Obesity Research & Clinical Practice (2017) **xxx**, xxx—xxx

ELSEVIER

Carbon dioxide in carbonated beverages induces ghrelin release and increased food consumption in male rats: Implications on the onset of obesity

Dureen Samandar Eweis, Fida Abed, Johnny Stiban*

Department of Biology and Biochemistry, Birzeit University, P.O. Box 14, Ramallah, West Bank 627, Palestine

Received 7 September 2016; received in revised form 15 January 2017; accepted 2 February 2017

KEYWORDS
Carbonated beverages;
Carbon dioxide;
Food consumption;
Weight gain;
Ghrelin

Summary
Background: The dangerous health risks associated with obesity makes it a very serious public health issue. Numerous studies verified a correlation between the increase in obesity and the parallel increase in soft drink consumption among world populations. The effects of one main component in soft drinks namely the carbon dioxide gas has not been studied thoroughly in any previous research.
Methods: Male rats were subjected to different categories of drinks and evaluated for over a year. Stomach ex vivo experiments were undertaken to evaluate the amount of ghrelin upon different beverage treatments. Moreover, 20 male students were tested for their ghrelin levels after ingestion of different beverages.
Results: Here, we show that rats consuming gaseous beverages over a period of around 1 year gain weight at a faster rate than controls on regular degassed carbonated beverage or tap water. This is due to elevated levels of the hunger hormone ghrelin and thus greater food intake in rats drinking carbonated drinks compared to control rats. Moreover, an increase in liver lipid accumulation of rats treated with gaseous drinks is shown opposed to control rats treated with degassed beverage or tap water. In a parallel study, the levels of ghrelin hormone were increased in 20 healthy human males upon drinking carbonated beverages compared to controls.

Abbreviations: CBs, carbonated beverages; CW, carbonated water; RCB, regular carbonated beverage; DCB, diet carbonated beverage; DgCB, degassed regular carbonated beverage; PBS, phosphate buffered saline; DMEM, Dulbecco's Modified Eagle Medium; CCK, cholecystokinin.

* Corresponding author. Phone: +970 59 245 1374/+972 54 392 1144; fax: +970 2 298 2084.
E-mail address: jstiban@birzeit.edu (J. Stiban).

http://dx.doi.org/10.1016/j.orcp.2017.02.001

Conclusions: These results implicate a major role for carbon dioxide gas in soft drinks in inducing weight gain and the onset of obesity via ghrelin release and stimulation of the hunger response in male mammals.

Introduction

A substantial deviation in the balance between energy intake and expenditure eventually leads to weight problems and one major example is obesity. Obesity is a great public health concern due to the numerous comorbidities associated with it such as type 2 diabetes, cardiovascular diseases and hypertension. A study at a global scale has shown that the percentage of overweight or obese adults has grown from 23% to 34% between 1980 and 2008 [1]. The worldwide problem of obesity also affects all age groups making it a serious issue [2].

Obesity causes are multifaceted; they include social, environmental and hereditary factors [3]. The major factor that eventually leads to obesity is excessive food consumption. Regulation of food intake and utilization is complex and requires a variety of hormones and enzymes. Ghrelin is a 28-amino acid peptide hormone that is released mainly from the stomach as a response to hunger. Ghrelin production in the body was mainly found to be in the stomach of rodents but it has also been identified in other tissues such as the gastrointestinal tract, pancreas, ovary and adrenal cortex. The secretion of this hormone depends greatly on the nutritional state of the body [4—9].

Another main factor shown to be correlated with the increase in obesity is the increased rate of consumption of carbonated beverages (CBs). CBs were first introduced in Europe in the 17th century in attempts of therapeutic use. Additional components were then introduced into the beverages allowing them to enter the commercial market [10]. CBs have also been upsised and extensively advertised, especially targeting children [11]. Since most CBs contain sugar as a key component, companies provide alternatives to sugary drink by replacing the sugar with artificial sweeteners. In this attempt to create diet CBs (DCB), the caloric content of the drinks is significantly reduced or even abolished.

Sugar substitutes from herbs, sugars and other naturally occurring substances are used to make artificial sweeteners. These substitutes give a more intense sweetness to the beverage compared to natural sugars. Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a widely used artificial sweetener which is water soluble. Once ingested it binds to T1R2 receptor on the tongue in order to give the sweet taste [12]. Several short-term animal studies have shown aspartame consumption to be relatively safe although a few other studies have suggested an increased risk of cancer and diabetes type 2 with artificial sweetener intake [13].

Nutritional studies indeed focused on the sweetener in the beverages, whether sugar or sugar substitute. There is, nonetheless, another dimension to the CBs complexity; the carbon dioxide gas. Whereas diet CBs contain the aforesaid artificial sweeteners, regular sodas contain sucrose. Both drinks, however, contain carbon dioxide which is introduced into the drink under pressure to add acidity and to sharpen the flavour of the drinks. The amount of carbonic acid produced in the CBs from the carbon dioxide depends on the pressure used to introduce the gas in the drinks. The gas also acts a preserver keeping the drink for a longer period of time. To our knowledge, the effect of the added carbon dioxide gas in the drinks has not been studied thoroughly in any previous research.

Here, the effects of carbon dioxide in CBs were studied in male rats, as well as on human subjects. Hunger stimulation as evident by elevated blood ghrelin concentration as a response to CBs was shown in rats and humans. Rats on CBs supplemented diet increased in size and consumed more food than control rats. This research is the first report to date to discuss the role of carbon dioxide in CBs as an inducer of hunger in mammals.

Materials and methods

Materials

Sprague Dawley white laboratory rats were bred in the animal unit facility at Birzeit University. Sixteen littermate rats (from two litters born on the same day) of similar size were assigned randomly into four different groups after weaning (23 days old). Upon the completion of the study, the animals were sacrificed by cervical dislocation and decapitation in accordance to animal treatment regulations at the institution.

Measurements of weight and food consumption

All rats were provided with standard diet Teklad Global 18% protein (2018SC from Harlan Laboratories). Rats of each group were supplemented with different beverages: (i) tap water, (ii) regular degassed CB (DgCB), (iii) regular CB (RCB) and (iv) diet CB (DCB); the aspartame content of one DCB can be around 180 mg/330 ml. Degassing of regular CB was performed by continuous stirring of the drink for a period of over 2 h. The weight of each rat was measured and recorded on a daily basis. Additionally, the amount of food ingested for each group was recorded every day to assess food consumption.

Please cite this article in press as: Eweis DS, et al. Carbon dioxide in carbonated beverages induces ghrelin release and increased food consumption in male rats: Implications on the onset of obesity. Obes Res Clin Pract (2017), http://dx.doi.org/10.1016/j.orcp.2017.02.001