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

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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.

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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.

Blood glucose and cholesterol determination

Six months after the experiment started, tail-blood samples were obtained from the rats and analysed for blood biochemistry at the University Clinic.

Ex vivo analysis of ghrelin secretion

Stomachs from well-fed 5-month old rats on a standard diet were excised post-euthanasia and food residues were disposed of and thoroughly washed off with ice-cold phosphate buffered saline (PBS). The washed stomach pieces were cut into four identical pieces per stomach then randomly separated into eight sterile Petri dishes. PBS was aspirated out and 1 ml of Dulbecco Minimal Eagle Medium (DMEM) was added to each stomach piece. To each Petri dish containing a stomach piece, 1 ml of the following beverage was added (tap water, RCB, DCB and DgCB). The dishes were incubated at 37° C for 10 min. Following the incubation, the solution from each Petri dish was collected and stored at −80° C until analysis for ghrelin. The same procedure was repeated using the other stomach incubating the dishes in 1 ml of beverages for 30 min.

Ghrelin hormone determination by ELISA kit

Blood samples (or ex vivo solution samples) obtained from rats undergoing the same tests were analysed for the ghrelin hormone content by using Rat/Mouse Ghrelin assay kit (EMD Millipore) according to manufacturer's instructions.

Dissection and determination of liver lipid accumulation

At the termination of the experiment (after 1 year), rats were euthanised and dissected and pictured. Livers were harvested from each rat. The livers were weighed and 1.5 g of each liver were boiled in 15 ml of saturated KOH and 15 ml of 95% ethanol for 3 h. The pH of each solution was adjusted using 6 N HCl (with 1 drop of bromocresol green as a pH indicator). In order to dissolve the lipids, 50 ml of chloroform was added to each solution, vortexed vigorously for 5 min then allowed to sit until the chloroform layer cleared. Different 10 ml aliquots of the chloroform layer were quantitatively removed and placed in a previously weighed flask. After evaporation of chloroform in a 65 °C degree oven, the weight of lipids was measured and multiplied by 5 to account for the total lipids.

Ghrelin concentration from human blood samples

A group of 20 male volunteers at Birzeit University was randomly selected. The students were all of Palestinian Arab origins and their age ranged between 18 and 23 years. The group was healthy and normal in height and weight (body weight average was 78 ± 5 kg and height average was 171 ± 8 cm). The group was asked to abstain from eating anything after 10 p.m. the night before the test. On the day of the test, at 9 a.m., all students were given the same breakfast consisting of a 300 g piece of cheese-filled baked bread. One hour after this light breakfast, the students were given 330 ml of water to drink. After 5 min, blood was collected and stored at −80 °C until ghrelin measurement. On different days, the same group of people repeated the experiment, using 330 ml of RCB, DCB, DgCB, or carbonated water (CW) following the light breakfast, followed by blood collection. All samples were then analysed for ghrelin using ghrelin ELISA kit per manufacturer's instructions.

Statistical analysis

Our results are presented as mean ± standard deviation of at least 3 experiments. Differences between groups were assessed using one-way ANOVA. Statistical significance was indicated with $p < 0.05$.

Results

Rats on CBs accumulate more weight than controls

In order to study the effects of CBs consumption on rats, male albino Sprague Dawley rats were allowed free access to standard rodent diet and beverages according to treatment group. The rats were measured daily and their food consumption recorded. The daily weights of rats in each of the treatment groups are shown over a period of 100 days (Fig. 1A). The average weight for each treatment at day 110 indicates that rats on tap water or regular DgCB (control groups) weighed significantly less than rats on RCB or DCB (Fig. 1B). There is a significant difference between food intake of rats on RCB and DCB compared to rats drinking water and DgCB. Initial weight gain rate for the first two months of the study indicates that water-treated rats grew the slowest (2.6 ± 0.4 g/day) compared to the other treatments. Interestingly, both RCB and DCB-treated animals amassed weights at a significantly higher rate (Fig. 1C).

Rats on CBs consume more food than controls because of increased ghrelin release

The cause of weight increase was probed. Rats, which were given access to RCB or DCB, significantly consumed more food daily, compared to rats which drank water or DgCB (Fig. 2A). This increased food consumption was due to a significant increase in ghrelin levels in the sera of rats on CBs (Fig. 2B). On the other hand, the levels of the satiety hormone, cholecystokinin (CCK), were assessed in these rats as well and the results showed no significant difference among different groups (data not shown).

Isolated rat stomach explants secrete ghrelin in response to CBs

In order to validate the previous findings, stomachs from normally fed rats were assessed for their ghrelin release after various drink treatments ex vivo. Ghrelin release

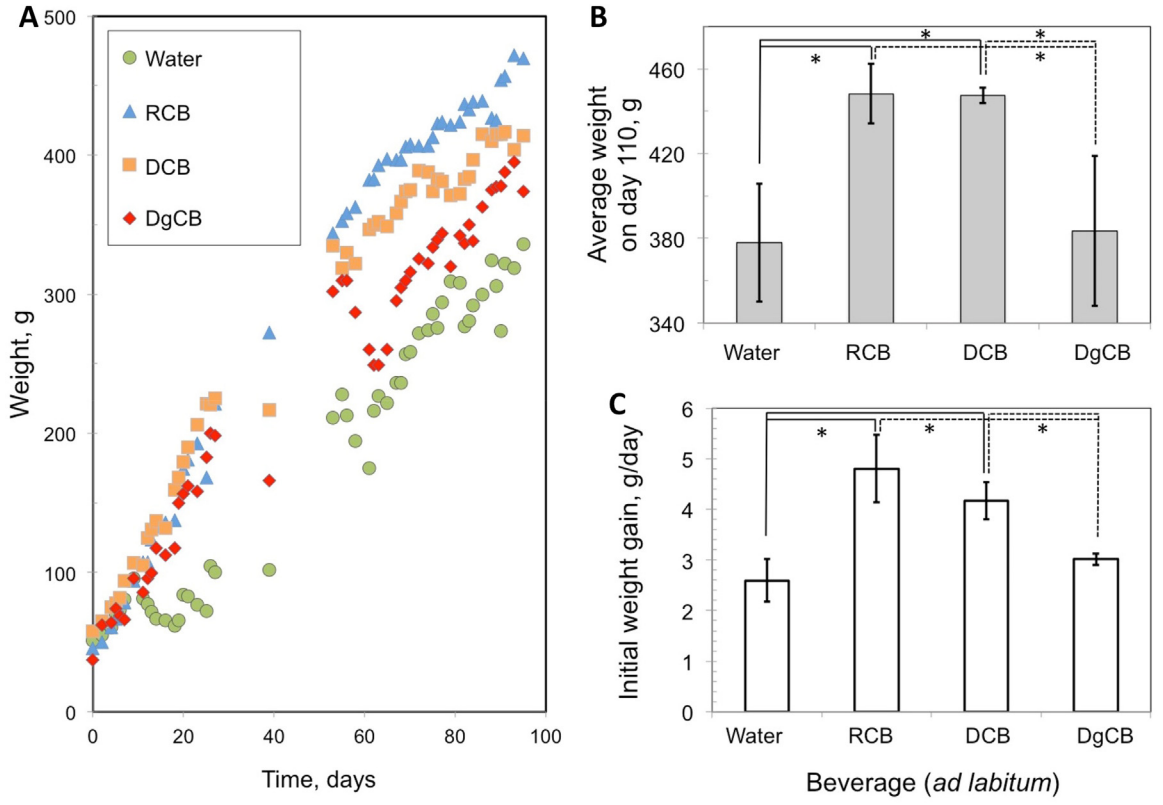

Figure 1 The rate of weight gain in male rats in each group is shown over a period of 100 days. (A) The average weight of rats in a group per day is monitored for 100 days. (B) Rats on different drinks showed significant differences in their average weights on day 110. (C) The rate of weight gain per day was determined by measuring the slope of the weight increase per unit time. RCB: regular carbonated beverage; DCB: diet carbonated beverage; DgCB: degassed regular carbonated beverage. All results are the average and standard deviation of biological triplicates (*$p < 0.05$). Solid lines show statistical significance between CBs and tap water, whereas dashed lines indicate the differences between CBs and DgCB.

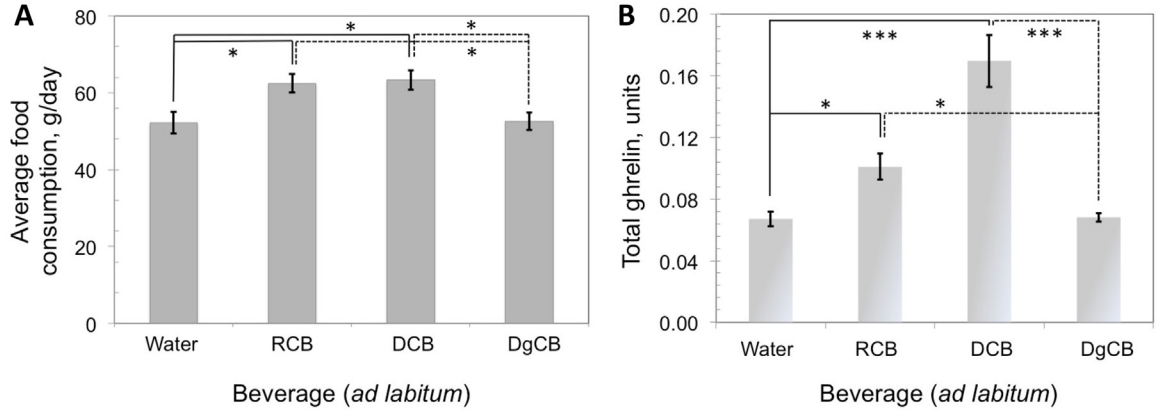

Figure 2 Rats on CBs consume more food daily due to a higher secretion of ghrelin. (A) The average food consumed per cage was monitored for all drinking groups. (B) Tail vein blood was collected from each rat to measure the levels of ghrelin hormone in the morning after the rats have been allowed to feed ad labitum (*$p < 0.05$; ***$p < 0.005$). Solid lines show statistical significance between CBs and tap water, whereas dashed lines indicate the differences between CBs and DgCB. RCB: regular carbonated beverage; DCB: diet carbonated beverage; DgCB: degassed regular carbonated beverage.

Table 1 Blood glucose and cholesterol levels in rats consuming different drinks.

Group	Glucose (mg/dl)	Cholesterol (mg/dl)
Water	157 ± 22	127 ± 3
RCB	187 ± 0.4	135 ± 3
DCB	172 ± 21	135 ± 5
DgCB	192 ± 5	138 ± 3

was significantly higher even at 10 min post exposure to CBs, compared to water and regular DgCB (Fig. 3, open bars). After 30 min, ghrelin release increased as expected in all treatments (Fig. 3, closed bars).

Obesity is induced in rats at a faster rate when consuming CBs

Six months into the study, blood biochemistry of all rats was performed to check early markers of obesity. In all rats, there appeared to be no significant changes in blood total cholesterol or fasting blood sugar (Fig. 4A). Nevertheless, in all samples, there was a small but steady increase in both cholesterol and glucose in all rats not drinking water, summarised in Table 1. At the end of the study, liver lipids were assessed for all rats, with rats on CBs amassing significantly more lipids than controls

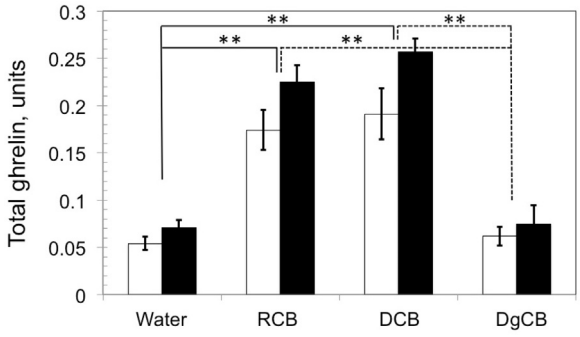

Figure 3 Stomachs secrete ghrelin in response to CBs ex vivo. Stomachs from control rats were extracted after cervical dislocation and excised into identical cuts and washed with PBS then incubated in DMEM medium. The stomachs were treated with different beverages for 10 (open bars) or 30 min (closed bars). Ghrelin level in the solution was measured afterwards. RCB: regular carbonated beverage; DCB: diet carbonated beverage; DgCB: degassed regular carbonated beverage. Results are the average and standard deviation of 3 replicates (**$p < 0.01$). Solid lines show statistical significance between CBs and tap water, whereas dashed lines indicate the differences between CBs and DgCB.

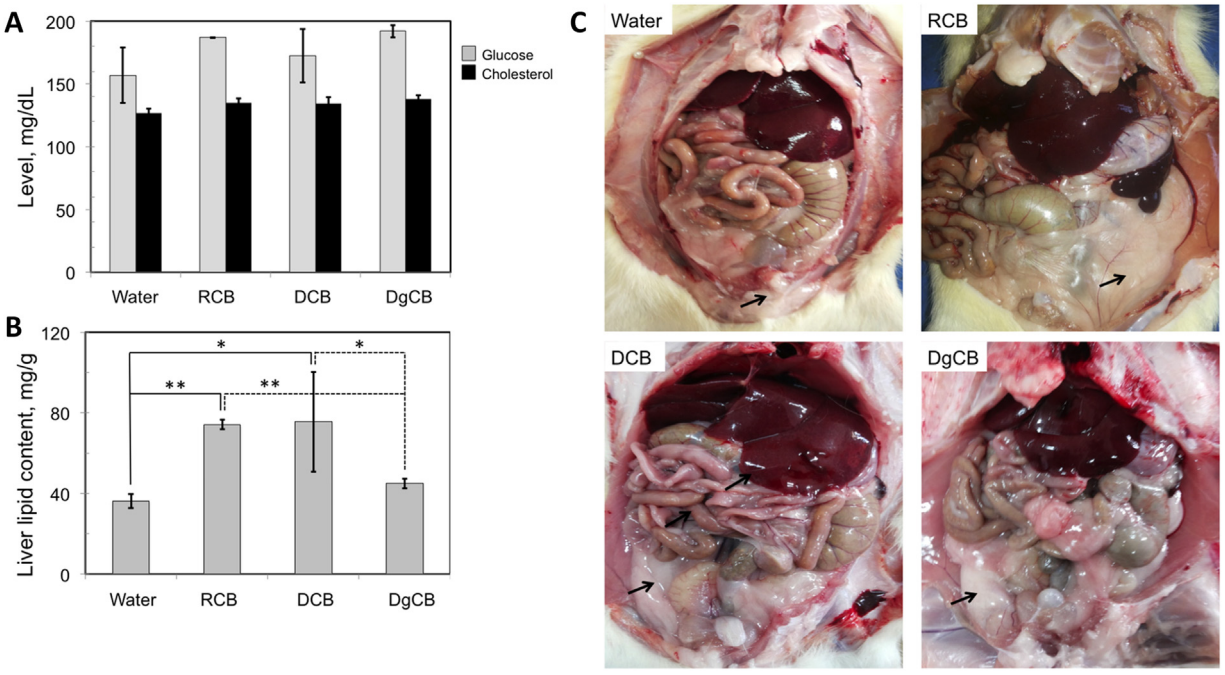

Figure 4 Late-onset, as opposed to early-onset, of obesity in rats was observed. (A) Blood was collected from rats after 6 months to measure obesity markers such as fasting blood sugar (grey bars) and total cholesterol (black bars). (B) At the conclusion of the study, accumulation of lipids in the livers of rats was measured as a marker of obesity. Livers were homogenised and total lipids extracted and weighed from all rats (*$p < 0.05$; **$p < 0.01$). (C) All rats, post-sacrifice were dissected to locate adipose depositions. Representative pictures from each group are presented here. The arrows indicate areas of lipid deposition. RCB: regular carbonated beverage; DCB: diet carbonated beverage; DgCB: degassed regular carbonated beverage. Solid lines show statistical significance between CBs and tap water, whereas dashed lines indicate the differences between CBs and DgCB.

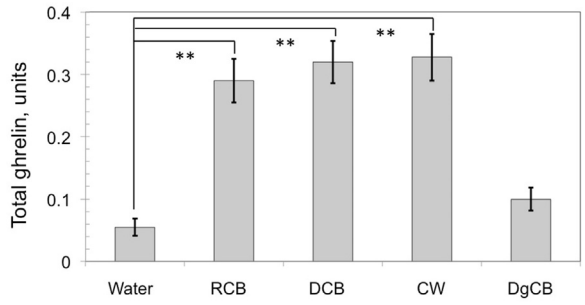

Figure 5 Ghrelin is released in human subjects upon ingestion of CBs. Twenty healthy students between the ages of 18 and 23 fasted overnight then broke their fast eating the same meal. One hour after breakfast, the students were given 330 ml of water (or other beverages) to drink and their blood was collected for ghrelin analysis. RCB: regular carbonated beverage; DCB: diet carbonated beverage; DgCB: degassed regular carbonated beverage; CW: carbonated water. Results are the average and standard deviation of 18—20 samples (**$p < 0.01$).

(Fig. 4B). Overall, all rats were checked for adipose tissue after dissection. Dissected representative rats from each group show lipid deposits around vital organs. Indeed, all rats that were not on water showed increased lipid deposits compared to water-treated controls, indicating an induction of obesity (Fig. 4C).

Carbon dioxide in CBs induces ghrelin release in human males

To extrapolate the study on humans, ghrelin levels were measured in male subjects after drinking any of the aforementioned beverages, in addition to CW (no caloric content, no sugar). Twenty students, over a period of 1 month, performed this experiment and the same individuals performed all tests. Individuals drinking CBs (including CW) an hour after meals had significantly higher circulating ghrelin levels compared to the same individuals on non-CB (water or DgCB). About 6-fold increase in ghrelin concentration was observed in the blood of subjects after consumption of CB, compared to water (Fig. 5). Moreover, compared to DgCB, a 3-fold increase in ghrelin was achieved when RCB, DCB or CW were used.

Discussion

Extensive research elucidates that weight gain and obesity are a leading cause of health risks worldwide [14]. A correlation between the consumption of soft drinks and increased weight gain and obesity has been shown in numerous studies [15—19]. Since the hazards of the obesity epidemic are increasingly concerning issues, many studies are carried out to help dissect and reduce this problem. Schulze et al. demonstrated an association between higher consumption of sugar-sweetened beverages and increased weight gain as well as heightened risk of type 2 diabetes development in women [19]. Similarly, overconsumption of high-fructose corn syrup in calorically sweetened beverages, including CBs, was shown to be a cause for the rise in the obesity epidemic [20]. Thus, CBs have been presented as a health hazard by alarming the public over the sugar content of these beverages. It is widely accepted that the sugar content of CBs and increased weight gain are tightly associated as research findings displaying such association are confirmed and date back to around 1983 [21]. In order to reduce the prevalence of obesity, Bray et al. proposed substituting caloric sweeteners with non-caloric alternatives [16]. Whereas some intervention studies show that consumption of CBs containing artificial, non-caloric sweeteners as substitutes for the sugar content have little association with obesity induction [22,23], there is accumulating evidence that the effect of consuming these drinks leads to reverse causation where drinking beverages that contain artificial sweeteners is correlated with higher food intake and weight gain [24,25] even though diet drinks were introduced to reduce weight gain. Additionally, the results of our study clearly demonstrate that the lack of sugar or calories in CBs still leads to increased food consumption and weight gain. Therefore, in this study, we demonstrate the previously unknown effect of the added carbon dioxide gas in CBs in inducing weight gain and obesity in mammals.

Confirming earlier literature, rats on either RCB or DgCB gain weight faster than water-treated rats due to the increased sugar content in the drink, even though the increase in DgCB group was not significant in comparison with water-drinking rats. While this finding is critical for the long-understood effect of CBs on increased weight gain, it was intriguing to find that drinking the calorie-free alternative (DCB) had even more pronounced effects. Since both RCB and DCB have similar effects, we conclude that the difference compared to water is not due to the sugar content but due to another key ingredient. Moreover, a significantly higher food intake was correlated with rats drinking CBs as opposed to rats on non-CBs (water and DgCB). This indicates that the rapid weight gain in rats drinking CBs was a consequence of higher food consumption. To our knowledge, the effects of the gas in CBs in relation to obesity had not been previously investigated although the majority of scientific studies investigating soft drink consumption primarily focus on the sugar ingested from these drinks and its association with weight gain [26—30]. In our study, we were able to assess the effects of the gas in soft CBs because of our experimental setup. Among all groups, the difference in the contents of the drinks, apart from tap water, are minimal. For instance, RCB differs from DgCB in having more carbon dioxide, whereas RCB has sugar instead of aspartame in DCB. The rest of the contents are the same since we used the same brand of drinks in this study. Hence, we were able to clearly differentiate between the effects of drinking CBs compared to non-CBs in terms of food consumption and weight gain.

To elucidate the role of carbon dioxide in CBs in weight gain, the mechanism of increased food consumption was then probed. Earlier studies implicated changes in the production and secretion of appetite-controlling hormones as probable mechanisms for increased weight gain from the caloric overconsumption from soft drinks [17,31]. In this study, the concentrations of the satiety hormone cholecystokinin (CCK) and the hunger hormone ghrelin were measured in blood samples collected from the rats to determine whether larger food consumption was due to the release of hunger hormones or the inhibition of release of satiety hormones. Endogenous

cholecystokinin (CCK), which is one of the various satiety hormones, has been shown to induce satiety and reduction of food intake [22,32—37]. CCK is secreted predominantly in the small intestine [38] and its release is stimulated more by the intake of lipids than ingestion of carbohydrates [39—41]. Whether the physiological satiating effect of CCK in humans is local or endocrinal is unclear, however, studies in rodents have shown that satiety is mediated by the vagus afferent nerve [42—44]. In our assessment, the concentration of CCK in blood samples collected from rats under different drink conditions was unchanged with respect to the type of drink administered. Ghrelin, which is considered a hunger hormone and is primarily secreted in the stomach, plays a key role in sensing nutritional status allowing for meal initiation and regulation of appetite [45]. Ghrelin has been shown to function in mediating energy balance via the hypothalamus [46—48]. Here, blood ghrelin concentration in rats was probed at the same time in the morning to avoid rhythmic changes of ghrelin levels [49]. Ghrelin levels in rats drinking CBs were significantly greater than its concentration in control rats (on non-CBs). There was almost a 1.5- or 2.5-fold increase in ghrelin concentration in rats drinking RCB or DCB, respectively, compared to rats drinking water and DgCB. A noteworthy observation is the significantly high ghrelin concentration (Fig. 2b; $p < 0.005$) in rats drinking DCB compared to rats drinking water or DgCB. This may be a consequence of the lack of sugar in diet drinks which probably induces rats to crave food to balance the non-nutritive aspect of diet drinks [50]. Therefore, whereas CCK levels were unchanged, ghrelin levels were different in our study. Ghrelin and CCK are only two of the regulators of appetite in mammals. While the physiological effects of gastrointestinal hormones on eating, food consumption and weight gain are very complex, our study confirms that at least one hormone is elevated upon consumption of CBs which is a novel finding.

In order to validate the in vivo data, ghrelin concentration was assessed ex vivo from harvested stomachs. Seoane et al. developed an ex vivo experiment where gastric explants were shown to be suitable models for testing ghrelin in vitro [51]. Upon treatment of isolated stomach pieces with different beverages, ghrelin was secreted with a 3 to 4-fold increase between stomachs treated with CBs compared to control drinks. This result represents direct evidence confirming that CBs induce ghrelin release upon interaction with stomach cells, as early as 10 min after incubation. Upon CB ingestion, pressurised carbon dioxide comes in contact with stomach walls inducing the release of ghrelin possibly through mechanosensation. Pressure on cellular membranes induces the release of key hormones such as serotonin (5-hydroxytryptamine) from gastrointestinal epithelial enterochromaffin cells [52,53] and parathyroid hormone-related peptide from coronary endothelial cells [54]. Gut emptying and food movement create pressure on multiple gastrointestinal sites that combine to produce a variety of feedback signals to terminate meals [55]. It was also recently shown that downstream effects of ghrelin are modulated via its interaction with serotonin 2C receptor [56]. Hence it is possible that CO_2 acts as a mediator of a similar mechanosensitive mechanism that ultimately leads to the release of ghrelin leading to increased food consumption and weight gain (Fig. 6).

To assess inclination of test subjects to develop signs of obesity, blood sugar and lipid levels were measured after 6 months of treatments. Rats drinking CBs showed slightly elevated levels of blood glucose in comparison to water treated rats. Even though the results are not statistically significant, they show an inclination of these rats to early onset of diabetes and obesity. It is noteworthy to mention that the results show that blood glucose is elevated in rats regardless of the type of drink they ingest (apart from water). This suggests that the presence of sugar and/or carbon dioxide in CBs leads to higher blood glucose. Cholesterol and triglycerides did not show significant differences in all rats suggesting that lipid metabolism malfunctions occur late in the progression of the disease.

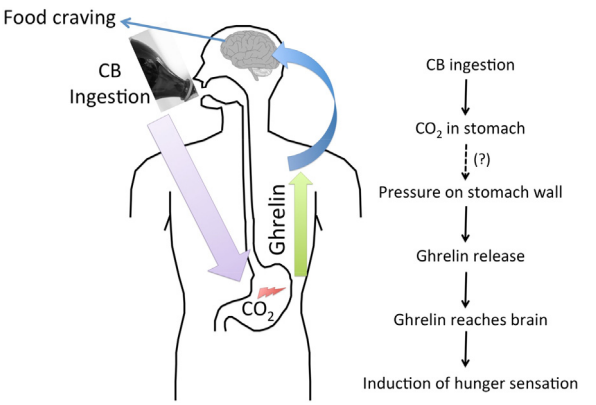

Figure 6 Putative mechanism of the effects of CO_2 in CBs on the induction of weight gain in male mammals. The ingestion of CBs will lead to an accumulation of pressured carbon dioxide gas in the stomach which may (dashed line and question mark) result in a mechanosensitive signal that results in the release of ghrelin from stomach cells. Once released, ghrelin can reach the brain and stimulate the sensation of hunger leading to increased food craving and food consumption.

Obesity is not the only condition caused by overconsumption of CBs, fatty liver disease is another concerning issue that may manifest due to increased sugar ingestion from CBs [31,57]. A correlation between obesity and fatty liver disease has been shown in a number of studies [58,59]. Therefore, to study the late onset of obesity, fat deposits on the liver (early onset of fatty liver disease) and other organs were monitored post-mortem. We found that there was a remarkable difference in the amount of accumulated liver fat between rats drinking CBs and rats drinking water and DgCB. Rats consuming RCB and DCB had more than 2-fold increase in liver lipid amount compared to water-drinking rats. Furthermore, rats on RCB or DCB accumulated liver lipids around 1.5-times more than rats on DgCB due to the presence of the gas in the aforementioned drinks. Moreover, DgCB rats accumulated lipids more than water controls due to the sugar content of the beverage that is metabolised and stored as fat droplets when the intake of food exceeds the energy needs of the organism. Studies have shown the dangerous effects of liver lipid accumulation on liver functionality and the risk of developing fatty liver disease [58—60]. Therefore, results from this study on liver lipid accumulation upon CB consumption add greater alarming importance to the effect of the gas ingested from these drinks.

The results of the rodent studies were then confirmed in human subjects. Twenty male students volunteered to undergo blood tests following ingestion of beverages. In agreement with previous results obtained for ghrelin concentration in rats, we found that the ghrelin concentration in human males consuming CBs was significantly greater than the ghrelin concentration in those drinking non-CBs. This result is remarkable since the same group of people was tested on different days after consumption of different drinks 5 min after ingestion of the drinks. Endogenous clocks of the circadian system allow for the alternation of certain metabolic, physiological and behavioural functions [49]. Taking into consideration that the secretion of ghrelin is governed by this clock, we collected samples from all human subjects at 9 a.m. In addition to the standard beverages we have been using throughout our experiments we added CW as a further control. Indeed, consumption of CBs induced a significant ghrelin release in human volunteers compared to the same group drinking non-CBs.

This is the first report that assigns new effects of carbon dioxide in CBs inducing ghrelin release and weight gain in mammals. We therefore recommend the regulation of CBs consumption by the general public to limit their damaging effects on consumers.

Conclusion

While it has become a consensus that consumption of soft drinks is directly linked with obesity due to their sugar content [18,19,61—64], our novel research delineates another aspect of consuming CBs. There has been no research on the effects of the added carbon dioxide in CBs on the onset of obesity. Hence, this study clearly shows discernible effect of the carbon dioxide gas in CBs on increased food ingestion and heightened risk of weight gain, obesity and fatty liver disease by inducing ghrelin release and increased food consumption in rodents and humans. Thus, the increased risk of weight gain as well as development of fatty liver disease seems to be amplified via the intertwined role of the carbon dioxide gas and the sugar content of the CBs. There is, therefore, another dimension in obesity induced by CBs that needs to be taken into account in the future.

Conflict of interests

The authors declare no conflict of interests regarding the publication of this article.

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