Published in "Obesity Reviews 16: 7–18, 2015"
which should be cited to refer to this work.

The Thermic Effect of Sugar-Free Red Bull: Do the Non-Caffeine Bioactive Ingredients in Energy Drinks Play a Role?

Jennifer L. Miles-Chan, Nathalie Charrière, Erik K Grasser, Jean-Pierre Montani, and Abdul G Dulloo

Objective: Consumption of energy drinks is increasing amongst athletes and the general public. By virtue of their bioactive ingredients (including caffeine, taurine, glucuronolactone, and B-group vitamins) and paucity of calories, sugar-free "diet" versions of these drinks could be a useful aid for weight maintenance. Yet little is known about the acute influence of these drinks, and specifically the role of the cocktail of non-caffeine ingredients, on resting energy expenditure (REE) and substrate oxidation. Therefore, the metabolic impact of sugar-free Red Bull (sfRB) to a comparable amount of caffeine was compared.
Methods: REE and respiratory quotient (RQ) were measured in eight healthy young men by ventilated-hood indirect calorimetry for 30 min baseline and 2 h following ingestion of 355 ml of either: sfRB + placebo, water + 120 mg caffeine, or water + placebo, according to a randomized cross-over design.
Results: sfRB and water + caffeine both increased REE to the same degree (+4%). Additionally, sfRB briefly increased RQ. Water + caffeine had no effect on RQ relative to water + placebo.
Conclusions: sfRB enhanced thermogenesis and marginally shifted RQ to favor carbohydrate oxidation. The stimulatory effects of sfRB on REE are mimicked by water + caffeine, indicating that the auxiliary ingredients do not influence this thermic effect. The metabolic effects of sfRB are primarily due to caffeine alone.

Introduction

"Energy" drinks generally refer to a class of beverages containing sugar and various combinations of ingredients purported to "energize" the body and mind, most commonly caffeine, taurine, and vitamins.

Typically the sugar content of energy drinks is comparable to or greater than conventional soft drinks, with ingestion leading to an intake of calories far exceeding the thermic effect of the drink. Coupled with increasing interest regarding the use of non-nutritive sweeteners as a weight-loss aid (1), the emergence of sugar-free "diet" energy drinks has raised the question of whether, by virtue of their paucity of calories and cocktail of bioactive ingredients, such drinks could increase thermogenesis and fat oxidation sufficiently to impact weight maintenance. Indeed, acute increases in energy expenditure (EE) and decreases in fat mass with regular consumption have been reported following ingestion of an energy drink containing caffeine (200 mg/336 ml), taurine, glucuronolactone, and extracts of guarana, green tea leaf, and ginger (2). Whilst different brands of energy drinks differ in terms of their alleged bioactive ingredients, Red Bull (RB), a popular commercial energy drink, and its sugar-free version, both contain a blend of caffeine, taurine, B-group vitamins, and glucuronolactone. Caffeine has been consistently shown to increase both EE and fat oxidation (3,4), yet little is known about the contribution of the cocktail of non-caffeine ingredients to the observed changes in resting EE (REE) and substrate oxidation. Nonetheless, taurine, another ubiquitous energy drink ingredient, has shown some promise as an anti-obesity tool. Supplementation with 3 g/day of taurine for 7 weeks was demonstrated to decrease serum triglycerides and body weight (5).

Energy drinks often contain a large quantity of B-group vitamins, frequently in doses many times the recommended daily intake for healthy individuals. Such micronutrients serve as essential coenzymes for cellular energy transformation (6), and as such may directly influence EE, with one study having shown lower fat mass and a tendency toward higher REE in men who regularly consumed multivitamins (7).

Glucuronolactone, a glucose metabolite also commonly contained within energy drinks, is suggested to delay the depletion of glycogen

Laboratory of Integrative Cardiovascular and Metabolic Physiology, Division of Physiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland. Correspondence: Jennifer L Miles-Chan (jenniferlynn.miles@unifr.ch)

Disclosure: The authors have no competing interests.
Author contributions: JLM-C, EKG, JPM, and AGD designed research; JLM-C and NC conducted research; JLM-C analyzed the data and wrote the initial draft of the manuscript. All authors contributed toward, read, and approved the final manuscript.
Additional Supporting Information may be found in the online version of this article.

stores (8), although no studies have investigated its effect on REE and substrate utilization.

Finally, the addition of artificial sweeteners to "diet" energy drinks (in the case of RB, by aspartame and acesulfame K) may also affect the metabolism, and the sensorial effect of the beverages must be considered—with early studies showing an increase in postprandial EE following a palatable meal as compared to an isocaloric unpalatable meal (9).

As the majority of investigations regarding the effect of energy drinks of metabolism and substrate utilization have consisted of a drink and placebo comparison, the relative role of caffeine versus the non-caffeine ingredients in such beverages is, as yet, unknown. Therefore, in the present study we tested the hypothesis that, due to the cocktail of bioactive ingredients contained within, the EE response would be greater following sugar-free RB (sfRB) consumption to that of a comparable amount of caffeine alone.

Methods

Subjects

Eight young, healthy men of European descent participated in the present study, with a mean (±SEM) age of 25.4 ± 1.3 years, weight of 75.6 ± 3.9 kg, and body mass index (BMI; in kg/m^2) of 24.4 ± 1.0. All subjects were weight-stable. Smokers, claustrophobic individuals, individuals taking medication, those with any metabolic disease, and caffeine naïve individuals were excluded. Daily caffeine intake (estimated by questionnaire) ranged between 100 and 350 mg/day (mean = 210 ± 30 mg/day). Each subject completed three separate experimental test days, according to a randomized crossover design, with at least a two day interval between any two test days. The study complied with the Declaration of Helsinki and was approved by the state ethical review board; all participants gave written consent.

Experimental design

On the day of testing, participants arrived at the laboratory at 8h00 following a 12 h overnight fast. EE and respiratory quotient (RQ) were measured using a ventilated hood system (Cosmed Quark RMR, Cosmed srl, Rome, Italy). A detailed description of this procedure is given in the Supporting Information. Briefly, participants were seated comfortably in a car seat adapted for calorimetric monitoring (10,11), with metabolic measurement conducted until stabilization of EE for at least 15 min, after half an hour of rest. The ventilated hood was then removed for 4 min while the subject ingested one of the three test substances:

1. 355 ml of distilled water + placebo capsule (W+P);
2. 355 ml of a degassed sfRB + placebo capsule;
3. 355 ml of distilled water + capsule containing 120 mg of caffeine (W+caff).

It should be noted that although the quantity of caffeine contained within the capsules (120 mg) was slightly higher than that stated by RB as being contained within its beverage (114 mg per 355 ml), it is the average value of caffeine content reported through independent analyses performed in recent years, which range from 115 mg to 124 mg per 355 ml serving (12-17). For more details on the composition of sfRB please refer to the Supporting Information.

The ventilated hood was replaced, and calorimetric monitoring continued for a further 120 min. Participants were permitted to watch a calm movie or a documentary throughout the metabolic measurements. All participants were blinded as to the order in which they would receive the test substances. Determination of baseline EE, and carbohydrate (CHO Ox) and fat (Fat Ox) oxidation rates is described in detail in the Supporting Information.

Data and statistical analysis

Values of the metabolic recordings were averaged in 10 min epochs for both the baseline and 2 h post-drink period. All data are presented as mean ± standard error of the mean (SEM). The statistical treatment of data, by repeated-measures ANOVA followed by Dunnett's multiple comparison tests, was performed using the computer software Prism (Version 5.02, GraphPad Software Inc, San Diego, CA).

Results

Baseline EE and RQ values are presented in the Supporting Information, and did not differ significantly between treatments. Similarly W+P did not have any significant effect versus baseline on any of the variables measured in this study.

The effect of each drink/capsule combination on resting EE is shown in Figure 1A. sfRB and W+caff ($P < 0.001$) both increased EE across the experimental time-period, in comparison to W+P. There was no significant difference between the effect of sfRB and that of W+caff. When expressed as percentage change in EE from baseline, the results were as follows: W+P −0.5%; sfRB 4.4%; W+caff 4.0%.

The thermic effect of sfRB (28.2 ± 9.4 kJ) amounted to 58% of calories consumed (48 kJ per 355 ml), equaling a mean energy surplus of 19.8 kJ over the 2 h of post-ingestion measurement. In contrast, W+caff contained no calories and thus created an energy deficit of 22.9 + 11.6 kJ.

The effect of each drink/capsule combination on RQ is shown in Figure 1B. During the first 30 min post-ingestion, sfRB increased RQ relative to W+P ($P < 0.01$), and returned to baseline values within 50 min of ingestion. During this time-period, RQ was also found to be significantly higher with sfRB than with W+caff ($P < 0.05$). W+caff had no effect on RQ relative to W+P.

Rates of CHO Ox and Fat Ox are shown in Figure 1C and D, respectively. Rates of CHO Ox mirrored delta RQ, with sfRB increasing CHO Ox rates during the first 50 min compared to W+P ($P < 0.01$) or W+caff ($P < 0.05$). In contrast, sfRB elicited a decrease in Fat Ox during the 50 min of the post-ingestion period ($P < 0.05$).

Additionally, in this study group (regular mild-moderate caffeine consumers), there was no significant correlation between estimated habitual caffeine consumption (by questionnaire) and EE or RQ response to any of the drink/capsule combinations (see Supporting Information).

Discussion

Despite their increasing popularity and consumption, to-date little research has investigated the effect of energy drinks on REE and

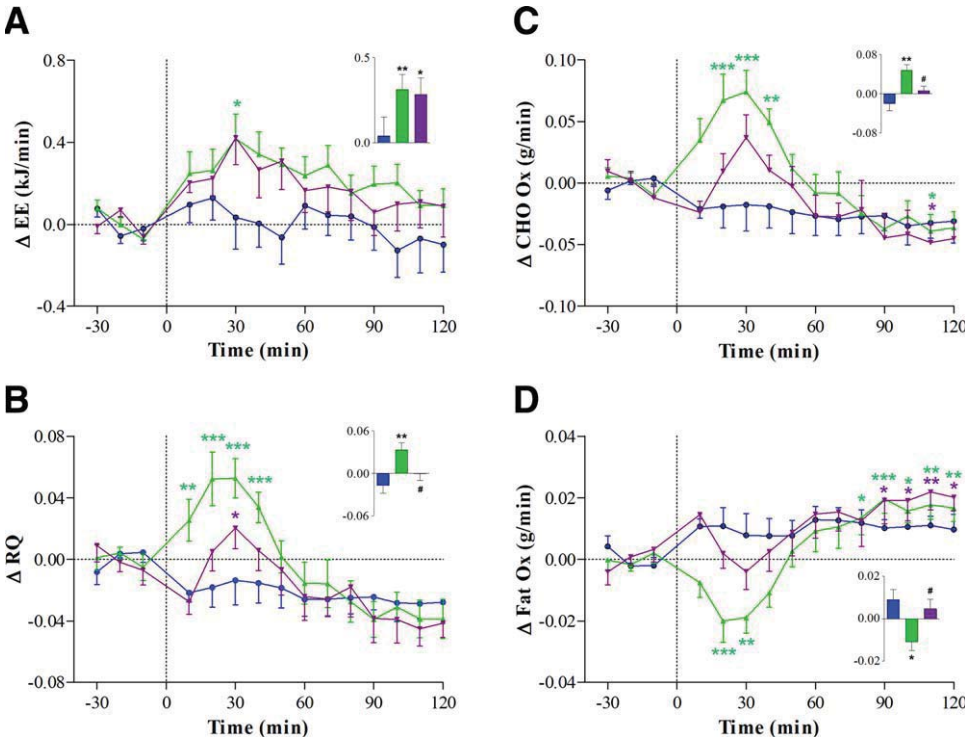

Figure 1 Changes in resting energy expenditure (ΔEE; kJ/min; Panel A), respiratory quotient (ΔRQ; Panel B), carbohydrate oxidation (ΔCHO Ox; g/min; Panel C), and fat oxidation (ΔFat Ox; g/min; Panel D) before and after ingestion of water + placebo capsule (blue), sugar-free RB + placebo (green), and water + 120 mg caffeine capsule (purple). Mean value was significantly different to baseline: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0001$.
Insets: average change during first 50 min relative to baseline. Values are means, with their standard errors represented by vertical bars. Mean value was significantly different from that of water + placebo: $^{*}P < 0.05$, $^{**}P < 0.01$. Mean value was significantly different from that of sugar-free RB $^{\#}P < 0.05$.

substrate utilization, and in particular the contribution of the non-caffeine bioactive ingredients. In the present study, we observed a 4% increase in resting EE from baseline following ingestion of sfRB, identical to that observed following a comparable amount (120 mg) of caffeine.

Caffeine alone is known to induce a thermogenic effect, with a single dose (100 mg), shown to increase resting EE in the order of 3–4% over 150 min (3), comparable to the 4% increase we observed here following both sfRB and W+caff ingestion. Most importantly, the effect of sfRB on EE was identical to that of W+caff, indicating that the non-caffeine, ingredients of sfRB (for example, taurine, glucuronolactone, and vitamins) have little or no effect at the quantities tested.

In addition to changes in EE, the present study also investigated changes in RQ (an index of substrate utilization). Interestingly, despite containing no sugar, sfRB acutely increased RQ for 30 min post-ingestion. This beverage contains both aspartame and acesulfame-K and hence, whilst there have been no investigations published to-date regarding the effect of artificial sweeteners on RQ, this acute shift may have been due at least in part to a combination of: (i) the direct stimulation of insulin secretion from the pancreatic islets by acesulfame-K (18); (ii) the metabolism of aspartame to the amino acids phenylalanine and aspartic acid; and/or (iii) sensorial effects due to the sweet taste of the beverage.

The effect of the other non-caffeine components of sfRB on RQ is also largely unknown (19), and may have contributed to the increase observed. However, one limitation of the present study was the mismatch in the taste between the sfRB and the other two beverages, meaning that the participants were not completely blinded to the treatment. Hence, the transient rise in carbohydrate utilization following sfRB ingestion may simply have represented a sensorial and/or psychological effect (through increased sympathoadrenal system activity) of the participant knowing/suspecting they were consuming caffeine. Decreases in RQ can be observed in both caffeine-containing conditions between 30 and 90 min post-ingestion, which correspond to the time to peak plasma concentration of caffeine (4) and may therefore represent a caffeine-induced increase in fat oxidation at a low dose.

In conclusion, sfRB enhanced thermogenesis, and marginally shifted RQ to favor carbohydrate oxidation. The stimulatory effects of sfRB on resting EE mimicked those of water+caffeine, indicating that the auxiliary ingredients (taurine, glucuronolactone, and B-group vitamins) are unlikely to possess thermogenic properties and hence have no role as aids for weight management. The metabolic effects of sfRB are primarily due to caffeine alone.

References

1. Anton SD. Can non-nutritive sweeteners enhance outcomes of weight loss interventions? *Obesity (Silver Spring)* 2014;22:1413-1414.
2. Dalbo VJ, Roberts MD, Stout JR, Kerksick CM. Effect of gender on the metabolic impact of a commercially available thermogenic drink. *J Strength Cond Res* 2010; 24:1633-1642.
3. Dulloo AG, Geissler CA, Horton T, Collins A, Miller DS. Normal caffeine consumption: influence on thermogenesis and daily energy expenditure in lean and postobese human volunteers. *Am J Clin Nutr* 1989;49:44-50.
4. Jeukendrup AE, Randell R. Fat burners: nutrition supplements that increase fat metabolism. *Obes Rev* 2011;12:841-851.
5. Zhang M, Bi LF, Fang JH, et al. Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects. *Amino Acids* 2004;26:267-271.
6. Groff JL, Gropper SAS. *Advanced Nutrition and Human Metabolism*, 3rd ed. Belmont, CA: West/Wadsworth; 2000.
7. Major GC, Doucet E, Jacqmain M, St-Onge M, Bouchard C, Tremblay A. Multivitamin and dietary supplements, body weight and appetite: results from a cross-sectional and a randomised double-blind placebo-controlled study. *Br J Nutr* 2008;99:1157-1167.
8. Tamura S, Tsutsumi S, Ito H, Nakai K, Masuda M. Effects of glucuronolactone and the other carbohydrates on the biochemical changes produced in the living body of rats by hard exercise. *Jpn J Pharmacol* 1968;18:30-38.
9. LeBlanc J, Brondel L. Role of palatability on meal-induced thermogenesis in human subjects. *Am J Physiol* 1985;248:E333-E336.
10. Miles-Chan JL, Sarafian D, Montani JP, Schutz Y, Dulloo AG. Sitting comfortably versus lying down: is there really a difference in energy expenditure? *Clin Nutr* 2014;33:175-178.
11. Miles-Chan JL, Sarafian D, Montani JP, Schutz Y, Dulloo A. Heterogeneity in the energy cost of posture maintenance during standing relative to sitting: phenotyping according to magnitude and time-course. *PLoS One* 2013;8:e65827.
12. Ali MM, Eisa M, Taha MI, Zakaria BA, Elbashir AA. Determination of caffeine in some Sudanese beverages by High Performance Liquid Chromatography. *Pakistan J Nutr* 2012;11:336-342.
13. Jenway Analysis. The Quantitative Determination of Caffeine in Beverages and Soft Drinks Using UV Wavelength Spectroscopy, 73 Series Spectrophotometer. Bibby Scientific Ltd, Staffordshire, UK; 2012. Application note A09-010A.
14. Hassan MI, Al-Abbad NA. Glutamate and caffeine intake of some snacks and drinks in Saudi Arabia. *Food Nutr Sci* 2011;2:162-167.
15. Nour V, Trandafir I, Ionică ME. Chromatographic determination of caffeine contents in soft and energy drinks available on the romanian market. *Sci Study Res Chem Chem Eng Biotechnol Food Ind* 2010;11:351-358.
16. Sereshti H, Samadi S. A rapid and simple determination of caffeine in teas, coffees and eight beverages. *Food Chem* 2014;158:8-13.
17. Vochyanova B, Opekar F, Tuma P. Simultaneous and rapid determination of caffeine and taurine in energy drinks by MEKC in a short capillary with dual contactless conductivity/photometry detection. *Electrophoresis* 2014;35:1660–1665.
18. Liang Y, Maier V, Steinbach G, Lalic L, Pfeiffer EF. The effect of artificial sweetener on insulin secretion. II. Stimulation of insulin release from isolated rat islets by Acesulfame K (in vitro experiments). *Horm Metab Res* 1987; 19:285-289.
19. McLellan TM, Lieberman HR. Do energy drinks contain active components other than caffeine? *Nutr Rev* 2012;70:730-744.