<th>Sugar g</th>
<th>Caloric Beverages ml</th>
<th>Total Fluid ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1377 ± 528</td>
<td>64.1 ± 36.5</td>
<td>296 ± 253</td>
<td>585 ± 515</td>
</tr>
<tr>
<td>Pineapple Soda</td>
<td>1488 ± 607</td>
<td>81.9 ± 54.2</td>
<td>426 ± 391</td>
<td>692 ± 467</td>
</tr>
<tr>
<td>Pineapple Diet Soda</td>
<td>1385 ± 611</td>
<td>66.4 ± 34.8</td>
<td>293 ± 218</td>
<td>495 ± 368</td>
</tr>
</tbody>
</table>

Table 6. Energy, Sugar, Caloric Beverages and Total Fluid Intake after ingestion of the preloads

Discussion

The main findings of this study were: 1) All beverages had a similar effect on thirst sensation, hydration status and plasma osmolality values; 2) Sweetened preloads led to lower hunger scores than water; 3) Pineapple soda promoted a more accentuate decrease in glycaemia values. A tendency to higher water ingestion in subsequent meal and higher energy, sugar and caloric beverages intake throughout day was also observed with sugary preload compared with water and diet soda.

The few studies that investigated the relationship between thirst and caloric beverages intake revealed a large inconsistency. Almiron-Roig et al [3] did not find differences in thirst sensation in the 2 hours following the intake of a chilled 590ml preload of either orange juice, low-fat milk (1%), regular cola or sparkling water. However, when data was analyzed separately for gender, water and orange juice satisfied thirst better than did the cola beverage among women, with the juice having less sugar (55g vs. 76.6g) than cola. Even so, water and 1% milk, the two beverages with less sugar (0g and 29.1g respectively) did not led to lower thirst ratings than the other two. A contradictory finding was described by Della Valle et al [4], with thirst ratings being lower after the consumption of 360g of chilled diet cola (40g sugar) than after the consumption of the same amount of orange juice (38g sugar) or 1% milk (18g sugar). Nevertheless, in this study the beverages were consumed with an ad libitum lunch, so it is very difficult to interpret the isolated effect
of beverage on thirst with this kind of protocol. Another study from Tsuchiya et al [8], described lower ratings of thirst with 400ml fruit drink (50g sugar) and dairy fruit drink (46.5g sugar) than the less sugary 378g liquid and semi-solid yogurts (both with 32.1g sugar). In study of Monsivais et al [5], no differences in thirst ratings were observed between 495ml of 1% milk (27.2g sugar), 475ml of diet cola (0g sugar) and 3 sugar sweetened colas with different formulations: 525ml sucrose cola (54.7g sugar), 475ml high-fructose corn syrup with 42% fructose (57.3g sugar) and 525ml high-fructose corn syrup with 55% fructose (57.7g sugar). Only one study [7] reported a less effective capacity of sugary lemonade (20g sugar) to satisfy thirst compared to water and aspartame sweetened lemonade (0g sugar), when these beverages were ingested with a meal and in a small portion (≈ 237 ml). However, these differences were less pronounced when the beverages were consumed as preloads and with a larger preload volume (≈ 473 ml), which corresponded to a greater amount of sugar (40g). Thus, with this lack of coherence between all these trials, we can conclude that a direct link between the amount of sugar in a beverage and a positive impact in thirst sensation cannot be established.

It should be mentioned that the main aim of all these cited studies was to investigate the role of sugars and caloric beverages in satiety and subsequent energy intakes, so, their design was not specifically appropriate to analyze the impact of these beverages and their sugar content in thirst sensation. For instance, the amount of fluid ingested (an indirect method to evaluate thirst sensation) in the subsequent meal was recorded but the amount of food available was indiscriminate which is a strong constraint to a correct interpretation of the results.

Thus, with a research protocol designed specifically to evaluate thirst sensation and fluid intake, our results are according with the literature that reveal a lack of effect of sugar content of a beverage and thirst. There is a variety of physiological stimuli for thirst sensation, such as variations on plasma osmolality, blood volume, blood pressure and hormonal release (e.g. angiotensin, ADH) [12], but no physiological link between sugar and thirst was described in healthy subjects. The assumption of the theory that the ingestion of sugary beverages results in an increased sensation of thirst due to their hyper osmolality relative to blood is somehow an academic hypothesis. In our study, although osmolality of PS (409 mOsm/kg) was higher than initial plasma osmolality (311 mOsm/kg), it led to a decrease in these values in the final evaluation (303 mOsm/kg). Besides that, no differences in plasma osmolality and thirst sensation ratings were noticed between PS and other two hypo-osmolar beverages. Plasma osmolality is also very sensitive to changes in sodium concentration, the main cation in extracellular fluids [13]. PS had a much lower sodium concentration (1.18mEq/l) than normal blood sodium levels (135-145 mEq/l), which can explain, at least in part, the lack of effect of this beverage on thirst even though it is hyperosmolar compared to blood. It must be also recognized that even with the absence of effect on thirst sensation, PS revealed a tendency to higher ad libitum water ingestion in subsequent meal compared to water preload (P=0.09) and promoted a more accentuated decrease in glycaemia. Excessive thirst is one of the classical symptoms of untreated/uncontrolled diabetic patients since the extreme values of blood sugar induce a state of hyperosmolality. Thus, in people with diabetes or other disease that cause an impairment in sugar metabolism, the intake of sugary beverages could (beyond other disadvantages) increase thirst as a reflex of augmented osmolality. However, in
healthy subjects, in response to a high sugar load, more insulin is produced in order to reestablish glycaemia values into their physiological narrow. Since we observed a greater decrease in glycaemia with the sugary preload, this fact can be explained by a higher insulin response. This could also provide an explanation for the lack of effect that this hyperosmolar beverage had on thirst and plasma osmolality, since glucose contributes to plasma osmolality and this higher decrease in blood sugar levels could balance a hypothetical short-term increase in osmolality in reaction to sucrose-sweetened soda.

There are some other causes that can provide an additional explanation of these results such as the non-physiological stimulus that encompass the ingestion of beverages. Their physical and chemical characteristics may explain the observed lack of effect of sugar and beverage’s sweetness in thirst sensation. At this level, beverage temperature has a role in thirst suppression, with cold beverages being more effective [14-16], perhaps due to a greater increase in saliva production with consequent decrease in mouth dryness [17] and for their higher reward effect and relief for thirst [18]. As in other studies, beverages in our trials were served at a low temperature (8 – 10°C), that reflects the usual way of consumption of soft drinks. Beverage carbonation has also been described as a sensory thirst-quenching property in beverages [19]. However, a study [6] that measured directly the impact of beverage carbonation on thirst did not confirm this assumption. Nevertheless, the beverages in our study were decarbonized to withdraw this effect, and also because there are non-carbonated soft drinks available. Regarding acidity, if sweetness is perceived as low thirst-quenching property, acidity has an inverse role being one of the most thirst-quenching properties described [20] mostly due to their direct relationship with saliva production [21]. In our study the two sodas had a lower pH than water (W: pH = 5.8; PS: pH = 3.5; PDS: pH = 3.8) which could be another reason for the lack of effect of beverage sweetness and sugar content on thirst. Since the discover of osmoreceptors and the role of hypothalamus paraventricular and supraoptic nuclei on thirst with the release of vasopressin in blood stream, some authors consider that this “dry mouth theory” is somehow outdated [22, 23]. However, these osmoreceptors detect variations in plasma osmolality [24, 25] and regarding this parameter there were no differences between the three beverages studied. Therefore, the peripheral stimuli to thirst, namely beverage properties and environmental factors, such as social expectancy or expected sensory stimulation [1], cannot be discarded in this analysis.

Although the effect of beverages on thirst related variables was the main objective of our study, other interesting results were also observed. An increase in hunger ratings was observed with W preload compared to PS and PDS. Additionally, a higher energy, sugar and caloric beverages intake throughout day was seen after PS preload but without statistical significance. The majority of the studies with similar design did not observed differences in hunger between caloric beverages [3-5], but higher hunger scores after water intake compared to other caloric beverages has already been described [3]. This lack of differences between sugary and artificial sweetened preloads on hunger ratings does not corroborate their reported effects on satiety. On one hand, sucralose has already demonstrated to be one of the artificial sweeteners more able to induce cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) secretion in cell lines [26]. On the other, in human studies, a less effective capacity of artificial sweeteners to induce the secretion
of satiety hormones like insulin, GIP and GLP-1, face to sugar is well described [27, 28]. Furthermore, when we analyzed the nutritional intake throughout the day, we could observe that when participants drank a sugary morning preload, they tended to eat more calories, sugars and drink more caloric beverages, with a total absence of energy compensation.

Some limitations have to be pointed in this study. The ingestion of 500ml of water on the evening before and on the morning of the trial, used to ensure a euhydration status could be seen as excessive and potentially attenuate differences in thirst. However, even with this procedure, we could notice that baseline plasma osmolality values were still increased (310 - 313 mOsm/Kg) compared to their physiological range (280 – 300 mOsm/Kg). So, it is unlikely that this recommendation may have affected our results. Similarly, a 500 ml preload may not be a physiological load and could have masked some differences in thirst sensation. Nevertheless, similar or even higher preload volumes were used in other studies [3-5, 29]. Moreover, the fluid portions were not adjusted to the same amount of water, with sugar replacing 5.5 g of water in PDS and 55g in PS; however this issue always occurs when beverages are consumed in a standardized portion. Additionally, the BIA method is not considered the gold standard to assess intracellular and extracellular water, nevertheless, the variation in hydration status was not the main aim of the study.

Conclusions

In summary, water, sugar and artificial sweetened beverages matched on volume did not differ in their effects on thirst, desire to drink, mouth dryness and osmolality values. Our data provide no support for the hypothesis that sugary or sweet beverages increase thirst sensation due to the increasing of blood osmolality values. However, there was a tendency to a decreased glycaemia and an increased water intake at the subsequent meal when the sugary sweetened soda was the preload beverage.

Acknowledgments

We thank to all participants of the study and to the Portuguese Institute of Hydration and Health for the financial support.
References


3.3
Effect of Beverage Sweetness on Thirst Sensation and Fluid Intake

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Submitted for publication

Abstract

Sweetness is associated with a low thirst-quenching ability and poorly correlated with the refreshing intensity of a beverage. The impact of non-nutritive sweeteners on appetite and energy intake is still inconsistent. This study compared the impact of water and two diet sodas with distinct sweetness levels on thirst, mouth dryness, hunger, water ingestion at subsequent meal as well as their impact on hydration status, glycaemia and blood osmolality. In a crossover design, twenty-seven participants (13 male, 18-35 y) consumed in three consecutive weeks, 500ml of water (W), or a soda with a 50% increase (High Sucralose - HS) or 50% decrease (Low Sucralose - LS) of its original amount of sucralose, 1h after a standardized breakfast. Participants rated beverages sweetness and thirst, desire to drink, mouth dryness, nausea, hunger and desire to eat, at baseline and at 30-min intervals until a standardized lunch with ad libitum water ingestion being served 2h30 after preload. Throughout the rest of day, participants record all food and fluid intake. Participants differentiated the sweetness level between the three beverages (W: 1.2 ± 0.4; HS: 7.4 ± 1.2; LS: 5.5 ± 1.6; P<0.001), but no differences in hydration status, plasma osmolality, thirst, mouth dryness and hunger were observed between beverages. Glycaemia decreased on all beverages and a tendency to higher water ingestion at subsequent meal was observed for the two sodas in comparison to water (P=0.09). No differences between HS and LS were observed for water intake at lunch and all beverages led to similar energy, fluid, sugar and caloric beverages intake throughout the day. These results reveal that soft drinks with different sweetness level seem to be as thirst-quenching as water, and indicate that non-nutritive sweeteners does not appear to increase appetite and energy intake in a real-life consumption pattern.

Keywords: Sucralose, soft-drinks, appetite, mouth dryness
Introduction

Thirst plays a key role in the regulation of body fluid homeostasis in a mechanism mainly regulated by the hypothalamus. Signals of fluid depletion such as increases in plasma osmolality, hypovolemia and hypotension are detected by osmo and baroreceptors in kidneys, arterial walls and cardiopulmonary system that send a neural input to the brain triggering thirst sensation [1]. However, these physiological changes are not the only stimulus for all drinking episodes [2], and there are other strong non-homeostatic influences on drinking behavior [3]. The act of drinking could also be driven by dry mouth sensations [4], or social cues [5] that goes beyond physiological drive. Furthermore, beverage properties can also interfere in thirst sensation and in drinking motivations. Although the concept of hedonic thirst has never been established, it must be acknowledged that beverage temperature [6, 7], carbonation [8-10], color [11, 12], acidity [13, 14], flavor [12], palatability and sweetness [13, 14] all seem capable to promote drinking behaviors not associated with fluid needs. Regarding beverage sweetness, this property has been inversely correlated with refreshing intensity [14], and considered a negative driver for thirst-quenching purposes [13]. Even so, Zellner et al [12] reported sweetness as a characteristic that 50% of American students expected in a thirst-quenching beverage, and sensory panel studies [15] already considered the sweetest beverages as the most thirst satisfying. Non-nutritive sweeteners have also a contradictory role on hunger [16] and their addition to non-energy-yielding products may even heighten appetite [17] however, to date, few studies [18] have analyzed the effect of beverage sweetness on short-term appetite and energy intake.

Thus, the main aim of this study was to evaluate the impact of beverages with different sweetness levels on thirst sensation, mouth dryness and other physiological parameters involved in thirst regulation and, as a secondary aim, analyze their influence on short-term appetite and nutritional intake.

Material and methods

Participants

Thirty participants were recruited at University of Porto through e-mail advertisings. During the study, 3 dropouts occurred so the final sample included 27 subjects (13 male). The eligibility criteria included: age between 18-35 years; body mass index (BMI) between 18.5 – 27.5 kg/m2; non-smokers; non-athletes; weight stable on the previous 6 months; not dieting to gain or lose weight; not using any kind of medication (except oral contraceptives in women) and not being pregnant and nursing. To minimize variability, each participant was asked to fast since 22.00 of the previous day and to abstain from alcohol, caffeine and strenuous physical activity on the day of the
trial and the day before. To ensure euhydration status and avoid mouth dryness, subjects were instructed to drink 500 ml of water the evening before the trial and 500 ml water on the morning of the trial immediately after wake up [19]. Participants who met these requirements and accepted to participate in this study were informed verbally and in writing regarding the experimental procedures before giving their written informed consent. Participants received a financial compensation upon submission of meal and transportation expenses resulting from travel to our lab.

**Study design**

The three trials followed a single blind, randomized cross-over mode with treatment order in counterbalanced design. Each participant came for three sessions, separated by a week, and lasting from 08h00 to 14h00. On all testing occasions was offered a standardized breakfast to each participant followed by a preload stimuli 1h30 after. A standardized lunch was served 2h30 after preload. The time interval set between preload and lunch was longer than the previous studies in this area [20-22].

**Preload stimuli**

The 3 beverages studied were Water (W), a decarbonized pineapple diet soda with a 50% increase of sucralose face to standard beverage - ["High Sucralose" (HS)], and the same soda but with a 50% decrease of sucralose face to standard - ["Low Sucralose" (LS)]. Energy, nutritional and chemical composition of the preloads are represented in Table 1. Beverages were presented chilled (8 – 10º C), but without ice, in 500 ml portions in opaque plastic containers. Participants were asked to consume the entire amount within 15 min. The sodas were previously decarbonized by the manufacturer to avoid any kind of interference of beverage carbonation [9, 10] and all beverages were analyzed previously in laboratory in order to obtain their pH, osmolality and sodium characteristics.

<table>
<thead>
<tr>
<th>Preload</th>
<th>Energy kcal</th>
<th>Carbohydrates g</th>
<th>Sugars g</th>
<th>Protein g</th>
<th>Fat g</th>
<th>Sucralose mg/L</th>
<th>Sodium mmol/L</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>5.8</td>
</tr>
<tr>
<td>High Sucralose*</td>
<td>25</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0.227</td>
<td>25</td>
<td>3.8</td>
</tr>
<tr>
<td>Low Sucralose*</td>
<td>25</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0.076</td>
<td>29</td>
<td>3.8</td>
</tr>
</tbody>
</table>

* Fastio ™, Portugal
* Sumol Zero Ananás™, Portugal

**Table 1.** Energy, nutritional and chemical composition of the 330ml preloads
Motivational Ratings

Participants rated their thirst, mouth dryness, desire to drink, hunger, nausea and desire to eat using a nine point Likert scale. The unipolar adjective scales were anchored at each end with labels 1 = *not at all* and 9 = *extremely*. Participants also rated their perceived sweetness of the beverage in a nine Likert scale, where 1 = *not sweet at all* and 9 = *extremely sweet*. All scales were marked on paper.

Meals Provided

Breakfast was served at 9h30 and consisted in 4 low fat cookies and 200 ml of orange juice. Participants had to consume the entire breakfast within 15 min. A lunch meal prepared without added salt was served at 13h00 and consisted in, approximately, 180 g of vegetable soup, 200 g of boiled rice, 150 g of grilled pork chop, 50g of lettuce, carrot and tomato salad and 100 g of apple (Table 2). Participants were allowed to drink ad libitum the water provided and the amount was measured afterwards. Identical meals were provided on each occasion and their energy and nutrient composition are represented in Table 2. Information was obtained from the label or from software Food Processor SQL Edition, version 10.0 (ESHA Research Inc., Salem, OR, USA).

<table>
<thead>
<tr>
<th>Food</th>
<th>Portion</th>
<th>Energy</th>
<th>Carbohydrates</th>
<th>Sugar</th>
<th>Protein</th>
<th>Fat</th>
<th>Energy Density</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BREAKFAST</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cookies</td>
<td>50</td>
<td>226.5</td>
<td>34.5</td>
<td>11</td>
<td>3.8</td>
<td>7.5</td>
<td>4.53</td>
</tr>
<tr>
<td>Fruit Nectar</td>
<td>200ml</td>
<td>86</td>
<td>21</td>
<td>19.2</td>
<td>0.4</td>
<td>0.01</td>
<td>0.43</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>312.5</td>
<td>55.5</td>
<td>30.2</td>
<td>4.2</td>
<td>7.5</td>
<td>1.25</td>
</tr>
<tr>
<td><strong>LUNCH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable Soup</td>
<td>180</td>
<td>39.6</td>
<td>8.7</td>
<td>2.9</td>
<td>1.4</td>
<td>0.54</td>
<td>0.22</td>
</tr>
<tr>
<td>Vegetable Salad</td>
<td>50</td>
<td>12</td>
<td>2.7</td>
<td>1.4</td>
<td>0.42</td>
<td>0.09</td>
<td>0.24</td>
</tr>
<tr>
<td>White Rice</td>
<td>200</td>
<td>260</td>
<td>57.2</td>
<td>1</td>
<td>4.8</td>
<td>8.42</td>
<td>1.3</td>
</tr>
<tr>
<td>Pork Chop</td>
<td>150</td>
<td>266.4</td>
<td>0</td>
<td>0</td>
<td>31.9</td>
<td>14.5</td>
<td>1.78</td>
</tr>
<tr>
<td>Apple</td>
<td>100</td>
<td>47.8</td>
<td>12.7</td>
<td>9.6</td>
<td>0.24</td>
<td>0.16</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>625.8</td>
<td>81.3</td>
<td>14.9</td>
<td>30.8</td>
<td>15.7</td>
<td>0.92</td>
</tr>
</tbody>
</table>

* Proalimentar, Milk and Cereals ™️, Portugal
* Compal Classic Tutti Frutti Nectar™️, Portugal

Table 2. Energy and nutrient composition of foods provided at breakfast and lunch

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Procedures

Participants arrived at the laboratory at 8h00 in order to collect their values of height in a stadiometer (SECA 220) and weight, total body water (TBW), intracellular water (ICW) and extracellular water (ECW) in a Segmental Multi Frequency Body Composition Monitor (TANITA MC 180 MA®). Then, a blood sample (8 ml) was collected from the antecubital vein by a trained phlebotomist for analysis of blood glucose (ABX Pentra 400; ABX Diagnostics, Montpellier, France) and osmolality (Löser Micro-Osmometer Type 15; Löser Messtechnik, Germany). During the session participants were allowed to read, listen to music with earphones, or use their portable computers with the exception of internet access to minimize visual cues (e.g., unwanted publicity for beverages or visiting Web sites showing pictures of food and drinks), which may have had effect on thirst. Breakfast was served at 9h30 on every occasion, the preload beverage was offered exactly 60 minutes after breakfast (10h30) and lunch was provided at 13h00. Motivational ratings were firstly obtained at the end of blood sampling (baseline or time 0) and every 30 minutes thereafter until lunch time (times 1 through 7). Before lunch, another blood sampling was collected and TBW, ICW and ECW were evaluated once again. Immediately after lunch (time 8), participants completed the last set of ratings and a food record was given to record all food and fluid intake from that moment until 00h00 of that day. Energy and nutritional intake were estimated by Food Processor SQL Edition, version 10.0 (ESHA Research Inc., Salem, OR, USA, added with nutritional information of Portuguese recipes and the following parameters were extracted: Energy, sugars, caloric beverages intake, and total fluid intake.

The procedures were approved by Ethics Committee of the Universidade do Porto (Nº30/CEUP/2011), and were registered in ClinicalTrials.gov with (NCT ID: NCT01771094).

Data Analysis

All statistical analyses were conducted using IBM Statistical Package for Social Sciences (SPSS) 20.0 for Windows. Normality was determined by the Kolmogorov-Smirnov test (normal if P > 0.05). When the assumption of normality was violated, it was assured that variables skewness was < 3 and kurtosis < 10 [23]. Sphericity was determined by Mauchly’s test (sphericity assumed when P > 0.05). When the assumption of sphericity was violated, univariate tests of within-subjects effects were subject to Greenhouse Geisser correction (when epsilon < 0.75) and to Huynh-Feldt correction (when epsilon > 0.75). General linear models (GLM) with repeated measures were used to analyze motivational ratings with beverage and time post-ingestion (times 3-7) as within-subjects factors and sex as the between-subjects factor. Analyses of glycaemia, plasma osmolality, extracellular, intracellular and total body water used a GLM with repeated measures with beverage and time as within-subjects factors and sex as between-subjects factor. When a main effect of time was observed in these variables, a t-student test was performed to find differences between moments for each beverage condition. Analyses of water intake at lunch and energy and nutritional intake after lunch used a GLM with repeated measures with beverage as within-subjects factors and sex as between-subjects factor. Bonferroni-adjusted pairwise comparisons were made when differences in GLM’s were found.
Only when there was a gender interaction, data were analyzed separately for each group. Data was expressed as mean ± standard deviation. The level of significance was set at $P < .05$.

## Results

The mean age (± SD) of participants was 22.2 ± 1.57 years for men, 21.9 ± 2.11 years for women, and 22.0 ± 1.84 years for the whole group. Mean body mass index (BMI; in kg/m²) was 23.7 ± 1.90 for men, 22.3 ± 1.95 for women, and 23.0 ± 2.03 for the whole group.

### Motivational Ratings

Thirst sensation ratings (Fig. 1) decreased after the preload consumption and gradually increased until lunch time, but it was not observed a main effect of beverage $[F (2, 50) = 0.25; P=0.78]$. Only a main effect of time was detected $[F (1.8, 45) = 29.9; P<0.001]$ and no beverage*sex $[F (2, 50) = 0.62; P=0.54]$, or beverage*time interaction $[F (8, 200) = 1.18; P=0.31]$ were observed.

![Figure 1. Temporal profile of thirst sensation by beverage](image)

In mouth dryness ratings (Fig. 2) the three beverages showed a very similar pattern with no main effects of beverage type $[F (2, 48) = 0.23; P=0.79]$. Only a main effect of time was detected $[F (1.75, 42.1) = 14.3; P<0.001]$. No sex $[F (2, 48) = 1.1; P=0.34]$, or beverage*time interaction $[F (8, 192) = 0.72; P=0.68]$ were observed.
In desire to drink ratings (Fig. 3), only a main effect of time was observed \[F (1.8, 44) = 33.2; P<0.001\]. There was no main-effect of beverage \[F (2, 50) = 0.41; P=0.66\], and no beverage*sex \[F (2, 50) = 1.37; P=0.26\] or beverage*time interaction-effect \[F (4.6, 116) = 0.94; P=0.45\] were noticed.

Figure 2. Temporal profile of mouth dryness by beverage

Figure 3. Temporal profile of desire to drink by beverage
In hunger ratings (Fig.4), no differences between beverages were seen \( F(2, 50) = 0.41; P=0.68 \). Only a main effect of time was observed \( F(2.2, 55.3) = 102; P<0.001 \) and no beverage*sex \( F(2, 50) = 1.84; P=0.17 \) or beverage*time interaction \( F(4.7, 117) = 0.66; P=0.65 \) were noticed.

Ratings of desire to eat and nausea were not affected by time or beverage, and no sex interaction was observed (data not shown).

![Figure 4. Temporal profile of hunger by beverage](image)

**Hydration Status and Biochemical Parameters**

Table 3 shows, for each preload condition, the TBW, ICW and ECW at the beginning of the trial and at the pre-lunch evaluation. No sex interaction was observed for all three parameters and there was not observed a significant main-effect of beverage in TBW: \( F(2, 50) = 0.88; P=0.42 \), ICW: \( F(2, 50) = 1.61; P=0.21 \) and ECW: \( F(2, 50) = 0.32; P=0.31 \), neither a significant main-effect of time.

<table>
<thead>
<tr>
<th>Preload Condition</th>
<th>Total Body Water (Kg)</th>
<th>Extracellular Body Water (Kg)</th>
<th>Extracellular Body Water (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Water</td>
<td>38.1 ± 7.47</td>
<td>38.1 ± 7.49</td>
<td>15.5 ± 2.66</td>
</tr>
<tr>
<td>High Sucralose Soda</td>
<td>38.3 ± 7.68</td>
<td>38.2 ± 7.60</td>
<td>15.5 ± 2.71</td>
</tr>
<tr>
<td>Low Sucralose Soda</td>
<td>38.2 ± 7.52</td>
<td>38.2 ± 7.47</td>
<td>15.5 ± 2.70</td>
</tr>
</tbody>
</table>

*Table 3. Hydration Status values for each preload condition.*
Table 4 expresses the variation of glycaemia and plasma osmolality during the trial. No major differences were observed in osmolality values over time \[F (1, 25) = 0.44; P = 0.51\]. Besides, no main-effect of beverage \[F (2, 50) = 0.47; P = 0.63\], nor interaction-effect between beverage*time \[F (1.7, 42.5) = 0.26; P = 0.74\] or beverage*sex \[F (2, 50) = 0.23; P = 0.79\] were observed.

Regarding glycaemia values, only a significant effect of time was observed leading to their decrease \[F (1, 25) = 25.5; P < 0.001\]. Nevertheless, a significant interaction beverage*time \[F (2, 50) = 0.40; P = 0.67\] was not observed, indicating that glycaemia decreased in a similar way in all conditions.

<table>
<thead>
<tr>
<th>Preload Condition</th>
<th>Osmolality (mOsm/Kg)</th>
<th>Glycaemia (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Water</td>
<td>322 ± 11.6</td>
<td>323 ± 3.3</td>
</tr>
<tr>
<td>High Sucralose Soda</td>
<td>323 ± 5.9</td>
<td>324 ± 4.7</td>
</tr>
<tr>
<td>Low Sucralose Soda</td>
<td>324 ± 6.5</td>
<td>324 ± 4.7</td>
</tr>
</tbody>
</table>

Table 4. Glycaemia and plasma osmolality values for each preload condition.

* - P < 0.001 vs. Initial Glycaemia

Sweetness Rating and Water Intake

Table 5 shows mean sweetness ratings of the three beverages with a main effect of beverage type \[F (2, 50) = 275.7; P < 0.001\]. Bonferroni comparisons detected differences between all pairs of beverages (P < 0.001), indicating that participants distinguished the differences in beverages sweetness.

<table>
<thead>
<tr>
<th>Preload</th>
<th>Sweetness Rating*</th>
<th>Water Ingestion (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.2 ± 0.4</td>
<td>269 ± 149</td>
</tr>
<tr>
<td>High Sucralose Soda</td>
<td>7.4 ± 1.2</td>
<td>312 ± 175</td>
</tr>
<tr>
<td>Low Sucralose Soda</td>
<td>5.5 ± 1.6</td>
<td>313 ± 161</td>
</tr>
</tbody>
</table>

Table 5. Sweetness Rating and Water ingestion at Lunch

* - P < 0.001. W vs HS; W vs LS; HS vs LS

Regarding the amount of *ad libitum* water intake at lunch for each preload condition, although a slight difference was observed between water and the other beverages, no main effect of beverage was detected \[F (2, 50) = 2.56; P = 0.09\]. Bonferroni pairwise comparisons between beverages almost revealed differences between W and LS (P = 0.067), but the increased levels of sweetness between LS and HS condition did not lead to a higher water ingestion at lunch (LS vs HS, P = 1.0).
Energy, sugars, caloric beverages and total fluid intake

Table 6 shows the energy, sugars, caloric beverages and total fluid intake since the end of the trial until the end of that day. Although a lower caloric intake was observed in HS condition, no main effect of beverage was detected \([F (2, 50) = 1.6; P=0.21]\). The sweetest beverage led to a lower sugar intake throughout day, although no beverage effect was observed \([F (2, 50) = 2.03; P=0.14]\). The caloric beverages intake was reasonably higher in LS face to HS condition, but without beverage main-effect \([F (2, 50) = 1.92; P=0.16]\), or statistically significant pairwise comparisons between HS and LS \((P=0.152)\). The volume of all fluids ingested was also higher in LS condition, but, as in other variables, no main effect of beverage was observed \([F (2, 50) = 0.98; P=0.384]\). No interactions beverage*sex were observed for all variables \((P>0.05)\).

<table>
<thead>
<tr>
<th>Preload</th>
<th>Energy</th>
<th>Sugars</th>
<th>Caloric Beverages</th>
<th>Total Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kcal</td>
<td>g</td>
<td>ml</td>
<td>ml</td>
</tr>
<tr>
<td>Water</td>
<td>1335 ± 523</td>
<td>65.8 ± 34.4</td>
<td>211 ± 210</td>
<td>500 ± 348</td>
</tr>
<tr>
<td>High Sucralose Soda</td>
<td>1265 ± 572</td>
<td>55.7 ± 36.3</td>
<td>144 ± 206</td>
<td>523 ± 312</td>
</tr>
<tr>
<td>Low Sucralose Soda</td>
<td>1485 ± 562</td>
<td>75.7 ± 52.0</td>
<td>249 ± 246</td>
<td>611 ± 431</td>
</tr>
</tbody>
</table>

Table 6: Energy, Sugars, Caloric Beverages and Total Fluid Intake after ingestion of the preloads
Discussion

The main findings of this study were: 1) Sweetening beverages with sucralose does not lead to higher feelings of thirst or mouth dryness; 2) All beverages had a similar effect on hydration status, glycaemia, plasma osmolality and nutritional intake throughout the day.

In our opinion, a key requirement to a correct interpretation of these results was to understand if participants distinguished the different sweetness level of beverages. In this context, the manipulation of sucralose amount in the two sodas was enough to promote statistically significant differences (P<0.001) in participants awareness of sweetness with HS having a higher rating (7.4 ± 1.2) than LS (5.5 ± 1.6) and both having higher scores than water (1.2 ± 0.4). Despite that, no differences between beverages were observed on thirst sensation and mouth dryness - the two variables that could be more affected by sweetness level.

Sweetness is a beverage property already considered as a negative driver of refreshing sensation in gels [14], beers [8] and beverages [13]. Acknowledging the fact that some studies reported sweetness as a characteristic expected in a thirst-quenching beverage [12], and as thirst-satisfying beverage characteristic [15], the results of our study are somehow unexpected since the “High Sucralose” version comprised 50% more sucralose than the soda commercially available, and this increase in sweetness was perceived by participants. Some possible explanations could be related with other beverage properties involved in their thirst-quenching capacity, such as temperature and acidity. The sodas were served chilled since this reflects their regular way of consumption and cold temperatures are unsurprisingly reported as the major sensory characteristic of refreshing beverages [12]. Behind this sensorial perception, there are some physiological mechanisms that can explain the fact of cold beverages being more thirst quenching than warm ones [7, 24, 25]. On one hand, they increase salivary flow rate [26] and, subsequently, decrease mouth dryness. On the other, trigeminal cold-sensing neurons have specific receptors localized on the tongue, nasal cavity and peripheral nervous system that are responsible for pre-absorptive thirst satiety even before changes in plasma osmolality [1, 25, 27]. So, the cold temperatures at which beverages were served possibly act as a thirst-quenching by itself not allowing a major role of beverage sweetness at this regard. With respect to beverage acidity, we can notice that sodas’ pH was very low (3.8). Aromas of acidic fruits such as oranges and lemons are associated as refreshing flavors by consumers [12], but acidity itself is a property independently related with thirst-quenching capacity as seen in the classical study of McEwan and Colwill [13]. In this study, a focus group identified seven sensory attributes which were important in thirst quenching properties of drinks (acid, astringent, fruity, strength of flavor, carbonation, sweetness and thickness) and acidity was the attribute more related to thirst-quenching ability (r=0.809) with sweetness being inversely related (r=-0.107). Similarly to cold temperatures, acid tastants are also responsible for an increase in saliva flow rate leading to a mouth-wetting effect which decreases the thirst sensation [28]. In fact, the addition of citric acid to enhance the acidity of a beverage is capable of reduce the perceived sweetness by taste suppressive interaction [29].
So, in our study, the high acidity of the two sodas likely counterbalanced the variations promoted in sweetness. Although participants were able to find differences in sweetness perception between these two beverages, these differences were not enough to promote changes in thirst sensation between them, and even with water. We have to recognize that, although thirst scale measurements do not differ between beverages, a tendency to a higher water intake at subsequent lunch, in both soda conditions was observed. No main effects of beverage were detected ($P=0.09$) but pairwise comparisons revealed an almost statistically significant higher water intake in LS face to W ($P=0.067$) which was a bit surprising since this was the less sweet soft drink. However the medium differences between beverages regarding water intake were $\approx 44$ mL which does not have a real significance. Other studies with similar design that evaluated the effect of sweet beverages on thirst sensation were inconclusive with more sugary beverages (and presumably the sweetest) leading to lower ratings of thirst than the less sugary ones [21, 30], or even no differences at all between beverages with different amounts of sugar [22]. This reinforces the importance of other beverage properties in thirst satiety.

The similar variation of hydration values, glycaemia and plasma osmolality values were somehow expected since the 3 beverages had similar amounts of sodium, and the low sugar content (7g) of the 500ml portion soda probably was not enough to promote a greater decrease in glycaemia than in water condition. Regarding hunger scores and nutritional intake throughout the day, the differences in these variables were not the main objective of this study, but in fact, our differentially sweetened sodas had similar results to water, something that is in agreement with a recent study in women that showed similar effects of sucralose and water on hunger and other physiological parameters [18].

Some limitations have to be pointed to this study. To ensure an euhydration status, participants had to ingest 500ml of water on the evening before and on the morning of the trial. This procedure could be seen as excessive and potentially attenuate differences in thirst, however, we could notice that baseline plasma osmolality values were still increased (322 - 324 mOsm/Kg) compared to their physiological range (280 – 300 mOsm/Kg). So it is unlikely that this recommendation may have affected our results. Similarly, a 500 ml preload may not be a physiological load and could possibly mask some differences in thirst sensation. However, similar or even higher preload volumes were used in other studies and not even a tendency to higher thirst ratings were observed for any of the beverages. The plasmatic sodium was not measured and the BIA method is not the gold standard to assess intracellular and extracellular water, even knowing that the variation in hydration status was not the main aim of the study.
Conclusions

In summary, beverages with different degrees of sweetness elicited similar effects on thirst, mouth dryness, hunger and water intake at subsequent meal. Our data do not confirm the low thirst quenching ability of sweet beverages.

Acknowledgments

We thank to all participants of the study and to the Portuguese Institute of Hydration and Health for the financial support.
References


3.4
The Impact of Different Energetic Beverages on Thirst Sensation

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FACULDADE DE DESPORTO, UNIVERSIDADE DO PORTO, PORTUGAL
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Abstract

The effect of sugar sweetened beverages on thirst sensation is a very controversial topic and very few studies involving different sugary drinks have been performed. In this study, we compared the effects of water (W), non-fat milk (NFM), orange juice (OJ) and iced tea (IT) intake on thirst, mouth dryness, desire to drink and water ingestion at subsequent meal as long as their impact on hydration status, glycaemia, blood osmolality and sodium. In a crossover design, thirty-two participants (17 male, 18-30 y) consumed in four consecutive weeks a standardized breakfast followed by a 330ml preload of water, non-fat milk, 100% orange juice and iced tea. They rated thirst, desire to drink, mouth dryness, nausea, hunger and desire to eat at baseline and at 30-min intervals until a standardized lunch with ad libitum water intake being served 2h30 after preload. Total, intra and extracellular body water, glycaemia, plasma osmolality and sodium were evaluated at baseline and before lunch. Until the end of the day, participants recorded all food and fluid intake. A beverage*sex interaction was observed in thirst sensation with NFM revealing a tendency to higher thirst ratings in men face to W and IT. Ad libitum water intake at lunch also tended to higher values in NFM in comparison with W and IT, with differences between NFM and IT being statistically significant in men (P=0.001). No major differences in hydration status, plasma osmolality and plasmatic sodium were observed, but glycaemia decreased in all beverages, although more pronouncedly in sugary beverages (P<0.001) than in water preload (P=0.007). Energy, sugar, calorific beverages and total fluid intake after lunch and until the end of the day does not differ significantly regardless of the morning preload. These results does not confirm a positive effect of soft drinks on thirst in comparison with water, but showed a clear tendency to an increase in thirst triggered by milk, particularly in men.

Keywords: Sugar, Osmolality, Water, Milk, Orange juice, Tea
Introduction

The effect of sugar and sugary beverages on thirst is a topic still badly understood. Unlike sodium, that seems to have a linear relationship with plasma osmolality values, hypertonic solutions of glucose are apparently as effective increasing osmolality but does not have the same effect on vasopressin secretion and thirst stimulation [1]. Our past investigations (unpublished data) already showed a lack of effect between the presence of sugar or non-energetic sweeteners in a beverage and an increase in thirst face to water. Likewise, we also demonstrate that the sweetness level of a soda, an attribute generally considered as low thirst-quenching [2, 3], may be superseded by their acid flavor and low temperature - attributes commonly associated with their way of consumption - being as effective as water in thirst satisfaction.

A few studies [4-8] analyzed the impact of different beverages such as milk, fruit juices and cola beverages on satiety and subsequent energy intake, with thirst assessment being only a secondary outcome. Even so, the results were contradicting with diet cola being more thirst suppressing than orange juice and milk in one study [4], with the opposite occurring in women of another trial [5]. Other studies reported no differences in thirst between milk and four cola beverages with distinct sweeteners [6], or between caloric and non-caloric orangeade [8]. To the best of our knowledge, the only study [4] that has compared simultaneously the effect of three beverages with distinct dietary sugars (sucrose, fructose, lactose) on thirst, did it in a context of an ad libitum meal, and no physiological issues such as glycaemia, plasmatic sodium and osmolality were evaluated.

Then, the main aim of our study is to determine the influence of different energetic beverages with different dietary sugars and osmolality, on thirst sensation, physiological parameters involved in its regulation and subsequent water intake.

Material and methods

Participants

Thirty two participants (17 male) were recruited at University of Porto through e-mail advertisings. The eligibility criteria included: age between 18-30 years; body mass index (BMI) between 18.5 and 25 kg/m2; nonsmokers; non-athletes; weight stable on the previous 6 months; not dieting to gain or lose weight; not using any kind of medication (except oral contraceptives in women) and not being pregnant and nursing. Each participant was asked to fast since 22h00 of the previous day and to abstain from alcohol, caffeine and strenuous physical activity on the day of the trial and the day before. To ensure euhydration status and avoid mouth dryness, subjects were
instructed to drink 500 ml of water the evening before the trial and 500 ml water on the morning of the trial immediately after wake up [9]. Participants who met these requirements and accepted to participate in this study were informed verbally and in writing regarding the experimental procedures before giving their written informed consent. Participants received a financial compensation upon submission of meal and transportation expenses resulting from travel to our lab.

Study design

The trials followed a single blind, randomized cross-over mode with treatment order in counterbalanced design in which each participant came for four separate sessions separated by a week and lasting from 08h00 to 14h00. On all testing occasions was offered to each participant a standardized breakfast followed by a preload stimuli 1h30 after. A standardized lunch was served 2h30 after preload. The time interval between preload and lunch was set to be greater than the previous studies in this area [5, 6, 10].

Preload stimuli

The 4 beverages studied were Water (W), Non-Fat Milk (NFM), Orange Juice (OJ) and Lemon Iced Tea (IT). Energy, nutritional and chemical composition of the preloads are represented in Table 1. Beverages were presented chilled (5 – 10º C), but without ice, in 330 ml portions in opaque plastic containers. Participants were asked to consume the entire amount within 15 min. The beverages were analyzed previously in laboratory in order to obtain their pH, osmolality and sodium characteristics.

<table>
<thead>
<tr>
<th>Preload</th>
<th>Energy (kcal)</th>
<th>Carbohydrates (g)</th>
<th>Sugars (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Energy Density (kcal/g)</th>
<th>pH</th>
<th>Osmolality (mOsm/Kg)</th>
<th>Sodium (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7.1</td>
<td>0</td>
<td>0.18</td>
</tr>
<tr>
<td>Mimosa Skimmed™</td>
<td>115.5</td>
<td>16.2</td>
<td>16.2</td>
<td>10.9</td>
<td>0.66</td>
<td>0.35</td>
<td>7.0</td>
<td>278</td>
<td>172.7</td>
</tr>
<tr>
<td>Compal Fresh Orange™</td>
<td>145.2</td>
<td>34.0</td>
<td>27.7</td>
<td>1.98</td>
<td>0.87</td>
<td>0.44</td>
<td>4.0</td>
<td>607</td>
<td>154</td>
</tr>
<tr>
<td>Nestea Lemon™</td>
<td>105.6</td>
<td>25.4</td>
<td>25.4</td>
<td>0</td>
<td>0</td>
<td>0.32</td>
<td>4.0</td>
<td>294</td>
<td>138.8</td>
</tr>
</tbody>
</table>

* Fastio ™, Portugal
  * Mimosa Skimmed™, Portugal
  * Compal Fresh Orange™, Portugal
  * Nestea Lemon™

Table 1, Energy, nutritional and chemical composition of the 330ml preloads
Motivational Ratings

Participants rated their thirst, mouth dryness, desire to drink, hunger, nausea and desire to eat using a nine point Likert scale. The unipolar adjective scales were anchored at each end with labels 1 = not at all and 9 = extremely. Participants also rated their acceptability for the beverage in a nine point hedonic preference scale, where 1 = dislike extremely and 9 = like extremely. All scales were marked on paper.

Meals Provided

Breakfast was served at 9h30 and consisted in 4 low fat cookies and 200 ml of fruit nectar. Participants had to consume the entire breakfast within 15 min. A lunch meal prepared without added salt was served at 13h00 and consisted in approximately 180 g of vegetable soup, 200 g of boiled rice, 150 g of grilled pork chop, 50g of lettuce, carrot and tomato salad and 100 g of apple. During lunch, participants were allowed to drink the water provided ad libitum and the amount was measured afterwards. Identical meals were provided on each occasion and their energy and nutrient composition are represented in Table 2. Information was obtained from the label, and, if not possible, from software Food Processor SQL Edition, version 10.0 (ESHA Research Inc., Salem, OR, USA).

<table>
<thead>
<tr>
<th>Food</th>
<th>Portion</th>
<th>Energy</th>
<th>Carbohydrates</th>
<th>Sugar</th>
<th>Protein</th>
<th>Fat</th>
<th>Energy Density kcal/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>BREAKFAST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cookies a</td>
<td>50</td>
<td>226.5</td>
<td>34.5</td>
<td>11</td>
<td>3.8</td>
<td>7.5</td>
<td>4.53</td>
</tr>
<tr>
<td>Fruit Nectar b</td>
<td>200ml</td>
<td>86</td>
<td>21</td>
<td>19.2</td>
<td>0.4</td>
<td>0.01</td>
<td>0.43</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>312.5</td>
<td>55.5</td>
<td>30.2</td>
<td>4.2</td>
<td>7.5</td>
<td>1.25</td>
</tr>
<tr>
<td>LUNCH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable Soup</td>
<td>180</td>
<td>39.6</td>
<td>8.7</td>
<td>2.9</td>
<td>1.4</td>
<td>0.54</td>
<td>0.22</td>
</tr>
<tr>
<td>Salad</td>
<td>50</td>
<td>12</td>
<td>2.7</td>
<td>1.4</td>
<td>0.4</td>
<td>0.09</td>
<td>0.24</td>
</tr>
<tr>
<td>White Rice</td>
<td>200</td>
<td>260</td>
<td>57.2</td>
<td>1</td>
<td>4.8</td>
<td>0.42</td>
<td>1.3</td>
</tr>
<tr>
<td>Pork Chop</td>
<td>150</td>
<td>266.4</td>
<td>8</td>
<td>0</td>
<td>31.9</td>
<td>14.5</td>
<td>1.78</td>
</tr>
<tr>
<td>Apple</td>
<td>100</td>
<td>47.8</td>
<td>12.7</td>
<td>9.6</td>
<td>0.24</td>
<td>0.16</td>
<td>0.48</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>625.8</td>
<td>81.3</td>
<td>14.8</td>
<td>30.7</td>
<td>15.7</td>
<td>0.92</td>
</tr>
</tbody>
</table>

a Proalimentar, Milk and Cereals ™, Portugal

b Compal Classic Tutti Frutti Nectar™, Portugal

Table 2. Energy and nutrient composition of foods provided at breakfast and lunch

Procedures

Participants arrived at the laboratory at 8h00 in order to collect their values of height in a stadiometer (SECA 220) and weight, total body water (TBW), intracellular water (ICW) and extracellular water (ECW) in a Segmental Multi Frequency Body Composition Monitor (TANITA MC 180
MA®). Then, a blood sample (8 ml) was collected from the antecubital vein by a trained phlebotomist for analysis of blood glucose, sodium (Olympus AU5400; Olympus, Melville NY, USA), and osmolality (Micro Osmometer 3300; Advanced Instruments, Inc., Norwood MA, USA). Throughout the session participants were allowed to read, listen to music with earphones, or use their portable computers with the exception of internet access to minimize visual cues (e.g., unwanted publicity for beverages or visiting Web sites showing pictures of food and drinks), which may have effect on thirst. Breakfast was served at 9h30 on every occasion, the preload beverage was offered precisely 60 minutes after breakfast (10h30) and lunch was provided at 13h00. Motivational ratings were firstly obtained at the end of blood sampling (baseline or time 0) and every 30 minutes thereafter until lunch time (times 1 through 7). Before lunch, another blood sampling was collected and TBW, ICW and ECW were estimated once again. Immediately after lunch (time 8), participants completed the last set of ratings and a food record was given to record all food and fluid intake from that moment until 24h00 of that day. Energy and nutritional intake were estimated by Food Processor SQL Edition, version 10.0 (ESHA Research Inc., Salem, OR, USA), added with nutritional information of Portuguese recipes and the following parameters were extracted: Energy, sugars, caloric beverages intake and total fluid intake.

The procedures were approved by Ethics Committee of the Universidade do Porto (Nº30/CEUP/2011), and were registered in ClinicalTrials.gov with (NCT ID: NCT01770327).

**Data Analysis**

All statistical analyses were conducted using IBM Statistical Package for Social Sciences (SPSS) 20.0 for Windows. Normality was determined by the Kolmogorov-Smirnov test (normal if P > 0.05). When the assumption of normality was violated, it was assured that variables skewness was < 3 and kurtosis < 10 [11]. Sphericity was determined by Mauchly's test (sphericity assumed when P > 0.05). When the assumption of sphericity was violated, univariate tests of within-subjects effects were subject to Greenhouse Geisser correction (when epsilon < 0.75) and to Huynh-Feldt correction (when epsilon > 0.75). General linear models (GLM) with repeated measures were used to analyze motivational ratings with beverage and time postingestion (times 3-7) as within-subjects factors and sex as the between-subjects factor. Analyses of glycaemia, plasma osmolality, sodium, extracellular, intracellular and total body water used a GLM with repeated measures with beverage and time as within-subjects factors and sex as between-subjects factor. When a main effect of time was observed in these variables, a t-student test was performed to find differences between moments for each beverage condition. Analyses of water intake at lunch and energy and nutritional intake after lunch used a GLM with repeated measures with beverage as within-subjects factors and sex as between-subjects factor. When there was a gender interaction, data were analyzed separately for each group. Data was expressed as mean ± standard deviation. The level of significance was set at P < .05.
Results

The mean age (± SD) of participants was 22.4 ± 1.46 years for men, 22.2 ± 2.48 years for women, and 22.3 ± 1.97 years for the whole group. Mean body mass index (BMI; in kg/m2) was 22.9 ± 2.30 for men, 21.2 ± 2.07 for women, and 22.1 ± 2.32 for the whole group.

Motivational Ratings

A decrease in thirst sensation (Fig. 1) was observed after the preload consumption with ratings increasing gradually until lunch time. A main-effect of beverage was not observed [F (3, 90) = 1.25; P=0.30], but a main interaction beverage*time [F (9.9, 296) = 2.38; P=0.01] and beverage*sex [F (3, 90) = 4.63; P=0.005] occurred.

Regarding the beverage*time interaction, W condition led to the higher differences in thirst ratings between time 3 (T3) and time 7 (T7) and NFM the lowest. However, all beverages reveal differences between T3 and T7 (P<0.001).

Due to the significant interaction-effect beverage*sex, the data was analyzed separately from gender. Here, we can observe that in men (Fig. 2) thirst ratings were consistently higher in NFM condition. In this group a main effect of beverage was observed [F (3, 48) = 4.18; P=0.01] and the pairwise comparisons revealed a positive but non-significant effect of NFM on thirst face to W (P=0.068) and IT (P=0.053).
In women (Fig. 3), no main effects of beverage were noticed but a beverage*time interaction occurred \(F(4.66, 65.2) = 2.53; P=0.041\). Analyzing the data separately from beverages we noticed that only in W and IT the differences between T3 and T7 were significant (\(P=0.001\) and \(P=0.014\), respectively), indicating that thirst ratings rose more in W and IT than in the other two conditions.
In mouth dryness ratings (Fig.4) the four beverages showed a very similar pattern with no main effects of beverage type \( [F (2, 48) = 0.23; P=0.79] \). Only a main effect of time was detected \( [F (1.75, 42.1) = 14.3; P<0.001] \) with mouth dryness ratings being higher in T7 face to T3 \( (P=0.014) \) and T4 \( (P=0.011) \). No sex \( [F (2, 48) = 1.1; P=0.34] \), or beverage*time interaction \( [F (8, 192) = 0.72; P=0.68] \) were observed.

Desire to drink ratings (Fig.5) showed a similar pattern to those of thirst. A main effect of beverage type was not observed \( [F (3, 90) = 1.51; P=0.217] \), but an interaction beverage*sex occurred \( [F (3, 90) = 3.4; P=0.021] \) with NFM leading to higher ratings face to W \( (P=0.06) \) and IT \( (P=0.003) \) in men. In women nor beverage main effects or beverage*time interaction occurred.

The ratings of hunger, desire to eat and nausea only revealed a main effect of time \( (P < 0.001) \). No main effects of beverage or interactions with time and sex were observed (data not shown).
Hydration Status and Biochemical Parameters

Table 3 shows, for each preload condition, the TBW, ICW and ECW at the beginning of the trial (T0) and at the pre-lunch evaluation (T7). A significant effect of beverage was not observed in TBW: $[F (3, 90) = 0.65; P=0.59]$, ICW: $[F (3, 90) = 0.50; P=0.69]$ and ECW: $[F (3, 90) = 0.84; P=0.48]$, but a significant interaction beverage*time was registered in ECW $[F (2, 50) = 0.32; P=0.31]$. T-Test revealed differences between the two moments in OJ ($P=0.004$) and IT ($P<0.001$), but not in W ($P=0.096$) or NFM ($P=1.0$).

Table 3. Hydration Status values for each preload condition.

* - $P<0.05$ vs Initial ECW
Table 4 expressed the values of plasma osmolality, sodium and glycaemia at the beginning of the trial (T0) and at the pre-lunch evaluation (T7). The osmolality values didn’t change over time [F (1, 30) = 0.492; P=0.488] and no differences between beverages were noticed [F (3, 90) = 1.287; P=0.284].

Regarding plasmatic sodium values, it was observed a significant effect of time on their increase [F (1, 30) = 11.6; P=0.002]. A significant interaction beverage*sex [F (3, 90) = 13.9; P<0.001] was also found. When analyzed the data separately from gender, a main effect of beverage was found in both genders with OJ leading to higher sodium values than W (P<0.001) and NFM (P=0.022) in men. In women, W led to higher sodium values than NFM (P=0.045), OJ (P=0.002) and IT (P=0.035).

In respect to blood glucose values, a decrease was observed during the trial [F (1, 30) = 39.5; P<0.001], with a main interaction beverage*time [F (3, 90) = 3.26; P=0.025], that revealed a more pronounced decrease of glycaemia values in NFM, OJ and IT (P<0.001), than in water (P=0.007).

Table 4. Variation of Plasma Osmolality, Sodium and Glycaemia for each preload condition

<table>
<thead>
<tr>
<th>Preload</th>
<th>Osmolality (mOsm/Kg)</th>
<th>Sodium (mEq/L)</th>
<th>Glycaemia (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Water</td>
<td>291.3 ± 4.52</td>
<td>290.8 ± 3.78</td>
<td>137.1 ± 1.56</td>
</tr>
<tr>
<td>Milk</td>
<td>291.1 ± 4.49</td>
<td>291.2 ± 3.58</td>
<td>136.8 ± 1.79</td>
</tr>
<tr>
<td>Orange Juice</td>
<td>291.0 ± 7.87</td>
<td>289.8 ± 4.87</td>
<td>137.1 ± 1.87</td>
</tr>
<tr>
<td>Iced Tea</td>
<td>290.8 ± 4.64</td>
<td>289.9 ± 3.50</td>
<td>136.8 ± 1.68</td>
</tr>
</tbody>
</table>

* - P < 0.001 vs. Initial Sodium
+ - P < 0.001 vs. Initial Glycaemia

Beverage Acceptability and Water Intake

Table 5 shows the amount of ad libitum water intake at lunch for each preload condition. There was a main effect of beverage [F (2.5, 72.5) = 3.91; P=0.018], although the pairwise comparisons between beverages revealed a non-significant difference between NFM and W (P=0.095), and IT (P=0.071). Since in men we previously observed a tendency to higher thirst ratings in NFM condition, we analyzed the data separately by gender, and we noticed that a beverage main effect was detected in men [F (3, 48) = 4.82; P=0.005] but not in women, with pairwise comparisons revealing differences between NFM and IT (P=0.001), but not between NFM and W (P=0.155).

Regarding beverages hedonic ratings, a main effect of beverage was observed [F (3, 90) = 171; P<0.001], with Iced Tea having higher scores than other three preloads (P<0.001).
Table 5. Beverage Acceptability and Water intake at Lunch

* - P < 0.001

Energy, sugars, caloric beverages and total fluid intake

Table 6 shows the energy, sugars, total fluid intake and intake of energetic beverages in the period from the end of the trial until the end of that day. Although some differences in this four variables could be pointed out, no main effects of beverage or interactions beverage*sex were noticed for any of them, revealing that the energy, sugars, caloric beverages and total fluid intake after the trial was the same regardless of the morning preload.

Table 6. Energy, Sugars, Caloric Beverages and Total Fluid Intake after ingestion of the preloads
Discussion

The main findings of this study were: Non-fat milk led to a tendency to higher thirst ratings in men and higher water intake at lunch face to Water and Iced Tea; All beverages elicit similar responses in plasma osmolality and decreased glycaemia with a more pronounced effect of water; Energy, sugar, caloric beverages and total fluid intake throughout day was similar for the four beverages.

This tendency to an increment in thirst ratings observed only in men was probably a casual occurrence because sex differences in thirst mechanism have not been described so far. It is a fact that in women, water regulation mechanisms are somehow dependent of the menstrual cycle phase. In this context, resting vasopressin is greater in men than women in the early follicular phase of their menstrual cycle, but not in the mid-luteal phase [12, 13]. However, in young and healthy women – like the ones in our study - the shift in osmoregulation appears to have only a minor effect on overall body water balance [14]. We have also to refer that, although a tendency to higher thirst ratings with NFM face to W and IT was observed only in men, when we analyze the volume of water intake at lunch, this tendency occurred in the same direction for the whole group.

Despite being non-significant, the increase of thirst sensation in men after milk consumption face to water and iced tea, was somewhat unexpected, since milk was the energetic beverage with less sugar and a lower osmolality, characteristics traditionally and empirically associated with a positive impact on thirst [15]. Moreover, the small difference in sodium of NFM face to IT should not be able to justify such result since all preloads induced an increase in plasmatic sodium during the trial, and the sex*beverage interaction-effect does not reveal a higher effect of milk in the increase of sodium values compared to other preloads. The few studies that have investigated the effects of milk and other caloric beverages in thirst sensation also does not reveal higher thirst reports after milk consumption. Almiron-Roig et al [5] does not show any difference in thirst sensation with the intake of 591ml of 1% milk, compared with the same amount of orange juice, cola and carbonated water. In that study, a significant gender interaction-effect was observed and a beverage main-effect was seen in women with water and orange juice satisfying thirst better than cola but no differences were observed for milk. The study of Monsivais et al [6] was also unable to show differences in thirst with the intake of 495ml of 1% milk face to four cola beverages with different sweeteners. More recently, in a trial [16] that compared the intake during 1h of approximately 2.2L of cow’s milk, soy milk, milk-based liquid meal supplement and a sports drink showed that ratings of overall thirst were not different between beverages. Only one study [10] reported similar results with a fruit drink suppressing thirst more than did a liquid and a semisolid yogurt, although in this case we have to recognize that thickness and viscosity of yogurt could had an influence in these results.

Still, one of the explanations for our results could be precisely the sensory characteristics of milk. The focus group study from McEwan and Colwill [3] classified strawberry milk as the less thirst quenching beverage between seven other drinks like carbonated lemon drink, orange juice,
orange squash, cola, isotonic drink, sparkling mineral water and diet cola. Furthermore, beverage thickness was the attribute more inversely correlated with thirst-quenching potential of a drink and strawberry milk was the beverage more associated with this attribute. In the same study, we could draw another important insight to our investigation. The beverage perceived as more thirst-quenching – carbonated lemon drink – was the beverage with higher scores in “acid” attribute and acceptability, very much as seen in our results with iced tea being the beverage with lower pH (together with orange juice) and higher acceptability face to all other preloads (P<0.001). Since IT led to a tendency to lower thirst ratings in men face to NFM (P=0.053) and to lower water ingestion at lunch (P=0.001), we can somehow relate these results and propose that acidity of a drink and its acceptability are important determinants of their thirst-quenching ability.

Another interesting finding of our study was the lack of effect that a highly hyperosmolar beverage such as orange juice (607 mOsm/Kg) had on thirst and even on plasma osmolality. This can be explained for the capacity of our digestive system to sustain the osmotic pressure of the intestinal contents equal to the plasma [17], with no sudden changes in the osmolality of the contents. The gastric emptying of hypertonic solutions is slowed via the enterogastric reflex, and this is triggered via osmoreceptors in the duodenum [15]. Regarding the absorption process, glucose and galactose, due to active sodium-glucose cotransporters (SGLT-1), are quickly absorbed (<2h) and water follows by osmosis [15, 17]. But fructose, the other sugar present in OJ, differ from glucose and galactose in the mechanism and speed of absorption with this process being mediated by passive carrier-mediated facilitated diffusion (GLUT 5) [18-20]. As fructose is absorbed along the entire small bowel, the absorption can take up to four hours and any water in fructose solution remains trapped until fructose is slowly absorbed [21]. So it is possibly that in our study, this process could have happen, but, since the interval between initial and final evaluation of plasma osmolality was >4h (08h00 – 12h30) meanwhile, these values have possibly returned to their regular narrow. Nevertheless, not always an increase in plasma osmolality induces an increase in thirst sensation and different solutes can have different roles. Sodium and its anions, which normally contribute >95% of the osmotic pressure of plasma, are the most potent solutes in terms of their capacity to stimulate AVP secretion and thirst. In contrast, increases in plasma osmolality caused by solutes such as urea or glucose cause little or no increase in plasma AVP levels in humans or animals [1, 22]. Thus, it is clear that osmoreceptor cells in the brain primarily respond to plasma tonicity rather than to total plasma osmolality which indicates that their primarily function is to preserve cell volume. Elevations of solutes such as urea and glucose, unlike elevations of sodium, do not cause cellular dehydration and consequently do not activate the mechanisms that defend body fluid homeostasis by preserving or increasing body water stores [23].

Concerning nutritional intake throughout the day of trials, the energy, sugars, caloric beverages and total fluid intake does not differ between preloads. Although the main objective was to evaluate if a hypothetical variation of thirst would have some effect in subsequent food behavior, it was interesting to observe that no energy compensation was made during the day with the caloric preloads. This finding is in agreement with other study with similar beverages that report no energy compensation at subsequent meal after consumption of sugary preloads [5], although, the study from Monsivais et al [6], showed that 1% fat milk led to a lower energy intake at subsequent
lunch compared with no-beverage condition and aspartame sweetened cola. In our study, in addition to a lack of effect in hunger ratings caused by non-fat milk, this preload does not led to lower energy intake during the day.

About the limitations of this study, we could see the ingestion of 500ml of water on the evening before and on the morning of the trial to ensure euhydration status as excessive and potentially mask some differences in thirst sensation. However, even with this procedure, we could notice that initial plasma osmolality values were between 290 – 291.3 mOsm/Kg, and within their physiological range (280 – 300 mOsm/Kg). So it is unlikely that this recommendation may have affected our results and a hypothetic dry-mouth state of some participants at baseline could have led to a bias in our results [24]. The BIA method is not the gold standard to assess intracellular and extracellular water, even knowing that the variation in hydration status was not the main aim of the study.

**Conclusions**

So, the ingestion of a 330 ml preload of three different sugary beverages does not led to substantial effects on thirst sensation compared to water. Only a tendency to higher thirst ratings in men was observed with milk preload face to water and iced tea and the same tendency remained for water intake at subsequent lunch but for the whole group. Water, non-fat milk, orange juice and iced tea promoted also equally responses in energy, sugars and caloric beverages intake throughout the day of the trials.

**Acknowledgments**

We thank to all participants of the study and to the European Hydration Institute for the financial support.
References


The findings of our studies allow us to conclude that:

. At similar temperature conditions, sugar-sweetened and diet beverages are as thirst-quenching as water, despite the hyperosmolality of sugary sodas face to plasma.

. Increasing sweetness level in cold and acid beverages does not seem to induce increments in thirst sensation compared to water.

. Beverages with different dietary sugars such as sucrose, lactose and fructose elicit a thirst response similar to water, acknowledging the fact that non-fat milk revealed a tendency to higher thirst ratings face to water and iced tea in men.