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which should be cited to refer to this work.

SUPPORTING INFORMATION

Methods

Measurement of EE and RQ

Prior to testing, participants visited the laboratory in order to complete a questionnaire regarding their lifestyle and medical history, and to familiarize themselves with the experimental procedure and equipment. All participants were requested to avoid strenuous physical activity, caffeine, and dietary supplements in the 24h prior to testing. Furthermore, in order to minimize the effect of physical activity on the morning of each test day, participants were requested to use motorized transport instead of walking or cycling to reach the laboratory. On the day of testing, participants arrived at the laboratory at 8h00 following a 12h overnight fast. After the participant voided their bladder, body weight and height were measured using a mechanical column scale with integrated stadiometer (Seca model 709, Hamburg, Germany).

During metabolic monitoring, participants remained seated comfortably at rest and were requested to relax, breathe normally. During the entire test, subjects were allowed to watch a documentary or a calm movie in order to reduce boredom and prevent sleeping; they were instructed not to talk and to refrain from fidgeting. Whilst studies examining the effects of movie watching on resting EE are scarce, Cooper *et al* showed no significant change in resting EE or RQ between no movie watching and "pleasant", "amusing" or "exciting" movies (1).

The room temperature was maintained in a zone of comfort for human beings wearing light clothes (between 22-24°C) with controlled hygrometry, and the environment quiet. EE and respiratory quotient (RQ) were measured using a ventilated hood system (Cosmed Quark RMR, Cosmed srl, Rome, Italy). The gas collection system is based on the air dilution technique. A pump is set to draw ambient air at a constant rate through the ventilated canopy, and then exhaled air is diluted with room air and shunted to a mixing chamber for analysis.

The O_2 analyzer is a paramagnetic oxygen sensor which offers a fast response time to measure oxygen changes within 120 ms; the range of O_2 measurement is from 0 to 30% in the canopy mode, with an accuracy of 0.02%. The CO_2 analyzer is an infrared digital sensor which offers a fast response time to measure CO_2 change within 120 ms; the range of CO_2 measurement is from 0 to 10%, and accuracy of 0.02%. Prior to each test, the Quark RMR was warmed up according to the manufacturer's instructions (10 min) and the gas analysers then calibrated using a certified gas mix. The bidirectional turbine flowmeter (18 mm diameter) has an accuracy of 2% and was calibrated prior to each test with a 3 L syringe. Flow rate during each test was fixed between 30 and 37 L/min so as to maintain expired CO_2 between 0.7 and 1.0%. In order to avoid baseline drift the Quark RMR system automatically re-calibrates every 5 min. During this autocalibration, no measurements are made by the system.

Variability in EE over time was addressed in a group of subjects (n=6) in whom resting EE (after overnight fast) was monitored for 2 hours without any intervention, and data averaged in 10 min epochs. The within-subject coefficient of variability in EE across time was found to be 2.3% during the first hour of measurement and 1.9% during the second (standard deviation of about 0.1 kJ/min). The within-subject coefficient of variability in RQ across time was 1.5% and 1.8% during the first and second hours respectively.

EE was calculated according to the Weir equation (2).

$$EE = 5.68\ VO_2 + 1.59\ VCO_2 - 2.17\ N_u,$$

where EE= energy expenditure in kcal/24h

VO_2 = O_2 consumption in mL/min (STPD)

VCO_2 = CO_2 production in mL/min (STPD)

N_u = Urinary nitrogen excretion in g/24h

N_u was assumed to be constant, and a fixed value of 13g/24h was entered in the Cosmed device.

RQ was calculated as the ratio of CO_2 produced to O_2 consumed (i.e., RQ = VCO_2/VO_2).

During monitoring, participants were seated comfortably in a car seat adapted for canopy calorimetry, as previously described (3, 4), with metabolic measurement conducted until stabilization of EE for at least 15 min, after half an hour of rest. Stabilization was defined as no more than 2% variability of EE, with no consistent upward or downward trend.

Following the test, carbohydrate (CHO Ox) and fat oxidation (Fat Ox) were calculated according to the following equations:

$$\text{CHO Ox (g/min)} = 4.59\ VCO_2\text{(L/min)} - 3.25\ VO_2\text{(L/min)} - 2.87\ N_u\ \text{(g/min)}$$

$$\text{Fat Ox (g/min)} = 1.69\ VO_2\text{(L/min)} - 1.69\ VCO_2\ \text{(L/min)} - 1.72\ N_u\ \text{(g/min)}$$

For each parameter, delta values were calculated for each subject by subtracting the baseline value established on each test day from the post-ingestion measure of that day. As shown in the table below, there were no statistical differences in any of the baseline values between treatments.

Table of mean baseline values

	W+P	sfRB	W+caff	**ANOVA**
REE (kj/min)	4.80 ± 0.24	4.78 ± 0.26	4.59 ± 0.23	p=0.79
RQ	0.847 ± 0.023	0.862 ± 0.022	0.861 ± 0.022	p=0.87
CHO Ox (g/min)	0.112 ± 0.020	0.129 ± 0.017	0.127 ± 0.026	p=0.83
Fat Ox (g/min)	0.049 ± 0.012	0.042 ± 0.010	0.038 ± 0.008	p=0.73

Composition of Sugar-free Red Bull (sfRB) energy drink (according to manufacturer)

Water, acidity regulator (sodium citrate, magnesium carbonate), carbonic acid, acidifying agent: citric acid, taurine (400mg/100ml), caffeine (32mg/100ml), glucuronolactone (24mg/100ml), inositol, vitamins, flavor, color (caramel, riboflavin), sweeteners (aspartame, acesulfame-K), thickener: xanthan.

Per 100ml: Energy 14kJ (3kcal), protein 0g, carbohydrates 0g, fat 0g, sodium 0.04g, vitamin B12 2µg, vitamin B6 2mg, niacin 8mg, pantothenic acid 2mg

The sfRB was degassed by pouring into an open container and leaving at room temperature for at least 12h prior to ingestion.

Results

Spearman correlation statistics for estimated habitual caffeine consumption versus delta EE

	estimated habitual caffeine intake *versus* delta EE	
	at 30 min post-ingestion	mean post-ingestion delta
W+P	r = 0.43, p = 0.30	r = 0.72, p = 0.88
sfRB	r = -0.14, p = 0.75	r = -0.21, p = 0.62
W+caff	r = -0.55, p = 0.17	r = -0.19, p = 0.66

Spearman correlation statistics for estimated habitual caffeine consumption versus delta RQ

	estimated habitual caffeine intake *versus* delta RQ	
	at 30 min post-ingestion	mean post-ingestion delta
W+P	r = 0.48, p = 0.24	r = 0.50, p = 0.22
sfRB	r = 0.23, p = 0.58	r = 0.13, p = 0.75
W+caff	r = 0.52, p = 0.20	r = 0.36, p = 0.39

Supporting References

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