British Journal of Nutrition (2013), **110**, 282–288
doi:10.1017/S0007114512005089

Effects of green tea extracts on non-shivering thermogenesis during mild cold exposure in young men

Chantal Gosselin[1,2] and François Haman[1,2]*

[1]*Unité de Recherche sur la Nutrition et le Métabolisme, Hôpital Montfort, 713, Chemin Montréal, Ottawa, ON, Canada K1K 0T2*
[2]*University of Ottawa, Ottawa, ON, Canada K1N 6N5*

(Submitted 4 May 2012 – Final revision received 16 October 2012 – Accepted 16 October 2012 – First published online 14 December 2012)

Abstract

The effects of epigallocatechin-3-gallate (EGCG) and caffeine on non-shivering thermogenesis (NST) during cold exposure is unknown. The purpose of the present study was to quantify the effects of co-ingesting EGCG and caffeine on the thermogenic responses of a 3 h cold exposure. A total of eight healthy males were exposed to mild cold, using a liquid-conditioned suit perfused with 15°C water, on two occasions and consumed a placebo or an extract of 1600 mg of EGCG and 600 mg of caffeine (Green tea). Thermic, metabolic and electromyographic measurements were monitored at baseline and during the cold exposure. Results showed that the AUC of shivering intensity over the cold exposure period was reduced by approximately 20 % in the Green tea (266 (SEM 6) % maximal voluntary contraction (MVC) × min) compared with the Placebo (332 (SEM 69) %MVC × min) (P=0·01) treatments. In contrast, the total AUC for energy expenditure (EE) was approximately 10 % higher in the Green tea (23·5 (SEM 1·4) kJ/kg × 180 min) compared with the Placebo (327 (SEM 74) kJ/kg 180 min) (P=0·007) treatments. The decrease in shivering activity combined with an increase in EE, following the ingestion of EGCG and caffeine during the cold exposure, indicates that NST pathways can be significantly stimulated in adult human subjects. The present study provides an experimental approach for human investigations into the potential role of diet and bioactive food ingredients in modulating NST during cold exposure. Stimulating NST pathways in such a manner may also provide important targets in the search of targets for the management of obesity and diabetes.

Key words: Epigallocatechin-3-gallate: Caffeine: Non-shivering thermogenesis

It is important to develop effective strategies to reduce energy intake and increase energy expenditure (EE) to fight the growing obesity epidemic. An intriguing strategy to increase EE is facultative thermogenesis induced by cold exposure[1]. Cold-induced shivering (ST) and non-shivering thermogenic (NST) processes produce heat as a result of sympathetic nervous system induction and the release of its neurotransmitter, noradrenalin (NA)[2]. In 2009, evidence showing the presence of brown adipose tissue (BAT) in adult human subjects renewed interest in the role of this tissue to contribute to total NST[3–5]. In this context, we showed for the first time that BAT is not only metabolically active, but can also have whole-body effects through modulating shivering activity[6]. Consequently, the question begs: could ingested agents increase the activation of NST in order to increase overall EE and/or reduce the contribution of ST?

In an attempt to stimulate thermogenesis, several studies have been conducted using green tea and its components[7–9]. One such study[8] showed that green tea extract (90 mg of epigallocatechin-3-gallate (EGCG) and 50 mg of caffeine) increased EE at rest over a 24 h period through sympathetic activation. The thermogenic properties of green tea reside primarily in the interaction between its high content in catechin polyphenols and caffeine and their effect on sympathetically released NA. When co-ingested in basal conditions, EGCG and caffeine synergistically stimulate thermogenesis by 4–8 %[8,9]. In contrast, during cold exposure, where BAT activity would be greatly increased, only one study has reported the effects of caffeine on thermogenic responses. MacNaughton *et al.*[10] reported a 30 % increase in EE when cold-exposed men (2 h in air at 5°C) ingested a gel capsule containing 385 mg (5 mg/kg) of caffeine. While this effect

Abbreviations: BAT, brown adipose tissue; CHO, carbohydrate; EGCG, epigallocatechin-3-gallate; EMG, electromyography; MVC, maximal voluntary contraction; NA, noradrenalin; NST, non-shivering thermogenesis; RMS, root-mean-square; ST, shivering thermogenesis; T_{rec}, rectal temperature.

* **Corresponding author:** F. Haman, fax +1 613 562 5149, email fhaman@uottawa.ca

alone is significant, the synergistical effect of a combined EGCG/caffeine ingestion on thermogenic processes have never been quantified in cold conditions.

Therefore, the purpose of the present study was to quantify the combined effects of EGCG and caffeine on processes of thermogenesis (NST and ST) during mild cold exposure. Using a combination of indirect calorimetry and electromyography (EMG), changes in total EE and muscle shivering intensity were quantified in non-acclimatised young men ingesting 1600 mg of EGCG and 600 mg of caffeine. It was hypothesised that, when given at the onset of cold exposure, these compounds would increase NST, thus reducing overall shivering intensity in large torso muscles known to contribute significantly to ST during cold exposure[6,11,12]. The stimulation of NST will be assumed if, when compared with a placebo: (1) EE increases when given EGCG + caffeine, despite a similar shivering intensity or (2) shivering intensity decreases when given EGCG + caffeine for a similar EE.

Materials and methods

Subjects

A total of eight healthy, non-cold acclimatised men volunteered for the present study conducted according to the guidelines laid down in the Declaration of Helsinki All procedures involving human subjects/patients were approved by the Faculty of Health Sciences ethics committee at the University of Ottawa. Written informed consent was obtained from all subjects. Exclusion criteria included the following: percentage body fat >15 %, smokers, consumption of >200 mg/d of caffeine and use of dietary supplements or stimulants. Anthropometric measurements (height, weight and percentage body fat) and peak oxygen consumption (VO_{2max}) were estimated 5–7 d before the first experimental session (Table 1).

Experimental protocol

Each subject participated in two experimental trials, separated by at least 7 d. The order of the trials was randomly assigned and followed by a single-blind, balanced, cross-over design. Participants ingested a gel capsule of green tea extracts consisting of 1600 mg of EGCG and 600 mg of caffeine (Green tea) or a placebo containing all-purpose white and bran flour (Placebo). Each trial consisted of a 60 min baseline period at ambient temperature (22·7 ± 0·4°C), followed by 180 min of cold exposure. Experiments were conducted from 07.30 to 14.30 hours. Participants refrained from consuming caffeine, alcohol and avoided physical activity 24 h prior to the trials. The last evening meal, ingested between 18.00 and 20.00 hours, was standardised (3220 kJ or 770 kcal, 42 % carbohydrates (CHO), 28 % fat and 30 % protein) and subjects were asked to report to the laboratory at 07.30 hours the next morning after a 12–14 h fast.

Table 1. Characteristics of male participants

(Mean values with their standard errors, *n* 8)

Characteristics	Mean	SEM
Age (years)	23	1
Mass (kg)	75	4
Height (cm)	177	4
Body fat (%)*	13·4	0·9
VO_{2max} (ml/kg per min)†	56·9	1·8

* Underwater weighing; Brosek *et al.* (1945).
† Canadian Society for Exercise Physiology incremental treadmill exercise test.

Upon their arrival to the laboratory, subjects were asked to empty their bladder and then, wearing only shorts, were fitted with a liquid-conditioned suit (three-piece high density; Allen-Vanguard, Inc.). They were instrumented with thermal probes, a heart rate monitor and EMG electrodes. Maximal voluntary contraction (MVC) measurements were carried out for each recorded muscle. Volunteers then remained seated for 60 min at ambient conditions (22·7 ± 0·4°C) under a canopy. Following the baseline period, subjects were asked to empty their bladder for a second time and ingested either the green tea extracts or placebo containing capsule. The liquid-conditioned suit was then perfused with 15°C water using a temperature-controlled circulation bath (Thermoscientific Neslab Refrigirated RTE-7 Bath Circulator). Previous work has shown that perfusion of water from approximately 4–18°C only results in a grade decrease in average skin temperature without any modification of core temperature, indicating that thermal balance is achieved; increases in heat loss are compensated by increases in metabolic heat production[11,13–19]. Thermal response, metabolic rate and muscle activity were measured continuously during baseline and during the 180 min cold exposure.

Thermal response

Rectal (T_{rec}) and mean skin temperature (T_{skin}) were monitored continuously prior to and during cold exposure using peadiatric rectal (Mon-a-therm general purpose; Mallinckrodt Medical, Inc.) and heat flux transducers (area-weighed equation from twelve sites: forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings and hand[20]). Heart rate and thermal comfort were measured every 15 min throughout the experiments. Heart rate was measured using a Polar heart rate monitor (Polar FS2C Fitness Heart Rate Monitor System) and thermal comfort was monitored using a thermal comfort scale, ranging from 5 (being the hottest) to −5 (being the coldest).

Metabolic measurements

Changes in whole-body EE and fuel selection were quantified by indirect calorimetry (Flow Generator/Controller, FoxBox Field Gas Analysis System, RH-300 Water Vapor Analyser; Sable systems). Total protein ($PROT_{ox}$), CHO (CHO_{ox}) and lipid ($Lipids_{ox}$) oxidation rates (in g/min) were derived from oxygen consumption rate ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) measurements corrected for the contribution of proteins, as determined from urinary urea excretion ($UREA_{urine}$). Calculations were made using the following equations[14–16,18,21,22]:

$$\mathrm{PROT_{ox}\,(g/min)} = 2{\cdot}9 \times \mathrm{UREA_{urine}\,(g/min)}, \quad (1)$$

$$\mathrm{CHO_{ox}\,(g/min)} = 4{\cdot}59 \times \dot{V}\mathrm{CO_2\,(litres/min)} - 3.23 \times \dot{V}\mathrm{O_2(litres/min)}, \quad (2)$$

$$\mathrm{Lipids_{ox}\,(g/min)} = -1{\cdot}70 \times \dot{V}\mathrm{CO_2(litres/min)} + 1.70 \times \dot{V}\mathrm{O_2(litres/min)}, \quad (3)$$

where $\dot{V}CO_2$ (litres/min) and $\dot{V}O_2$ (litres/min) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1·010 and 0·843 l/g, respectively). $UREA_{urine}$ was measured in urine collected over the entire 4 h of the experiment using a urea assay kit (BioAssay Systems). Energy potentials of 16·3 kJ/g for CHO, 40·8 kJ/g for lipids and 19·7 kJ/g for proteins were used to calculate total EE and the relative contributions of each fuel to total EE[23,24]. During mild cold exposure, EE relies almost entirely on aerobic metabolism for ATP production, as the relative change in metabolic rate does not exceed twice the RMR (see Haman[25] for a review). AUC was used to represent total EE and fuel selection using middle Riemann sum over 30 min intervals.

Determination of shivering intensity

Shivering intensity was measured using surface EMG (Myomonitor, Delsys, Inc.). EMG sites were located on the right side of the upper body on the following muscles: *trapezius*, *pectoralis major* and *rectus abdominis*. These muscles have been shown to contribute significantly to total shivering activity during mild cold exposure[11,12]. Raw EMG signals were analysed with the use of custom-designed MATLAB algorithms (Mathworks). EMG signals were filtered to remove spectral components below 20 Hz and above 500 Hz, as well as 60 Hz contamination (and associated harmonics). Shivering intensity of individual muscles and their mean (EMG_{shiv}) was determined from root mean square values (RMS) rectified from EMG signals using a 50 ms overlapping window (50 %). Baseline RMS values ($RMS_{baseline}$: 5 min RMS average measured before cold exposure) were subtracted from RMS shivering (RMS_{shiv}) as well as RMS_{mvc} values. EMG_{shiv} was then normalised to RMS_{mvc} by using the following equation:

$$\frac{\mathrm{EMG_{shiv}\,(\%MVC)} = \mathrm{RMS_{shiv}} - \mathrm{RMS_{baseline}}}{\mathrm{RMS_{mvc}} - \mathrm{RMS_{baseline}}} \times 100. \quad (4)$$

AUC was used to represent total shivering intensity and was calculated for all muscles separately. The sum of their mean was used to represent AUC for total shivering intensity.

Statistical analysis

Changes in T_{rec}, T_{skin}, EE and shivering intensity were assessed by a two-way ANOVA for repeated measures to study the effects of time and treatment, as well as their interaction (SPSS for Mac version 18.0; SPSS, Inc.). Significant differences in time were followed up by a Bonferoni *post hoc* test. Differences in AUC between the two treatments for EE, shivering intensity and fuel selection were determined using a paired *t* test. The statistical power of our tests was calculated for key parameters (EE, fuel selection and shivering EMG signals), and it ranged from 0·9 to 1·0. Statistical differences were considered significant when $P < 0{\cdot}05$. All values given are means with their standard errors.

Results

Thermal responses

Changes in thermal responses (T_{rec} and T_{skin}) at baseline and during cold exposure are presented in Fig. 1(a). T_{rec} remained constant at 37·1 ± 0·1°C during the first 120 min for both conditions. No significant difference in T_{rec} was found between the two conditions. T_{skin} decreased by 19 % in both conditions during cold exposure (from 34·1 ± 0·2°C to 28·5 ± 0·5°C in Placebo and from 34·1 ± 0·2°C to 28·7 ± 0·4°C in Green tea; $P < 0{\cdot}001$). Thermal comfort during cold exposure remained constant between treatments averaging −2·1 (SEM 0·3).

Metabolic responses

Changes in EE at baseline and during cold exposure are presented in Fig. 1(b). EE increased by 1·8-fold from baseline values in the Placebo treatment (from 63·2 (SEM 1·4) to 116·2

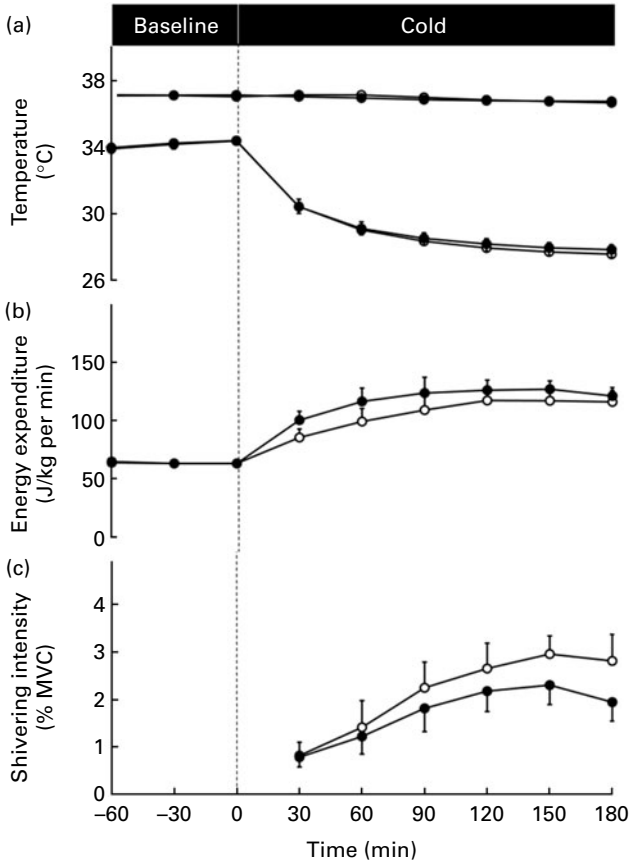

Fig. 1. Changes in (a) core temperature and mean skin temperature, (b) energy expenditure and (c) mean shivering intensity measured in men exposed to mild cold exposure following the ingestion of 1600 mg of epigallocatechin-3-gallate and 600 mg of caffeine (Green tea, –○–) or a placebo (Placebo, –●–). MVC, maximal voluntary contraction.

(SEM 8·3) J/kg per min) and by 2-fold in the Green tea treatment (from 63·5 (SEM 1·2) to 123·7 (SEM 7·0) J/kg per min) ($P = 0·001$). However, no overall difference in metabolic rate was found between the treatments during the 180 min cold exposure ($P = 0·16$). Mean heart rate remained unchanged in the cold and was not affected significantly by the treatment (64 (SEM 4) beats/min for Placebo *v.* 62 (SEM 3) beats/min for Green tea).

Differences in AUC for EE and fuel selection are presented in Fig. 2(a) and (b). AUC for EE was 10 % higher for the Green tea (23·5 (SEM 1·4) kJ/kg × 180 min) than for the Placebo treatment (21·5 (SEM 1·6) kJ/kg × 180 min) ($P=0·007$). In addition, no significant differences in absolute rate or relative contributions of CHO, lipid and protein oxidation to total EE were observed. Fig. 2(b) presents AUC for all metabolic fuels over the 180 min cold exposure period; the relative contributions of CHO (54 (SEM 5) %EE for Placebo and 45 (SEM 4) %EE for Green tea; $P=0·23$), lipids (34 (SEM 5) %EE for Placebo and 39 (SEM 5) %EE for Green tea; $P=0·39$) and proteins (12 (SEM 3) %EE for Placebo and 17 (SEM 3) %EE for Green tea; $P=0·41$) to total EE were also the same between treatments.

Shivering responses

Changes in shivering intensity (%MVC) at baseline and during cold exposure are presented in Fig. 1(c). ST intensity increased continuously during cold exposure, but no overall difference was observed between the Placebo and the Green tea treatments. However, when values were compared in the last 30 min, shivering intensity was 26 % higher in the Placebo than in the Green tea treatment (2·9 (SEM 0·5) *v.* 2·1 (SEM 0·4) %MVC, respectively; $P=0·03$).

Differences in AUC between treatments for overall shivering intensity and for the relative contribution of individual muscles to total ST are presented in Fig. 2(c) and (d), respectively. AUC for overall shivering intensity was 20 % lower for the Green tea (266 (SEM 57) %MVC × 180 min) than for the Placebo treatment (332 (SEM 69) %MVC × 180 min) ($P=0·01$). No significant differences in the AUC was found in the relative contribution of individual muscles to total shivering intensity; it was not different between treatments for *trapezius* (51 (SEM 5) % Shivering for Placebo and 47 (SEM 8) % Shivering for Green tea; $P=0·44$), *pectoralis major* (39 (SEM 6) % Shivering for Placebo and 43 (SEM 8) % Shivering for Green tea; $P=0·55$) and *rectus abdominis* (10 (SEM 2) % Shivering for Placebo and 10 (SEM 4) % Shivering for Green tea; $P=0·80$).

Discussion

The present study is the first to quantify the effects of green tea extracts on changes in shivering intensity and EE during mild cold exposure. When 1600 mg of EGCG and 600 mg of caffeine were ingested at the onset of a 3 h mild exposure to 15°C, total shivering intensity was reduced by approximately 20 %, while EE increased by approximately 10 % compared with the Placebo (Fig. 2). Together, these results confirm both predictions for determining an increase in NST. It also indicates that the contribution of both thermogenic processes to total heat production can be modulated during mild cold exposure. This modulation occurred without modifications

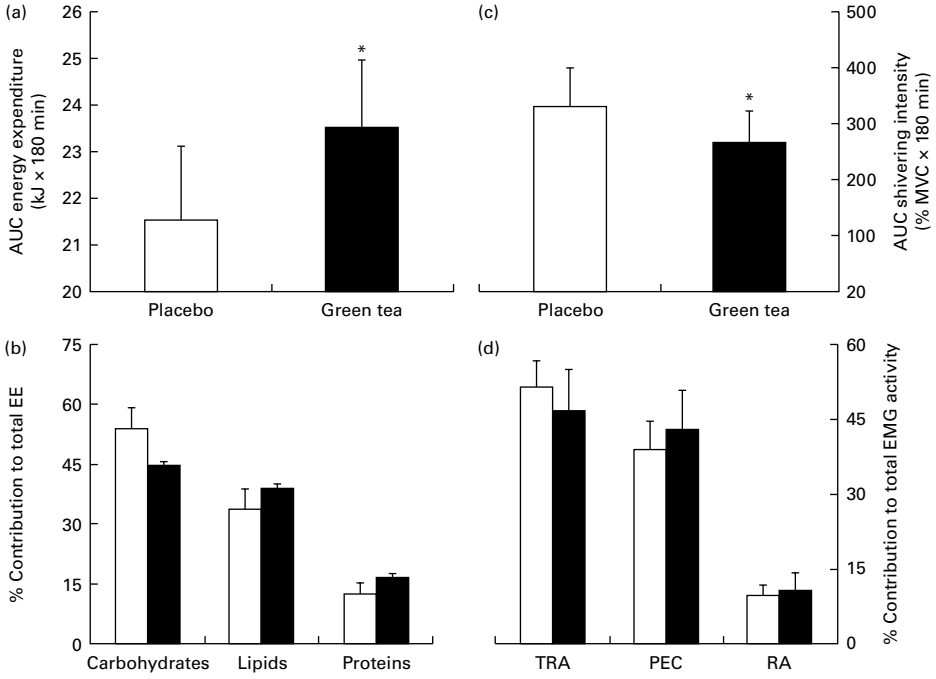

Fig. 2. AUC for (a) energy expenditure (EE), (b) relative contribution of carbohydrates, lipids and proteins to total EE, (c) average shivering intensity and (d) the contribution of respective muscles to total shivering intensity measured over 3 h of mild cold exposure in men following the ingestion of 1600 mg of epigallocatechin-3-gallate and 600 mg of caffeine (Green tea, ■) or a placebo (Placebo, □). EMG, electromyography; TRA, *trapezius*; PEC, *pectoralis major*; RA, *rectus abdominis*. Values are means, with their standard errors represented by vertical bars. *Mean value significantly different from that of placebo ($P < 0.05$).

in fuel selection and in the relative contribution of individual muscles (Fig. 2).

During cold exposure, the increase in whole-body thermogenic rate is accounted for by the combined activation of NST and ST. Because NST could not be measured directly in the present study, effects of the combined ingestion of EGCG and caffeine on activation of this process were assumed by measuring changes in EE and ST intensity. This indirect methodological approach for quantifying the contribution of NST in cold conditions was suggested almost 40 years ago by Jansky[26] and recently by Cannon & Nedergaard[27]. In the present study, the present results showed that during mild cold exposure, the ingestion of a green tea extract not only reduces shivering activity, but also increases overall thermogenic rate (Fig. 2). While this finding clearly indicates an increased contribution of NST to total heat production, this whole-body methodological approach does not allow us to discriminate on the exact pathways involved in providing this additional amount of heat.

Most NST pathways have one commonality; their activation by cold-induced sympathetic stimulation leads to a release in NA, subsequently triggering the activation of cyclic AMP-responsive pathways. Polyphenols found in teas have the potential to inhibit cathecol-*O*-methyltransferase, the enzyme that breaks down NA in the synaptic cleft[28]. Such an increase in synaptic NA creates an overflow into the circulation[8] and the action of NA on target tissues is therefore amplified. Additionally, intracellular cyclic AMP is increased with the inhibition of phosphodiesterase by caffeine[29]. As shown by Dulloo *et al.*[30], when NA was released (by adoeralin), EGCG and caffeine increased respiratory rate of *in vitro* inter-scapular BAT. The effect was even greater when EGCG and caffeine were given together, compared to caffeine alone. Indeed, the thermogenic effect of green tea goes beyond its content in caffeine *per se*[8]. Conversely, EGCG must be given with a minimal amount of caffeine in order to get the desired thermogenic effect[31]. Together, EGCG and caffeine exert a synergistic effect on tissues, such as BAT, contributing to NST.

Even though changes in BAT activity were not measured in the present study, we can still speculate that the combined ingestion of EGCG and caffeine would have stimulated cold-induced NST by increasing the contribution of BAT to total thermogenesis, as suggested by Dulloo *et al.*[30]. Because BAT is sympathetically innervated[32,33], the inhibition of cathecol-*O*-methyltransferase by EGCG would increase the action of cold-released NA on adrenergic receptors of BAT. Simultaneously, caffeine would increase intracellular cyclic AMP, therefore activating the release of fatty acids and inducing heat production from activated uncoupling protein 1. Skeletal muscle may also be a viable target for this NST stimulation through Ca^{2+} cycling. The leakage of Ca^{2+} in the cytosol (through ryanodine receptors) creates a gradient imbalance and Ca^{2+}-ATPase is activated to restore the equilibrium. The pumping of ions through Ca^{2+}-ATPase requires the hydrolysis of ATP and results in heat dissipation[34,35]. This mechanism could greatly contribute to NST, as skeletal muscle represents approximately 42% of body mass in adult human subjects[36]. Indeed, a study by Astrup *et al.*[37] showed a greater thermogenic contribution from adoeralin-stimulated skeletal muscle compared with BAT. In their study, adoeralin increased oxygen consumption by 40 ml/min in skeletal muscle compared with 10 ml/min in perirenal BAT. Just like cold exposure, adoeralin stimulates the release of NA[38]. This thermogenic response could be increased with EGCG by maintaining a higher level of NA in the circulation or by prolonging its action in the synaptic cleft.

Another heat-producing mechanism that could be stimulated by EGCG and caffeine is TAG and NEFA (TAG/NEFA) cycling. The release of NEFA is also stimulated by NA and activated through cyclic AMP. Vallerand *et al.*[39] speculated that the ingestion of a caffeine–adoeralin mixture in cold conditions (air at 10°C) had an effect on increasing TAG/NEFA cycling. Their results showed an increase in plasma glycerol and TAG in the caffeine–adoeralin condition, with practically unchanged NEFA levels and no increase in lipid oxidation compared with placebo. This goes along the lipolysis-enhancing effect of caffeine in thermoneutral condition[40]. Caffeine enhances lipolysis of TAG and the release of NEFA, but not necessarily their oxidation[41]. Later, Vallerand *et al.*[42] showed that a mild cold exposure increased TAG/NEFA cycling through the activation of the sympathetic nervous system and release of NA. Caffeine given with EGCG has the potential of increasing TAG/NEFA cycling in the cold through the release of NA, thus increasing NST. Possible sites for this to occur would be white adipose tissue[43] and the liver[36,44]. Based on the results obtained in the present study, it is possible to say that NST is potentiated during cold exposure when consuming EGCG and caffeine, but the contributing pathways can only be assumed. When examining the specific mechanisms contributing to cold-induced NST, nuclear imaging techniques as well as indirect calorimetry coupled with stable isotope techniques may provide important estimates. In future studies, it would be of importance to investigate the pathways that contribute to this increase in NST.

In conclusion, the present study shows that the contribution of NST towards total EE can be stimulated by the combined ingestion of EGCG and caffeine in cold-exposed human subjects. When ingested before a mild cold exposure, EGCG and caffeine increased total EE and decreased shivering intensity compared with control. This increase in EE accompanied by a similar decrease in EMG activity demonstrates the replacement of ST by NST without modifying whole-body substrate utilisation. Possible contributing mechanisms to NST could be UCP-1-mediated mitochondrial uncoupling in BAT, Ca^{2+} cycling in skeletal muscle and TAG/NEFA cycling in white adipose tissue and liver. The ability of EGCG to inhibit cathecol-*O*-methyltransferase and of caffeine to inhibit phosphodiesterase increases the NA- and cyclic AMP-dependent mechanisms responsible for NST. Although some inter-individual differences were observed, EGCG and caffeine seem to stimulate NST either by a notable increase in EE or a decrease in shivering intensity. The present study provides an experimental approach for human investigations into the potential role of diet and bioactive food ingredients in modulating NST during cold exposure. Stimulating NST pathways in

such a manner may not only prove to be important for improving fine motor performance and survival in cold-exposed human subjects, but may also provide important targets in the search of targets for the management of obesity and diabetes.

Acknowledgements

The authors would like to acknowledge the contribution and assistance provided by Marie-Andrée Imbeault, Denis Blondin, Jean-François Mauger and Bernard Pinet. They would also like to thank the subjects of the present study for their participation. The present work was funded by grants and scholarships from the Natural Sciences and Engineering Research Council of Canada (NSERC Canada) to F. H. and C. G. as well as from Ontario Graduate Scholarship (OGS). C. G. conducted the experiments, analysis and participated in the writing of the present paper under the supervision of F. H. The authors have no conflict of interest in relation to the present study.

References

1. Yoneshiro T, Aita S, Kawai Y, *et al.* (2012) Nonpungent capsaicin analogs (capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. *Am J Clin Nutr* **95**, 845–850.
2. Morrison SF & Nakamura K (2011) Central neural pathways for thermoregulation. *Front Biosci* **16**, 74–104.
3. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, *et al.* (2009) Cold-activated brown adipose tissue in healthy men. *New Engl J Med* **360**, 1500–1508.
4. Virtanen KA, Lidell ME, Orava J, *et al.* (2009) Functional brown adipose tissue in healthy adults. *New Engl J Med* **360**, 1518–1525.
5. Cypess AM, Lehman S, Williams G, *et al.* (2009) Identification and importance of brown adipose tissue in adult humans. *New Engl J Med* **360**, 1509–1517.
6. Ouellet V, Labbe SM, Blondin DP, *et al.* (2012) Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J Clin Invest* **122**, 545–552.
7. Berube-Parent S, Pelletier C, Dore J, *et al.* (2005) Effects of encapsulated green tea and Guarana extracts containing a mixture of epigallocatechin-3-gallate and caffeine on 24 h energy expenditure and fat oxidation in men. *Brit J Nutr* **94**, 432–436.
8. Dulloo AG, Duret C, Rohrer D, *et al.* (1999) Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutr* **70**, 1040–1045.
9. Rudelle S, Ferruzzi MG, Cristiani I, *et al.* (2007) Effect of a thermogenic beverage on 24-hour energy metabolism in humans. *Obesity (Silver Spring)* **15**, 349–355.
10. MacNaughton KW, Sathasivam P, Vallerand AL, *et al.* (1990) Influence of caffeine on metabolic responses of men at rest in 28 and 5 degrees C. *J Appl Physiol* **68**, 1889–1895.
11. Haman F, Legault SR, Rakobowchuk M, *et al.* (2004) Effects of carbohydrate availability on sustained shivering II. Relating muscle recruitment to fuel selection. *J Appl Physiol* **96**, 41–49.
12. Bell DG, Tikuisis P & Jacobs I (1992) Relative intensity of muscular contraction during shivering. *J Appl Physiol* **72**, 2336–2342.
13. Blondin DP, Depault I, Imbeault P, *et al.* (2010) Effects of two glucose ingestion rates on substrate utilization during moderate-intensity shivering. *Eur J Appl Physiol* **108**, 289–300.
14. Haman F, Peronnet F, Kenny GP, *et al.* (2004) Effects of carbohydrate availability on sustained shivering I. Oxidation of plasma glucose, muscle glycogen, and proteins. *J Appl Physiol* **96**, 32–40.
15. Haman F, Péronnet F, Kenny GP, *et al.* (2002) Effect of cold exposure on fuel utilization in humans: plasma glucose, muscle glycogen, and lipids. *J Appl Physiol* **93**, 77–84.
16. Haman F, Peronnet F, Kenny GP, *et al.* (2005) Partitioning oxidative fuels during cold exposure in humans: muscle glycogen becomes dominant as shivering intensifies. *J Physiol* **566**, 247–256.
17. Blondin DP, Maneshi A, Imbeault MA, *et al.* (2011) Effects of the menstrual cycle on muscle recruitment and oxidative fuel selection during cold exposure. *J Appl Physiol* **111**, 1014–1020.
18. Blondin DP, Peronnet F & Haman F (2010) Effects of ingesting [^{13}C]glucose early or late into cold exposure on substrate utilization. *J Appl Physiol* **109**, 654–662.
19. Blondin DP, Peronnet F & Haman F (2012) Coingesting glucose and fructose in the cold potentiates exogenous CHO oxidation. *Med Sci Sports Exer* **44**, 1706–1714.
20. Hardy JD & Dubois EF (1938) The technic of measuring radiation and convection. *J Nutr* **15**, 461–475.
21. Blondin DP, Depault I, Imbeault P, *et al.* (2010) Effects of two glucose ingestion rates on substrate utilization during moderate-intensity shivering. *Eur J Appl Physiol* **108**, 289–300.
22. Haman F, Legault SR & Weber JM (2004) Fuel selection during intense shivering in humans: EMG pattern reflects carbohydrate oxidation. *J Physiol* **556**, 305–313.
23. Elia M (1991) Energy equivalents of CO_2 and their importance in assessing energy expenditure when using tracer techniques. *Am J Physiol Endocrinol Metab* **260**, E75–E88.
24. Péronnet F & Massicotte D (1991) Table of nonprotein respiratory quotient: an update. *Can J Sport Sci* **16**, 23–29.
25. Haman F (2006) Shivering in the cold: from mechanisms of fuel selection to survival. *J Appl Physiol* **100**, 1702–1708.
26. Jansky L (1973) Non-shivering thermogenesis and its thermoregulatory significance. *Biol Rev Camb Philos Soc* **48**, 85–132.
27. Cannon B & Nedergaard J (2011) Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J Exp Biol* **214**, 242–253.
28. Borchardt RT & Huber JA (1975) Catechol *O*-methyltransferase. 5. Structure–activity relationships for inhibition by flavonoids. *J Med Chem* **18**, 120–122.
29. Dulloo AG, Seydoux J & Girardier L (1992) Potentiation of the thermogenic antiobesity effects of ephedrine by dietary methylxanthines: adenosine antagonism or phosphodiesterase inhibition? *Metab Clin Experimen* **41**, 1233–1241.
30. Dulloo AG, Seydoux J, Girardier L, *et al.* (2000) Green tea and thermogenesis: interactions between catechin–polyphenols, caffeine and sympathetic activity. *Int J Obes Relat Metab Disord* **24**, 252–258.
31. Gregersen NT, Bitz C, Krog-Mikkelsen I, *et al.* (2009) Effect of moderate intakes of different tea catechins and caffeine on acute measures of energy metabolism under sedentary conditions. *Brit J Nutr* **102**, 1187–1194.
32. Bartness TJ, Vaughan CH & Song CK (2010) Sympathetic and sensory innervation of brown adipose tissue. *Int J Obes (Lond)* **34**, Suppl. 1, S36–S42.

33. Bartness TJ, Kay Song C, Shi H, *et al.* (2005) Brain–adipose tissue cross talk. *Proc Nutr Soc* **64**, 53–64.
34. van Marken Lichtenbelt WD & Daanen HA (2003) Cold-induced metabolism. *Curr Opin Clin Nutr Metab Care* **6**, 469–475.
35. Block BA (1994) Thermogenesis in muscle. *Annu Rev Physiol* **56**, 535–577.
36. Rolfe DF & Brown GC (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev* **77**, 731–758.
37. Astrup A, Bulow J, Madsen J, *et al.* (1985) Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *Am J Physiol* **248**, E507–E515.
38. Dulloo AG & Miller DS (1984) Thermogenic drugs for the treatment of obesity: sympathetic stimulants in animal models. *Brit J Nutr* **52**, 179–196.
39. Vallerand AL, Jacobs I & Kavanagh MF (1989) Mechanism of enhanced cold tolerance by an ephedrine–caffeine mixture in humans. *J Appl Physiol* **67**, 438–444.
40. Hetzler RK, Knowlton RG, Somani SM, *et al.* (1990) Effect of paraxanthine on FFA mobilization after intravenous caffeine administration in humans. *J Appl Physiol* **68**, 44–47.
41. Acheson KJ, Gremaud G, Meirim I, *et al.* (2004) Metabolic effects of caffeine in humans: lipid oxidation or futile cycling? *Am J Clin Nutr* **79**, 40–46.
42. Vallerand AL, Zamecnik J, Jones PJ, *et al.* (1999) Cold stress increases lipolysis, FFA Ra and TG/FFA cycling in humans. *Aviat Space Environ Med* **70**, 42–50.
43. Garofalo MA, Kettelhut IC, Roselino JE, *et al.* (1996) Effect of acute cold exposure on norepinephrine turnover rates in rat white adipose tissue. *J Auton Nerv Syst* **60**, 206–208.
44. Kim J, Saidel GM & Kalhan SC (2008) A computational model of adipose tissue metabolism: evidence for intracellular compartmentation and differential activation of lipases. *J Theor Biol* **251**, 523–540.
45. Brozek J, Grande F & Anderson JT, *et al.* (1963) Densitometric analysis of body composition: revision of some quantitative assumptions. *Ann NY Acad Sci* **110**, 113–140.