



## Short Communication

# Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity

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The thermogenic effect of tea is generally attributed to its caffeine content. We report here that a green tea extract stimulates brown adipose tissue thermogenesis to an extent which is much greater than can be attributed to its caffeine content *per se*, and that its thermogenic properties could reside primarily in an interaction between its high content in catechin-polyphenols and caffeine with sympathetically released noradrenaline (NA). Since catechin-polyphenols are known to be capable of inhibiting catechol-O-methyl-transferase (the enzyme that degrades NA), and caffeine to inhibit transcellular phosphodiesterases (enzymes that break down NA-induced cAMP), it is proposed that the green tea extract, via its catechin-polyphenols and caffeine, is effective in stimulating thermogenesis by relieving inhibition at different control points along the NA–cAMP axis. Such a synergistic interaction between catechin-polyphenols and caffeine to augment and prolong sympathetic stimulation of thermogenesis could be of value in assisting the management of obesity.

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## Introduction

Current strategies to stimulate thermogenesis in assisting the management of obesity focus upon agents that would mimic the thermogenic action of catecholamines released by the sympatho-adrenal system—an important physiological regulator of thermogenesis.<sup>1</sup> While the pharmaceutical approach concentrates on the development of drugs that would target  $\beta_3$ -adrenoreceptors, believed to be the pivotal adrenoreceptors via which sympathetically released noradrenaline (NA) activates thermogenesis,<sup>2</sup> there is also considerable interest in the nutritional/nutraceutical areas for screening foods and/or dietary ingredients with potential thermogenic properties by virtue of their mode of action by interference with the sympathoadrenal system.<sup>3</sup> Indeed, interest in coffee/caffeine as a potential promoter of thermogenesis followed the realization that, in relatively small amounts, caffeine is effective in potentiating thermogenesis induced by drugs than enhance NA release from sympathetic nerve endings, e.g. ephedrine.<sup>4</sup> These findings have led to clinical trials which showed that a combination of caffeine and ephedrine was a safe anti-obesity drug

cocktail which was more effective than either ephedrine alone, caffeine alone or placebo in inducing losses in body weight and body fat in obese patients.<sup>5,6</sup>

To elucidate the mechanisms of interaction between caffeine and sympathetic control of thermogenesis, we have previously utilized an *in vitro* system of rat brown adipose tissue (BAT), a tissue which has a rich sympathetic innervation and whose respiration rate is a sensitive index of thermogenesis. Using this system, we demonstrated that the effect of low doses of caffeine on thermogenesis in this tissue is entirely dependent on intact sympathetic innervation,<sup>7</sup> and that caffeine potentiates the effect of sympathetically released NA on thermogenesis primarily by inhibiting phosphodiesterase enzyme activities, and to a much lesser extent by antagonizing adenosine receptors.<sup>8</sup> Since the physiological actions of sympathetically released NA are in part modulated by negative feedback mechanisms operating in the synaptic clefts (e.g. through adenosine, prostaglandins), as well as intracellularly through enhanced breakdown of NA-induced cAMP phosphodiesterase enzymes activities, the net effect of caffeine—by inhibiting adenosine and phosphodiesterases—is therefore to prolong the intracellular levels of cAMP, a critical intracellular mediator for the thermogenic effects of NA. The level of NA at the synaptic junction and its interaction with adrenoreceptors is also likely to be negatively modulated through its enzymatic degradation by COMT, i.e. catechol-O-methyl-transferase,<sup>9</sup> thereby providing an additional target for pharmaco-

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logical interference aimed at prolonging the effect of NA and hence increasing cAMP and thermogenesis. Interestingly, there is evidence that the enzyme COMT can be inhibited by certain plant polyphenols,<sup>10</sup> and notably by those in the class of catechins which are found in high quantities in tea prior to its fermentation, *i.e.* tea.<sup>11</sup> Consequently, green tea seems to contain pharmacologically active compounds (catechin-polyphenols and caffeine), which by virtue of their ability to relieve inhibition along the NA–cAMP axis at different control points, might confer it with quantitatively important thermogenic properties. With the use of our *in vitro* system for measuring the respiration rate of BAT, we report here investigations aimed at testing the hypothesis that a green tea extract, by virtue of its high content both in these catechin-polyphenols and caffeine, possesses thermogenic properties that are greater than can be accounted for by its caffeine content *per se*, and that is thermogenic potential resides at least in part in an interaction between catechin-polyphenols and caffeine with the sympathetically released NA.

## Materials and methods

### Animals and diets

All studies were conducted on 7–8-week-old male Sprague–Dawley rats (Tierzucht, Zurich, Switzerland), which were housed in a temperature controlled room (23°C) with a 12:12 h light–dark cycle. Before each experiment the animals were adapted for 5–7 days to room and cage conditions, and had free access to water and a standard laboratory chow diet (Provimi-Lacta, Cossonay, Switzerland) with the following macronutrient content (w/w): 20% protein, 5% fat and 60% carbohydrates. The study was approved by the institution's ethical committee for animal experimentation, and was conducted according to its guidelines and regulations.

### Chemical denervation

Chemical sympathectomy was performed using 6-hydroxydopamine (6-OHDA; Sigma, St Louis, MO) dissolved in distilled water containing 0.001 M HCL and equilibrated with nitrogen. Rats ( $n=5$ ) were injected subcutaneously with 6-OHDA (50 mg/kg body wt) twice in a day (8:00 a.m. and 5:00 p.m.) and were killed 15 h after the second injection.

### Tissue preparations

Animals were killed by decapitation between 7&30 and 8:00 a.m. and two fragments 10–12 mm long, ~1 mm thick, 10–14 mg wet weight, of interscapular brown adipose tissue (IBAT) were rapidly dissected out from the middle part of the fat pad. The tissues were perfused with Krebs–Ringer bicarbonate buffer

of the following composition (in mmol/l): 116.8 NaCl, 25 NaHCO<sub>3</sub>, 5.9 KCl, 1.2 MgSO<sub>4</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 1.25 CaCl<sub>2</sub>, and 5 glucose. The medium was gassed continuously with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> and was maintained at a set temperature of 30±0.2°C.

### Measurement of tissue respiration rate

The respiratory rates of IBAT fragments were measured by a method involving repeated O<sub>2</sub> uptake determinations, as described by Barde *et al.*<sup>12</sup> The O<sub>2</sub> partial pressure (PO<sub>2</sub>) of a bubble-free liquid phase enclosed in a thick-walled Lucite chamber was measured by a Clark O<sub>2</sub> electrode connected to a polarographic circuit, whose output voltage is directly proportional to PO<sub>2</sub>. All values for O<sub>2</sub> uptake rate (MO<sub>2</sub>) were taken during steady-state respiration and after 40–90 min when drug administration resulted in changes in MO<sub>2</sub>.

### Green tea extract and drug administration

The green tea extract, under the code name of AR25, is obtained by alcohol extraction from dry tea leaves of unfermented *Camellia sinensis*, standardized at 8.35% caffeine and 24.7% catechins (~70% as (–)-epigallocatechin gallate), and commercialized in capsular form under the name of EXOLISE (Arkopharma Laboratories, Nice, France). The green tea extract was added in the perfusion buffer medium, and the resulting suspension was automatically filtered before entering the respiratory chambers. Analysis of the filtrate from several samples of the green tea extract for caffeine and (–)-epigallocatechin gallate (EGCG, the most abundant of tea catechins) was performed by liquid chromatography with electrochemical detection, and yielded values, as percentage dry weight of extract, in the range of 5–10% for caffeine and 16–19% for (–)-epigallocatechin gallate. L-Ephedrine hydrochloride, caffeine and (–)-epigallocatechin gallate (Sigma, St Louis, MO, USA) were added to the medium by means of motor-driven syringes connected to needles set at the inlet of each chamber. It is to be noted that, at high concentrations, the green tea extract and (–)-epicallocatechin gallate interfere with oxygen in the buffer medium since they had an effect on the rate of O<sub>2</sub> consumption in the absence of the biological tissue. Consequently, the utilization of both the green tea extract and (–)-epigallocatechin gallate were limited to concentrations below which they had no impact on the pO<sub>2</sub> of the buffer medium.

### Statistics

Data are presented as mean±s.e. ( $n=5-7$ ). Statistical analysis was performed as follows:

(a) by analysis of variance with repeated measures on *absolute* MO<sub>2</sub> values (*i.e.* a two-factor Anova with sequential treatments as one factor and individual

rats as the other factor) for the effect of sequential administration of increasing concentrations of a given compound (caffeine, green tea, or EGCG), and also for the effect of sequential administration of two or more compounds; these analyses were then followed by *post-hoc* pair-wise comparisons by the Newman–Keul’s test, with the level for establishing significant differences taken at  $P < 0.05$ ; (b) by unpaired *t*-test on *absolute*  $\text{MO}_2$  values for comparisons between compounds at a given concentration of caffeine or EGCG, the level of significance denoted as  $*P < 0.05$ ,  $**P < 0.001$  and  $***P < 0.001$ ; and (c) by unpaired *t*-test on *changes* in  $\text{MO}_2$  relative to basal  $\text{MO}_2$  for comparisons between tissues from intact vs sympathectomized animals or between unstimulated vs stimulated states (with ephedrine).

## Results and discussion

Using this *in vitro* IBAT system, we have previously shown in dose–response studies<sup>7,8</sup> that the administration of either caffeine at 2–5 mM or ephedrine at 1–10  $\mu\text{M}$  resulted in marked stimulation of IBAT respiration rate (> 5-fold basal  $\text{MO}_2$  values). However, at low subthreshold concentrations of caffeine (100–250  $\mu\text{M}$ ) and ephedrine (0.05–0.1  $\mu\text{M}$ ), i.e. at concentrations at which neither drug alone stimulated IBAT  $\text{MO}_2$ , their administration in combination resulted in a synergistic effect on IBAT  $\text{MO}_2$ , with increases of 2–3-fold above basal values. The findings that this synergistic effect was prevented by pretreatment of the animals with 6-OHDA (a procedure that destroys the sympathetic nerve endings and depletes the NA stores) suggested that the interaction between caffeine and ephedrine requires intact sympathetic neural innervation.<sup>7,8</sup> Thus, at subthreshold concentrations, ephedrine enhance the release of NA (hence mimicking *in vitro* an increase in sympathetic activity), but at levels that do not overcome the effects of the negative modulators both in the synaptic cleft and at the tissue level. At such concentrations, therefore, ephedrine alone had no net effect on basal tissue respiration. Consequently, the permissive effects of caffeine in allowing a subthreshold dose of ephedrine to activate thermogenesis is explained by ephedrine’s enhancement of sympathetic neuronal release of NA, together with the ability of caffeine to inhibit phosphodiesterase activity, thereby resulting in an elevated cellular level of cAMP, leading to increased thermogenesis.

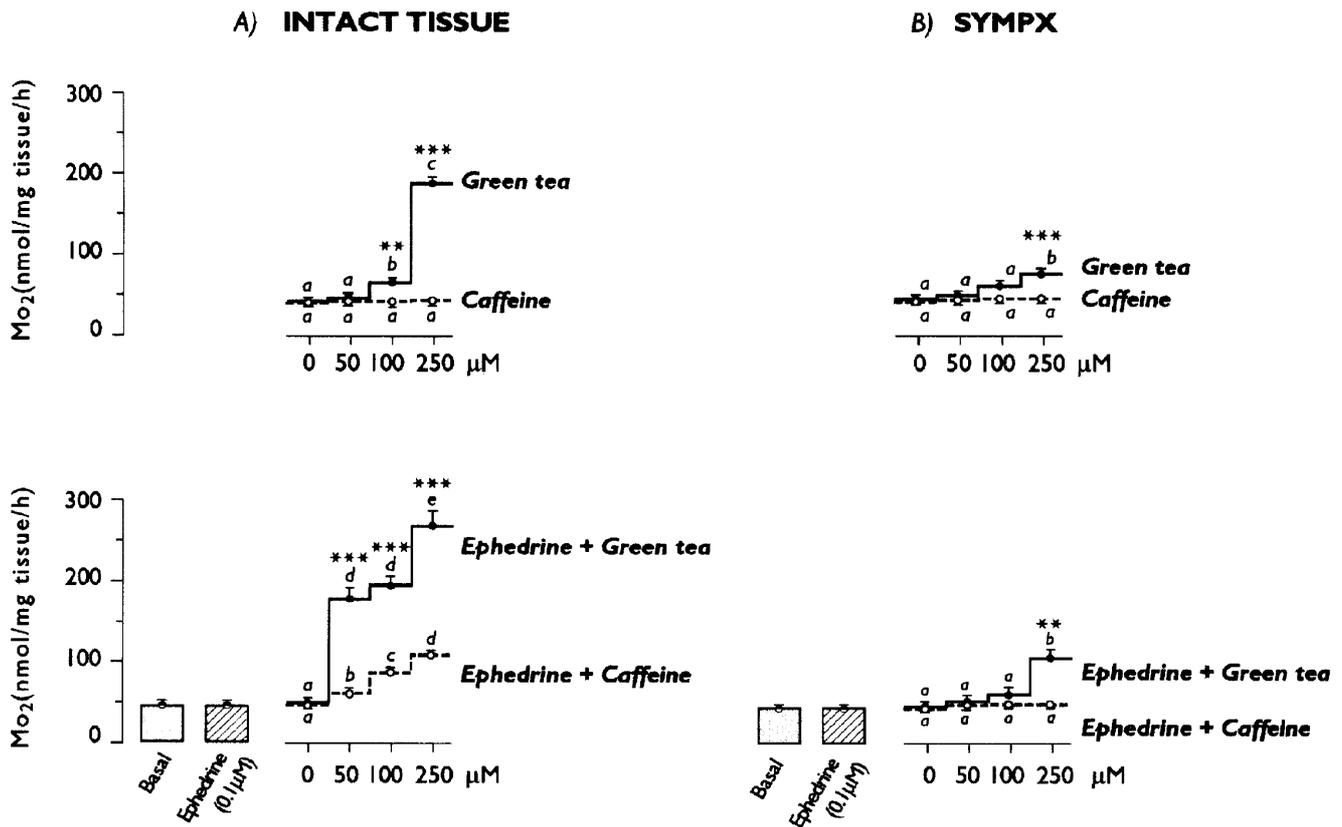
### Comparison between green tea extract and caffeine on thermogenesis

In the first study reported here, we repeated this approach to compare caffeine and green tea containing isomolar concentrations of caffeine on IBAT  $\text{MO}_2$ , under two conditions, namely: (i) in the unstimulated state, and (ii) in the stimulated state, using a

subthreshold concentration of ephedrine (0.1  $\mu\text{M}$ ) as a pharmacological tool to release NA from sympathetic nerve terminals, and hence to mimic a small increase in sympathetic activity. The results, shown in Figure 1 (panel A), indicate that caffeine in its own right does not increase basal IBAT  $\text{MO}_2$  (in line with previous findings<sup>7</sup> that caffeine has to be at a millimolar concentration to stimulate IBAT respiration rate). By contrast, the administration of green tea containing isomolar concentrations of caffeine resulted in significant increases in IBAT  $\text{MO}_2$  above basal levels in a dose-dependent fashion ( $P < 0.001$ ; Figure 1, top of panel A), namely by 28% at 50  $\mu\text{M}$ , 77% at 100  $\mu\text{M}$ , and by more than 5-fold at 250  $\mu\text{M}$ , with *post-hoc* pairwise comparison across concentrations indicating statistically significant increase with the green tea at 100 and 250  $\mu\text{M}$  of caffeine equivalents. Comparison between green tea and caffeine indicates statistically higher  $\text{MO}_2$  values with the green tea than with caffeine at 100  $\mu\text{M}$  ( $P < 0.01$ ) and at 250  $\mu\text{M}$  ( $P < 0.001$ ).

The effects of green tea and caffeine on tissue  $\text{MO}_2$  were then compared when added in combination with ephedrine (Figure 1, bottom of panel A). In line with our previously reported findings,<sup>7</sup> the addition of low doses of caffeine (50–250  $\mu\text{M}$ ) enabled an otherwise ineffective (subthreshold) dose of ephedrine (0.1  $\mu\text{M}$ ) to enhance IBAT  $\text{MO}_2$  in a dose-dependent fashion ( $P < 0.001$ ). However, this synergistic effect between ephedrine and caffeine was much more pronounced when the caffeine was substituted by green tea containing equivalent amounts of caffeine: whereas the combination of ephedrine + caffeine increased basal  $\text{MO}_2$  significantly by about 38%, 2-fold and 2.5-fold at caffeine concentrations of 50, 100 and 250  $\mu\text{M}$ , respectively ( $P < 0.001$ ), the combination of ephedrine + green tea increased basal  $\text{MO}_2$  by 4.5-fold, 4.8-fold, and 6.8-fold with green tea containing 50, 100 and 200  $\mu\text{M}$  of caffeine, respectively ( $P < 0.001$ ). The synergistic interaction between ephedrine + green tea is evident with green tea containing 50, 100 and 250  $\mu\text{M}$  of caffeine, since the combination of ephedrine + green tea resulted in increases in  $\text{MO}_2$  above basal values (136, 150, 255 nmol  $\text{O}_2/\text{mg}$  tissue/h at the respective concentrations), which were all significantly greater ( $P < 0.01$ ) than the additive effects of green tea alone (10, 28, 150 nmol  $\text{O}_2/\text{mg}$  tissue/h at the respective concentrations) and ephedrine alone (no significant increase in this experiment). Comparison between ephedrine + green tea and ephedrine + caffeine at 50, 100 and 200  $\mu\text{M}$  of caffeine indicates that the higher  $\text{MO}_2$  values with ephedrine + green tea were statistically significant ( $P < 0.001$ ) at all three concentrations.

To examine the extent to which the greater effect of green tea than caffeine (in the unstimulated state or in the stimulated state induced by ephedrine) is mediated via interference with NA released from the sympathetic nerves innervating this tissue, the experiments



**Figure 1** Respiration rates ( $MO_2$ ) of interscapular brown adipose tissue (IBAT) from intact rats (A) and from rats chemically sympathectomized (B) in response to caffeine or to green tea extract containing isomolar concentrations of caffeine. The data are presented in the absence (top parts) or presence of a low concentration ephedrine (an enhancer of NA release). Data are presented as mean  $\pm$  s.e. ( $n=5-7$ ). For 'horizontal' comparison across either caffeine concentration or for green tea (with isomolar concentration of caffeine), values not sharing a common superscript are significantly different from each other ( $P < 0.05$ ), as assessed by *post-hoc* pairwise comparison after Anova with repeated measures indicates significant differences. For 'vertical' comparisons between green tea and caffeine at a specific isomolar concentration of caffeine, the level of statistical significance of differences is indicated as asterisks: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

above were repeated in IBAT tissues from animals chemically sympathectomized by pretreatment with 6-OHDA, an agent that destroys sympathetic nerve endings and markedly reduces the endogenous NA levels by 90% or more in IBAT.<sup>13</sup> The results, shown in Figure 1 (panel B), indicate that the effect of green tea on  $MO_2$  or its synergistic interactions with ephedrine in increasing basal  $MO_2$  were considerably blunted, these being particularly evident for green tea containing 250  $\mu M$  caffeine and for ephedrine + green tea containing 50, 100 or 250  $\mu M$  of caffeine, in which cases the values of  $MO_2$  in BAT from sympathectomized animals were all markedly lower than in BAT from intact controls ( $P < 0.001$ ). It is to be noted that, at the end of each measurement with chemically sympathectomized IBAT, exogenous administration of NA (0.1  $\mu M$ ) to the same IBAT tissue resulted in a 6–8-fold increase in  $MO_2$  (results not shown). The lack of or blunted effect of ephedrine + caffeine, ephedrine + green tea or green tea alone on BAT thermogenesis in sympathectomized animals cannot therefore be attributed to postsynaptic tissue damage following 6-OHDA treatment, but is due to the depletion of NA stores following chemical sympathectomy. Indeed, previously reported studies<sup>7,14</sup> of

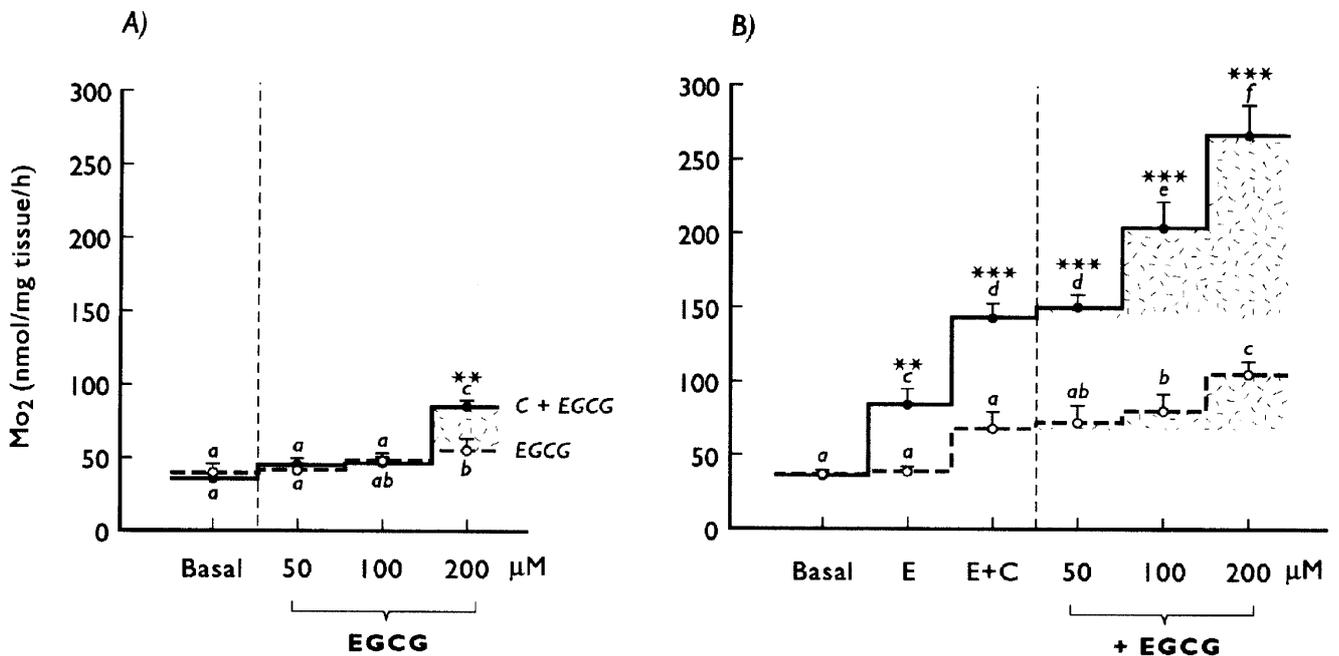
NA dose–response curve with IBAT of rats treated with similar doses of 6-OHDA and a similar injection protocol as utilized here indicated that the sensitivity and maximal thermogenic response to NA with IBAT from sympathectomized animals are not reduced but actually increased—this most probably arising from the development of denervation supersensitivity and from the fact that NA-reuptake mechanisms (and hence inactivation of NA) are also disrupted following chemical sympathectomy. These data thereby suggest that the effect of green tea in activating IBAT thermogenesis (in absence of ephedrine) requires an intact sympathetic neural innervation, and consequently its effects on thermogenesis are likely to be highly dependent upon the release of endogenous NA, rather than by direct effects on the tissue *per se*. This dependency of the stimulatory effect of the green tea extract upon sympathetic innervation even in the absence of ephedrine implies that, in the isolated BAT, there is a basal tonic release and reuptake of NA in the synaptic cleft—a contention which is supported by the fact that, in our *in vitro* BAT system, the basal respiratory rate of IBAT has previously been found to be reduced (by 20%) in response to the non-selective  $\beta$ -adrenoreceptor

blocker, propranolol (unpublished data) and to be increased (by 25%) in response to the NA-reuptake blocker, desipramine.<sup>15</sup> Taken together, these findings are consistent with the hypothesis that the ability of the green tea extract to stimulate thermogenesis cannot be explained solely by its content in caffeine *per se*. To what extent the effect of the green tea extract on BAT thermogenesis may also be attributed to an interaction between its high content both in catechin-polyphenols and caffeine with sympathetically released NA is the subject of investigations reported below.

**Interactions between epigallocatechin gallate, caffeine and ephedrine on thermogenesis**

The catechin-polyphenols in green tea exist in several isoforms, namely (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin (EC) and (–)-epicatechin gallate (ECG), with EGCG constituting > 50% of the total amount of tea catechins, and which is believed to be pharmacologically the most active tea catechin.<sup>11,16</sup> To obtain direct evidence that catechin-polyphenols contribute to the efficacy of the green tea extract in potentiating thermogenesis, we have therefore tested the effect of the EGCG on the *in vitro* respiration rate of IBAT. The results, shown in Figure 2 (panel A), indicate that

EGCG alone has no effect on IBAT MO<sub>2</sub> at doses of 50 and 100 μM, but at 200 μM it induced a small, though statistically significant, increase in MO<sub>2</sub> relative to basal values (40%, *P* < 0.05). In combination with 100 μM of caffeine (which, as shown in the study above, has no effect on IBAT MO<sub>2</sub> in its own right), EGCG also had no effect at 50 and 100 μM, but at 200 μM of EGCG the combination of EGCG + caffeine enhanced IBAT MO<sub>2</sub> to a greater extent than EGCG alone (2.4-fold versus 40% relative to basal values, respectively, *P* < 0.01). In another study (Figure 2, panel B), the combination of EGCG (50–200 μM) and caffeine (100 μM) on IBAT MO<sub>2</sub> was studied under stimulated conditions (i.e. increased NA release) induced by the administration of ephedrine either at 0.1 or 0.25 μM. The data of this study are divided into two subsets, according to whether administration of ephedrine *per se* did not increase or increase IBAT MO<sub>2</sub>. In the data subset when administration of ephedrine had no effect on basal MO<sub>2</sub> (broken lines), the addition of caffeine to ephedrine resulted in an 84% increase in MO<sub>2</sub> above the basal level (*P* = 0.06), and subsequent addition of EGCG increased IBAT MO<sub>2</sub> further, such that with EGCG at 200 μM, the combination of E + C + EGCG resulted in a significant 2.8-fold increase in MO<sub>2</sub> relative to basal values or in a 70% increase in MO<sub>2</sub> relative to that for E + C (*P* < 0.001). In the data subset when



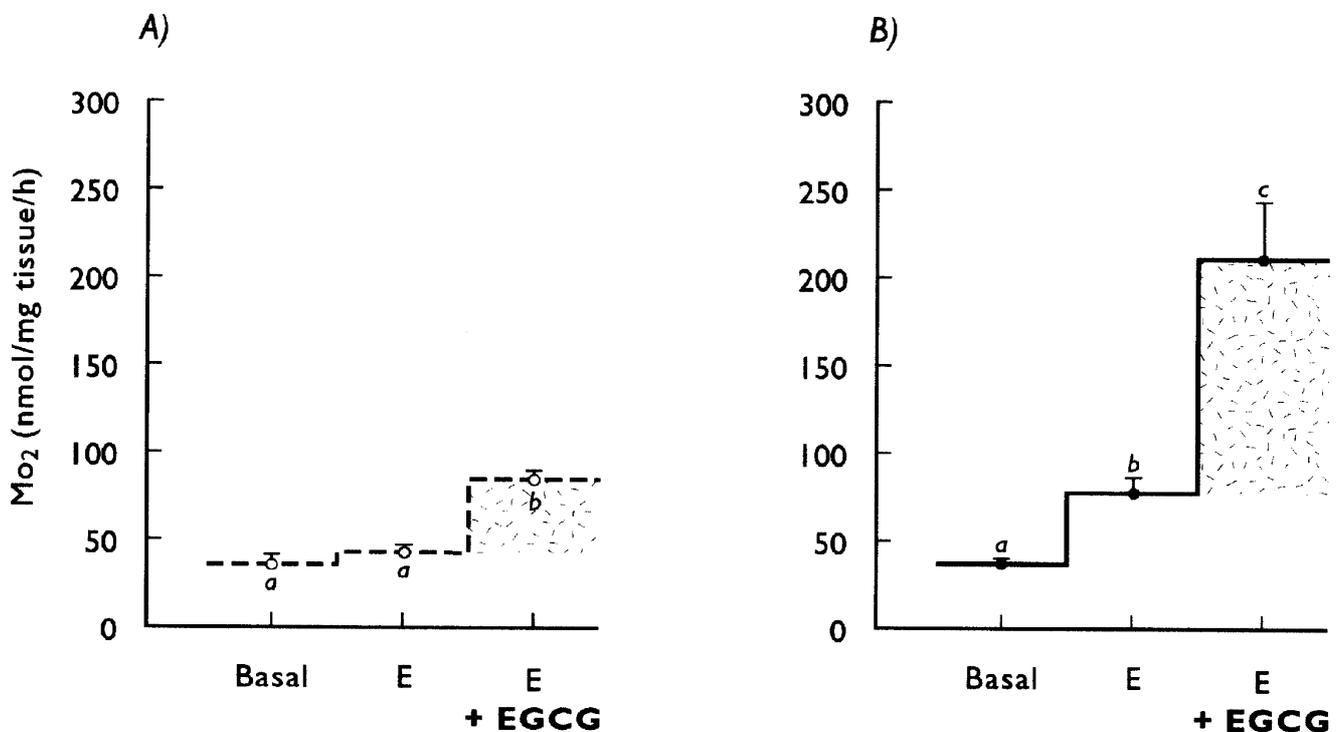
**Figure 2** Respiration rates (MO<sub>2</sub>) of interscapular brown adipose tissue (IBAT) in response to: (i) epigallocatechin-gallate (EGCG) alone, or in combination with caffeine (C) in unstimulated state (Panel A); and (ii) epigallocatechin-gallate (EGCG) in the stimulated state induced by low doses of ephedrine (E) and caffeine (Panel B). In all measurements, caffeine (C) was administered at 100 μM (a low subthreshold concentration which does not increase MO<sub>2</sub> in its own right), and in the study shown in panel B, ephedrine (E) was administered at 0.1 or 0.25 μM, which in its own right either did not increase basal MO<sub>2</sub> (broken line) or significantly increased basal MO<sub>2</sub> between 50 and 100% (solid line). The shaded areas represent the synergistic increases in tissue thermogenesis in response to the interaction between epigallocatechin gallate and caffeine (EGCG + C) in the absence (panel A) or in presence (panel B) of an increase in NA release (induced by ephedrine, E). Data are presented as mean ± s.e. (*n* = 5–7). For comparisons across either EGCG treatment and C + EGCG treatment (panel A) or across E + C + EGCG treatment (panel B), values not sharing a common superscript are significantly different from each other, as assessed by *post hoc* comparison with Anova with repeated measures. For ‘vertical’ comparisons between various treatments (e.g. between ephedrine + green tea and ephedrine + caffeine at a specific isomolar concentration of caffeine), the level of statistical significance of differences is indicated as follows: \**P* > 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

administration of ephedrine alone resulted in a significant stimulation of IBAT  $MO_2$  by 50–100% (solid line), the addition of caffeine to ephedrine resulted in a 4-fold increase in IBAT  $MO_2$  above basal values. Subsequent addition of EGCG resulted in further synergistic increases in IBAT  $MO_2$  (shaded zone), such that with EGCG at 100 and 200  $\mu M$ , the combination of E + C + EGCG increased basal  $MO_2$  values by 5.7-fold and 7.4-fold, respectively, or by 40% and 90% respectively relative to that for E + C ( $P < 0.001$ ). Thus although the effects of EGCG *per se* or EGCG + caffeine on IBAT thermogenesis are either absent or relatively small (50%) in the unstimulated state (i.e. in the absence of ephedrine), it is shown that in the stimulated state (i.e. during increased NA release) induced by ephedrine, the combination of EGCG + caffeine led to marked synergistic effects in stimulating IBAT  $MO_2$  by several fold. Similarly, in another study presented in Figure 3, it is shown that EGCG alone at 100  $\mu M$ , a concentration at which it is ineffective in increasing IBAT thermogenesis, is nonetheless capable of potentiating thermogenesis in the presence of low concentrations of ephedrine (i.e. either at 0.1 or 0.25  $\mu M$ ), and that this potentiating effect between EGCG and ephedrine is more marked in the subset of data when administration of ephedrine alone resulted in a significant stimulation of IBAT  $MO_2$  by about 2-fold (solid line), as compared to the other subset (broken line) when

ephedrine *per se* had no significant effect on basal  $MO_2$ . Taken together, these studies therefore suggest that the efficacy of the green tea extract to potentiate BAT thermogenesis, to a large extent, resides in an interaction between its high content in caffeine and EGCG (and probably also other catechin-polyphenols) with sympathetically released NA. Indeed, a consequence of the inhibitory action of catechins on COMT would be a reduction in enzymatic degradation of NA and hence a prolongation of action of sympathetically released NA on adrenoceptors. In turn, the increased NA-induced cAMP activation in the cell would be prolonged by caffeine's ability to inhibit phosphodiesterase-induced degradation of intracellular cAMP. Thus, on the basis of the inhibitory actions of the two main ingredients of green tea extract—catechins and caffeine—on two different enzyme systems that diminished the half-life and action of NA, the green tea extract is more effective than caffeine *per se* in potentiating sympathetic activation of thermogenesis.

## Concluding remarks

The thermogenic properties of green tea are of particular interest since it has been widely consumed in China and in Japan for many centuries, and hence is



**Figure 3** Respiration rates ( $MO_2$ ) of interscapular brown adipose tissue (IBAT) in response to epigallocatechin-gallate (EGCG) in the stimulated state induced by relatively low doses of ephedrine (E). In all measurements, EGCG was administered at 100  $\mu M$  (a low subthreshold concentration which does not increase  $MO_2$  in its own right), and ephedrine (E) was administered at 0.1 or 0.25  $\mu M$ , which in its own right either did not increase basal  $MO_2$  (broken line, panel A) or significantly increased basal  $MO_2$  by about 2-fold (solid line, panel B). The shaded areas represent the synergistic increases in tissue thermogenesis in response to the interaction between EGCG and ephedrine. Data are presented as mean  $\pm$  s.e. ( $n = 5-7$ ); values not sharing a common superscript are significantly different from each other, as assessed by *post-hoc* comparison after Anova with repeated measures.

regarded as safe. In fact, the potential therapeutic value of green tea is well recognized, and currently, because it possesses outstanding anti-oxidant properties, its ability to confer a protective role against free-radical mediated diseases (including coronary heart disease and cancer) is an active area of medical research.<sup>16–18</sup> According to current concepts, such anti-oxidizing effects, as well as its reportedly capillary-strengthening, anti-bacterial and antidepressant effects,<sup>19</sup> are primarily attributed to its remarkable content of caffeine and catechin-polyphenols (notably EGCG). Our studies, showing that the green tea extract—rich in catechin-polyphenols and caffeine—is a more effective potentiator of sympathetically mediated thermogenesis than caffeine *per se*, raise the possibility that the therapeutic potential of the green tea extract, or indeed a combination of EGCG and caffeine, may be extended to the management of obesity. However, a main limitation in the extrapolation of *in vitro* to *in vivo* responses resides in our lack of knowledge in the fate of polyphenols or caffeine from ingestion to an effective concentration at the critical sites (e.g. within the sympathetic neural cleft) that may lead to thermogenic stimulation. Although, the presence of substantial amounts of EGCG and other catechin isoforms has recently been demonstrated in the plasma of human volunteers after ingestion of a green tea powder, the peak plasma concentrations of catechins (non-conjugated) or indeed those of caffeine are low (<0.5 and <25 µM, respectively), and in the case of catechins, this corresponded only up to 3% of the ingested dose.<sup>20</sup> However, the distribution of the latter is not known and its interaction with sympathetic activity may have been reached despite low circulating levels. Indeed, the recent findings in humans<sup>21</sup> that administration of capsules containing the green tea extract (but not an equivalent amount of caffeine *per se*) resulted in a significant increase in 24 h energy expenditure, thermogenesis, fat oxidation, and 24 h urinary noradrenaline relative to placebo, is consistent with the present findings in IBAT *in vitro* that the effect of green tea on thermogenesis and fat oxidation may be attributed to an interaction between its high content in catechin-polyphenols and caffeine on sympathetic activity.

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