STIMULATING NONSHIVERING THERMOGENESIS IN COLD EXPOSED HUMANS: EMPHASIS ON THE ACTIONS OF GREEN TEA EXTRACTS

par / by

Chantal Gosselin

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Author: Chantal Gosselin, B.Sc. (Activité Physique)

Thesis advisor: François Haman, Ph.D., Department of Human Kinetics, Faculty of Health Sciences, University of Ottawa

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SUMMARY

Two mechanisms can contribute to heat production when humans are exposed to cold; shivering thermogenesis (ST) and nonshivering thermogenesis (NST). The stimulation of NST by the sympathetic nervous system requires the release of noradrenaline (NA). It has been shown that epigallocatechin-3-gallate (EGCG) and caffeine naturally present in green tea can stimulate thermogenesis in a thermoneutral environment. This thermogenic effect of EGCG and caffeine requires the release of NA via the activation of the sympathetic nervous system. The purpose of this thesis is to demonstrate that green tea extracts can stimulate cold-induced NST. More precisely, that ECGC and caffeine given in combination to men exposed to mild cold will increase NST and decrease the contribution of ST towards total heat production. Changes in NST can be assumed by simultaneously measuring total body oxygen consumption (VO_2) and shivering intensity. The study presented in CHAPTER 2 has shown that the ingestion of EGCG and caffeine increased total VO_2 by ~25% during a 3h mild cold exposure. This increase in VO_2 was accompanied by a decrease of ~21% in total shivering intensity. The mechanisms assumed to be responsible for the activation of NST are also discussed in this chapter. Although EGCG and caffeine has shown to stimulate NST by increasing VO_2 and decreasing shivering in most subjects, interindividual differences in these responses were observed. Lastly, as a general conclusion, CHAPTER 3 will review the effects of EGCG and caffeine given orally to men during a mild cold exposure. The limits and application of the study will also be presented in this final chapter.

RÉSUMÉ

Lorsque l'être humain est exposé à des conditions froides, deux mécanismes physiologiques sont déclenchés: la thermogénèse du au frissonnement (TF) et la thermogénèse sans frissonnement (TSF). Le système nerveux sympathique est responsable de la réponse par TSF et nécessite le relâchement de l'hormone noradrénaline (NA). Il a été démontré que l'épigallocatechine-3-gallate (EGCG) et la caféine présents dans le thé vert ont un effet thermogénique à des conditions ambiantes. Cet effet thermique déclenché par l'EGCG et la caféine est accentué lorsque la NA est relâchée. Le but de la présente thèse est de démontrer qu'un extrait de thé vert peut stimuler la TSF induite par le froid. Plus précisément, que l'EGCG et la caféine, ingérer simultanément chez des hommes exposés au froid, stimulera la TSF et diminuera la contribution de la TF tout en maintenant une production de chaleur adéquate. Une augmentation de la TSF peut être assumée indirectement en mesurant la consommation d'oxygène (VO_2) et l'intensité du frissonnement. L'étude présentée dans le CHAPITRE 2 démontre que l'ingestion d'EGCG et de caféine augmente la VO_2 total ~25% lors d'une exposition au froid de 3hr. Cette augmentation est accompagnée d'une diminution de l'intensité du frissonnement total d'environ 21%. Les mécanismes responsables de l'augmentation de la TSF sont également discutés dans ce chapitre. Même si l'EGCG et la caféine ont tendance à stimuler la TSF par une augmentation de la VO_2 et par une diminution de l'intensité du frissonnement, certaines différences interindividuelles ont été observées et seront rapportées. Finalement, en guise de conclusion générale, le CHAPITRE 3 fera un résumé des effets de l'EGCG et de la caféine lorsqu'administrés à des hommes dans des conditions froides. Les limites et les applications de cette étude seront également présentées dans ce chapitre.

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LIST OF ABREVIATIONS AND SYMBOLS

Abbreviations

Ad: Adenosine

ATP: Adenosine triphosphate

AUC: Area under curve

β-AR: beta-adrenergic

BAT: Brown adipose tissue

Ca^{2+}: Calcium

Ca^{2+}ATPase : Calcium adenosine triphosphatase

cAMP: Cyclic adenosine monophosphate

COMT: Cathec-*O*-Methyltranspherase

CON: Control condition (Placebo)

EC: Epicatechin

ECG: Epicatechin-gallate

EGC: Epigallocatechin

EGCG: Epigallocatechin-3-gallate

EMG: Electromyography

EXP: Experimental condition (1600mg EGCG + 600mg caffeine)

FFA/TG: Free fatty acid/Triglyceride

Gp : G protein

H_{prod} : Heat Production

MVC: Maximal voluntary contraction

NA: Noradrenaline

NST: Nonshivering thermogenesis

PD: Phosphodiesterase

PET/CT: Positron emission tomography/computed tomography

RMR: Resting metabolic rate

RyR: Ryanodine receptor

SNS: Sympathetic nervous system

ST: Shivering thermogenesis

T3: Triiodothyronine

T4: Thyroxine

TD2: thyroxin 5'deiodenase

$\mathbf{T_{rec}}$: Rectal temperature

$\overline{T}_{skin}$: Skin temperature

UCP-1: Uncoupling Protein-1

VO_2: Oxygen consumption

$\dot{V}O_2$: Oxygen consumption rate

Symbols

bpm: beats per minute

°C: Celsius

kg: kilograms

kJ: kilojoule

l: litters

min: minutes

mg: milligrams

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* Underwater weighing; Brosek *et al.* (1963).

† CSEP incremental treadmill exercise test.

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*Significantly different from baseline.

† Effect of interaction of time and condition.

*Significantly different from baseline.

† Significantly different between conditions.

*Significantly different from baseline.

† Significantly different between conditions.

Figure 2.4. Area under curve (AUC) for total shivering intensity and oxygen consumption (VO_2) during a 3 hour mild cold exposure using a liquid condition suit (water at 15°C), when ingesting a placebo (CON) and 1600mg EGCG + caffeine (EXP). AUC for shivering intensity is represented for (TRA & PEC) muscles individually, and their mean ($\overline{x}$), and AUC VO_2 is represented for whole body.

*Significantly different between the two conditions.

Figure 2.5. Area under curve (AUC) for total heat production (H_{prod}) during a 3 hour mild cold exposure using a liquid condition suit (water at 15°C), when ingesting a placebo (CON) and 1600mg EGCG + caffeine (EXP).

*Significantly different between the two conditions.

Figure 2.6. Interindividual variability to treatment (1600mg EGCG and 600mg caffeine) and cold response for VO_2 and shivering intensity (shiv). The differences between the two conditions are expressed as percent change (%), relative to CON for each subject individually and their mean ($\overline{x}$). A positive value means that the variable increased in EXP condition compared to CON.

CHAPTER 1: GENERAL INTRODUCTION

INTRODUCTION

Since the beginning of mankind, humans have been confronted to extreme temperature fluctuations and yet they have managed to survive. When exposed to cold environments, specific physiological processes occur within the organism to maintain homeostasis. In mammals, optimal thermal balance ranges between 36°C to 38°C (Schmidt-Nielsen 1990). Core body temperature must be maintained within this range otherwise tissue damage, impaired function or even death may occur. In order to assure that proper core temperature is maintained during cold exposure, two main physiological responses take place. One of them is a reduction in heat lost through vasoconstriction of peripheral vessels, which prevents heat dissipation by keeping warm blood closer to the vital organs and away from the extremities. The other is expressed by an elevation in heat production, which can be achieved through two mechanisms: shivering thermogenesis (ST) and non-shivering thermogenesis (NST). ST is a sympathetic response of involuntary, asynchronous skeletal muscle contractions aimed at producing the most amount of heat in cold exposed adult humans (Hemingway 1963). Shivering can vary from different intensities up to 40% of maximal oxygen consumption [or 5 times resting metabolic rate (RMR)] (Eyolfson *et al*. 2001). On the other hand, NST is a heat-production mechanism liberating chemical energy through processes that do not involve muscular contractions (Jansky, 1973). NST can be produced through diverse processes such as: proton leak through uncoupling protein UCP-1 in brown adipose tissue (BAT) (Cannon & Nedergaard 2004); Calcium (Ca^{2+}) cycling in skeletal muscle (Himms-Hamgen 2004); triglyceride and free fatty acid (TG/FFA) cycling in white adipose tissue (Garofalo *et al*. 1996) and possibly in the liver (Rolf & Brown 1994; Kim, Saidel & Kalhan 2008). All of these can be sympathetically activated by noradrenaline

(NA) through cyclic adenosine monophosphate (cAMP)-dependent mechanisms. A review by Leppäluoto *et al*. (2005) reported that NA is the main hormone released in the cold and plays a central role in maintaining temperature homeostasis. NA activates sympathetically innervated tissues like BAT (Cannon & Nedergaard 2004; Bartness & Song 2005; Bartness, Vaughan & Song 2010) and thyroid gland (Sundler *et al*. 1989), therefore contributes to cold stimulated NST. Since this hormone seems to be of such importance in activating NST mechanisms, finding a way to increase its contribution towards thermogenesis would be of great relevance in cold exposed humans.

Many studies have examined the possibility of stimulating the sympathetic nervous system in order to increase energy expenditure. Substances like green tea, capsaicin, ephedrine and caffeine have been reported to increase thermogenesis in humans in thermoneutral conditions (Astrup *et al*. 1985; Dulloo *et al*. 1999; reviewed by Kovac & Mela 2006; Westerterp-Plantenga *et al*. 2006; Belza, Frandsen & Kondrup, 2007) but only caffeine, ephedrine and capsaicin were tested during a cold stress (Vallerand, Jacobs & Kavanagh 1989; Glickman-Weiss *et al*. 1998; MacNaughton *et al*. 1990). Previous evidence suggests that one such untested compound, green tea, could be a great potential to stimulate NST in the cold. The thermogenic effects of green tea may reside in the presence of catechins and caffeine in the leaves. The most abundant and active of all catechins, epigallocatechin-3-gallate (EGCG), has the potential of inhibiting catecol-*O*-methyltransferase (COMT) (Borchardt & Huber 1975). This enzyme is responsible for degrading NA in the synaptic cleft (Chinet & Durand 1979), therefore increasing the action of NA on target tissues. On the other hand, caffeine has the potential of inhibiting phosphodiesterase, the enzyme that degrades cAMP (Dulloo, Seydoux & Girardier, 1992).

Caffeine is also an antagonist of adenosine, which is responsible for reducing adipose tissue sensitivity to NA and for inhibiting the release of NA by sympathetic terminals (Fain & Malbon 1979; Szillat & Bukowiecki 1983; Schimmel & McCarthy 1984). Together, EGCG and caffeine act synergistically by increasing NA and intracellular levels of cAMP and by blocking adenosine. This interaction amplifies the release of NA and its action on target tissues and could potentially lead to an increase in cold-induced NST. Therefore, the importance of NST would be greater and this could result in a decrease in the contribution of ST towards total heat production.

This review will focus on the potential role played by dietary phytoelements in stimulating NST mechanisms in cold exposed humans. Specific emphasis will be placed on the thermogenic action of catechins and caffeine obtained from green tea extracts. The bioavailability and the pharmacokinetics parameters of these compounds will be reviewed in order to investigate how the dosage and timing of administration of these molecules would influence thermogenic responses. Finally, potential methodologies for estimating increases in NST during cold exposure in humans will be suggested.

NONSHIVERING THERMOGENESIS

Brown adipose tissue

Even though a number of metabolic pathways can produce heat without shivering (described below), BAT remains the primary source of NST in rodents and newborn humans (Heaton 1972; Cannon & Nedergaard 2004). Newborns depend mostly on BAT for their survival, since they do not possess proper muscle mass to produce adequate amounts of heat by ST alone. In adult humans, skeletal muscles represent nearly half of total body mass

(Rolf & Brown 1994). As such, adults depend mostly on ST for heat production (Haman *et al.* 2010). Even though shivering is by far the most to total heat production, NST still provides some heat to compensate for increases in heat loss in the cold (Jansky 1973). In this context, new evidence (Cypess *et al.* 2009; Saito *et al.* 2009; van Marken Lichtenbelt *et al.* 2009; Virtanen *et al.* 2009) strongly suggests that the presence and activation of BAT, even in small amounts, cannot be overlooked when it comes to its potential contribution to total thermogenic rate. If the activity of BAT in humans would be similar to rat, maximal activation of this tissue could provide up to 25% of all the heat produced during low to moderate intensity shivering (Haman *et al*. 2010).

BAT is activated in the cold *via* sympathetically released NA. The synaptic ending releases NA in the synaptic clef, which binds with β–adrenergic receptors of BAT. Following this, a cascade of pathways is activated. These include the activation of G protein, adenylyl cyclase, cAMP, lipolysis and fatty acid release in the BAT cell. Fatty acids open the mitochondrial channel protein UCP-1, leading to influx of protons into mitochondria and to production of heat instead of ATP (Cannon & Nedergaard, 2004). Additionally, UCP-1 expression can be increased by local triiodothyronine (T3) in BAT, resulting in an elevation in UCP-1 and its activation (Bianco & Silva, 1987). This is accomplished through the enzyme thyroxin 5'deiodenase (TD2). TD2, activated by cAMP, converts thyroxine (T4) into T3. The latter amplifies the effects of the sympathetic nervous system on mechanisms such as lipolysis and the stimulation of the UCP-1 gene (Silva 2006). Given the presence of TD2 in BAT of newborn infants (Houstek *et al*. 1993), it could be assumed to exist in adult humans, but this still requires confirmation. The contribution of BAT towards total NST also remains unknown. Other potential cold-induced NST mechanisms exist in humans and

their involvement also requires more research.

Ca^{2+} homeostatis in skeletal muscle

Skeletal muscles represent approximately 42% body mass in adult humans (Rolf & Brown, 1997) and can increase the thermogenic rate up to 5x RMR during shivering (Eyolfson *et al.* 2001) and up to 12-15x from basal metabolic rate during exercise (Haman *et al*. 2010). In view of the anatomical abundance and metabolic capacity of this tissue, the presence of NST in skeletal muscles, even at low rates, could potentially be of great significance at the whole body level. A possible NST mechanism, other than BAT in humans, has been suggested in skeletal muscle (Himms-Hagen 2004). For example, the activation of NST could be linked to the turnover of energy required to maintain the ion gradient of Ca^{2+} between the cytosol and the sarcoplasimc reticulum. This equilibrium in gradient is achieved through Ca^{2+}ATPase, which actively pumps Ca^{2+} back in the reticulum after its release by active muscle (Simonide *et al*. 2001) or leakage through the ryanodine receptor (Nelson 2002). Activation of the ATPase requires ATP and heat is released through its action (Clausen, Van Hardeveld & Everts 1991; Block 1994).

Ca^{2+} cycling can be sympathetically stimulated in skeletal muscle. It has been shown that skeletal muscles possess the enzyme TD2 (Salvatore *et al.* 1996; Hosoi *et al.* 1999), which is expressed through β-receptors. The transformation of T3 by TD2 results in more Ca^{2+}leakage through the ryanodine receptor (Conelly *et al*. 1994). The alteration in intracellular Ca^{2+} homeostasis by increasing calcium leakage through the sarcoplasmic reticulum membrane results in the activation of ATPase in order to restore equilibrium, thus Ca^{2+} cycling is amplified. Possibility arise that Ca^{2+}cycling could be stimulated in the cold

through circulating NA and cAMP-dependent mechanisms (Himms-Hagen 2004), since they are responsible for the activation of TD2 through β-receptors (Hosoi *et al*. 1999).

The production of intracellular T3 by TD2 requires circulating T4. Sundler *et al.* (1989) found that the thyroid gland in rats, thus the site of T4 synthesis and release, is innervated in part by sympathetic fibers containing NA. There is also evidence that sympathetic innervations may have a direct stimulatory effect on the thyroid gland in humans (Silva 2006; Spratt, Pont & Miller 1982). Moreover, a review by Leppäluoto *et al*. (2005) shows that thyroid hormones (T3 and T4) are released during most cold exposures. Therefore, Ca^{2+} cycling has the potential of being activated by NA through the conversion of T4 in T3 by TD2 within the cell.

Heat dissipated through Ca^{2+} cycling in humans exposed to cold requires more investigation. However, scientific evidence clearly shows that this process of heat production cannot be overlooked. For example, Ca^{2+} cycling is present in birds (Bicudo, Vianna & Chaui-Berlinck 2001) and in fish (Block 1994). Additionally, increase leakage of Ca^{2+} in humans suffering from malignant hyperthermia points toward the existence of such a mechanisms in humans (O'Sullivan, McIntoch & Heffron 2001). Besides Ca^{2+} cycling, another ATP consuming cycle can be stimulated in the cold.

FFA/TG cycling in adipose tissue and liver

Substrates cycling are regulatory processes occurring within the body that result in heat dissipation. One such cycle is FFA/TG during which FFA are released via lypolisis and subsequently re-esterified rather than oxidized. This process requires ATP, thus resulting in heat dissipation. Several studies have pointed out the possibility of ATP consuming free

fatty acid cycling mechanisms induced by cold release NA. A study by Steinberg *et al*. (1964), first demonstrated that NA had an effect on plasma FFA release and oxidation at ambient temperature. In their study, infused NA in young adult humans increased FFA turnover rate by 74% and oxygen consumption by 24%. Garofalo *et al*. (1996) demonstrated that white adipose tissue possessed possible thermogenic properties similar to BAT through its increased activation in the cold via NA. In their study, they compared epydidimal and retroperitoneal white adipose tissues to intra-scapular brown adipose tissue (IBAT) of rats. Their results showed that the cold induced a reduction of endogenous NA content of 50% in IBAT and 30% in both white adipose tissues. They also found that compared to rats kept at ambient temperature, fractional rates of NA turnover were 5 times higher in IBAT and 2.3 times higher in white adipose tissues. This increase in NA turnover was also accompanied by an increase in FFA release. Their study showed that NA stimulated lypolisis exists in WAT as well as in IBAT. Stimulation of TG/FFA cycling by the cold could also occur in the liver. In their review, Rolf & Brown (1994) mentioned that the potential for substrate cycling is probably larger in the liver that in most tissues at ambient temperature. Therefore, it would not be surprising if TG/FFA cycling would be stimulated in the liver by cold. Vallerand *et al.* (1999) confirmed that TG/FFA cycling rate is increased in the cold. In their study, the rate of appearance of FFA in the cold was 2-fold higher than fat oxidation, suggesting an enhanced non-oxidative disposal of fatty acids like TG/FFA cycling. This increase in TG/FFA could be attributed to a greater enhancement in β–adrenergic stimulation (Wolf, Herndon & Jahoo 1987; Barbe *et al.*1996) by cold released NA. Indeed, cold increased extracellular TG/FFA cycling rate by 2.5 fold, but intracellular cycling was unaffected. This suggests that white adipose tissue is responsible for the increase in TG/FFA

cycling.

Taken together, these studies show that lypolisis and the release of FFA are activated by cold, and the release of NA, creating an overflow of circulating FFA and therefore inducing their re-esterification when they are not oxidized. This recycling of FFA results in heat dissipation through inefficient ATP demanding mechanisms. Like in UCP-1 in BAT and Ca^{2+} cycling in skeletal muscle, the release of FFA requires adrenergic and cAMP-dependent signaling (Robinson, Butcher & Sutherland 1968; Fain & Shepherd 1979; Honnor, Dhillon & Londos 1985). Since NA and cAMP seem to play a central role in cold induced NST, finding a way to amplify their potential for heat production would be of great relevance.

STIMULATING NST IN THE COLD

As previously mentioned, physiological responses in the cold are stimulated through the activation of the sympathetic nervous system. Most of the mechanisms contributing to NST are activated through the release of NA and cAMP-dependent mechanisms. In order to increase thermogenesis, these pathways must be stimulated. Certain agents like capsaicin, caffeine, ephedrine and green tea have the potential to induce an increase in energy expenditure through sympathetic activation (Astrup *et al.* 1985 & 1990; Dulloo *et al*. 1999; Kovac & Mela 2006; Westerterp-Plantenga *et al.* 2006; Belza, Frandsen & Kondrup 2007). Their consumption, in thermoneutral conditions, has been shown to stimulate thermogenesis. Yoshioka *et al.* (1998) demonstrated that capsaicin ingested with a high fat meal increases diet-induced thermogenesis and lypolisis. Caffeine has also been shown to stimulate thermogenesis and impact lypolisis. In a study by Astrup *et al*. (1990), caffeine given at

doses of 100, 200 and 400 mg stimulated thermogenesis in a linear dose dependent manner; with the highest dose resulting in a 4 times increase in energy expenditure compared to placebo. Additionally, TG levels in plasma increased significantly with the 400 mg dose compared to placebo. Acheson *et al*. (2004) obtained similar results. Energy expenditure increased 13% compared to placebo and turnover of lipids doubled, of which 24% were oxidized and 76% were recycled. Moreover, adrenoceptor blockade with propranolol decreased caffeine-stimulated energy expenditure and lipid turnover. This indicates that some of the metabolic effects of caffeine were mediated by the sympathetic nervous system. Ephedrine was also reported to increase oxygen consumption (Evans & Millens 1977) and thermogenesis through β–adrenergic stimulation (Astrup *et al.* 1985). In another study, ephedrine given in combination with caffeine increased oxygen consumption of IBAT compared to baseline values (Dulloo Seydoux & Girardier 1992). Astrup *et al.* (1991) also obtained a thermogenic effect of caffeine and ephedrine when given in combination. The highest dose of ephedrine/caffeine ingested (20 mg/200 mg) increased energy expenditure significantly compared to placebo (64%) and compared to lower doses. Together the ephedrine/caffeine mixture resulted in a greater increase in thermogenesis compared to ephedrine and caffeine given alone in the same dosage. Compounds in green tea also work synergistically in order to induce a thermogenic effect (Phung *et al.* 2009). Together, catechins and caffeine naturally found in green tea have been shown to increase energy expenditure (Duloo *et al*. 1999; Dulloo *et al.* 2000; Bérubé-Parent *et al.* 2005; Rudelle *et al.* 2007). Indeed, Dulloo *et al.* (1999) demonstrated that green tea induced a thermogenic effect through sympathetic activation of thermogenesis (see details below - *Interaction between catechins & caffeine on energy expenditure and thermogenesis*).

All of these compounds (capsaicin, caffeine, ephedrine and green tea) seem to be potent stimulants of the sympathetic nervous system and capable of inducing thermogenesis. First, it should be emphasized that in contrast to the mentioned studies, the desired increase in thermogenesis would occur during cold exposure and not in thermoneutral conditions. The question therefore remains as to which substance is the best at increasing cold-induced NST?

Capsaicin increases heat production, but also increases heat loss by inducing cutaneous vasodilatation (Szolscansyi 1983; Kobayashi *et al.* 1998). The potential heat produced through stimulated NST by capsaicin would be lost through this vasodilatation. Therefore, the selection of capsaicin would be ruled out. As for ephedrine, it would not be needed in cold conditions because it exerts its peripheral thermogenic effect entirely *via* the release of NA from sympathetic nerve terminals (Dulloo Seydoux & Girardier 1992). Since NA is already being released in the cold (Leppäluoto *et al.* 2005), ephedrine would not be needed. Additionally, ephedrine has been shown to have negative cardiac effects by increasing blood pressure (Astrup *et al*. 1985; 1991) and heart rate (Kowalczyk *et al*. 2006; Astrup *et al*. 1991). In contrast, green tea does not possess any short-term adverse cardiovascular consequences. Dulloo *et al.* (1999) reported no side effects and no differences in heart rate across their EGCG and caffeine treatments. Similarly, Bérubé-Parent *et al.* (2005) reported no increase in systolic blood pressure when a maximal dose of 1600 mg of EGCG and 600 mg of caffeine were given orally over a 24 h period. This said, it seems evident that a green tea mixture of EGCG and caffeine would be a good selection to increase cold-induced NST. Additionally, Duloo *et al.* (1999) emphasized that the results of their study, in combination with a previous one (Dulloo *et al.* 2000), raise the possibility that

the effect of a green tea extract on BAT could be greater under conditions of elevated sympathetic tone and NA release (e.g. cold exposure).

Other evidences suggest that ECGC and caffeine would stimulate cold-induced NST. Studies performed in the cold using caffeine and ephedrine (2.5 and 1.0mg/kg body mass respectively) (Vallerand *et al.* 1989) or caffeine only (5mg/kg body mass) (MacNaughton *et al.* 1990), showed an increase in oxygen consumption compared to placebo during cold exposure. However, the treatment increased heat production, but shivering intensity was not measured. Without electromyography (EMG) to measure involuntary muscle contractions, we cannot determine if caffeine or caffeine/ephedrine increased the metabolic response through stimulated NST and/or ST. Clearly, quantifying the effects of green tea and its underlying compounds on the stimulation of NST in adult humans during cold exposure would be a great interest. The following section will describe green tea and its composition. It will also explain more in depth the mechanisms through which EGCG and caffeine could potentially stimulate NST. Bioavailability and pharmacokinetic characteristics of these compounds will also be discussed in order to select the appropriate dosage and estimate their time of ingestion in the cold.

GREEN TEA

Tea (*Camellia sinensis*, Family Theaceae) is one of the most popular beverages in the world. The majority of tea beverage is prepared from three types of manufactured tea: green tea, oolong tea, and black tea (Shixian *et al*. 2006). Green tea is prepared from fresh leaves and is a non-oxidized product; oolong is partially oxidized and black tea is fully oxidized (Kovacs & Mela 2006). Because of this low oxidation process, green tea contains larger

amounts of catechin polyphenols, which is found to be the most active of its compounds (Westerterp-Plantenga *et al.* 2006) and constitutes about 30-42% of green tea leafs dry weight (Shixian *et al.* 2006). One cup of green tea contains about 100-150 mg of catechins (Nakagawa *et al.* 1999). The most important of the tea catechins are epicatechin (EC; 2%), epicatechin-3-gallate (ECG; 2-3%), epigallocatechin (EGC; 6-10%), and epigallocatechin-3-gallate (EGCG; 10-15%), with the last one being the most abundant (Kovacs & Mela 2006) and most active (Chantre & Lairon 2002). Besides catechins, there are also considerable amounts of caffeine in green tea extracts (Klaus *et al*. 2005); about 3-6% of dry tea leafs (Shixian *et al*. 2006) and about 35-85 mg/cup of infused green tea (Reto *et al*. 2007).

Interaction between catechins & caffeine on energy expenditure and thermogenesis

Both caffeine and catechins are believed to be responsible for the acute effects of green tea on increasing energy expenditure (Dulloo *et al*. 1999; Rudelle *et al*. 2007). Thermogenic properties of green tea reside primarily in the interaction between its high content in catechin-polyphenols and caffeine with sympathetically released NA. The thermogenic effect of catechins comes from their ability to inhibit COMT, the enzyme that degrades NA in the synaptic clef (Brochard & Hubert 1975). This results in prolonging the action of NA on target tissues such as BAT (Dulloo *et al.* 2000; Shixian *et al*. 2006). In addition, the thermogenic action of caffeine resides through the inhibition of phosphodiesterase activity, an enzyme that breaks down NA-stimulated cAMP (Wolfram, Wang & Thielecke 2006). cAMP is a critical intracellular mediator (second messenger) for the actions of catecholamines on thermogenesis (Dulloo, Seydoux & Girardier, 1992).

Caffeine is also an antagonist of adenosine, which is responsible for reducing adipose tissue sensitivity to NA and for inhibiting the release of NA by sympathetic terminals (Fain & Malbon 1979; Szillat & Bukowiecki 1983; Schimmel & McCarthy 1984). Both caffeine and catechins, especially EGCG, are believed to be responsible for the acute effects of green tea on increasing thermogenesis (Dulloo *et al*. 1999; Rudelle *et al*. 2007).

Several studies have been conducted with the objective of reducing obesity and optimizing weight management using different types and doses of catechins and caffeine. In one such study, Dulloo *et al*. (1999) compared green tea (catechins + caffeine), caffeine alone and placebo and showed that a green tea extract (90 mg EGCG + 50 mg caffeine) given at three repeated times (with meals) during a single day increased 24 h energy expenditure by 4% (about 328 kJ/d). They also demonstrated an increase in urinary NA concentration for the total 24 hr of treatment (vs placebo and caffeine alone). Urinary catecholamine excretion is a simple method for estimating the degradation at sympathetic nerve endings and the renal excretion of NA (Gregersen *et al*. 2009). The results from Dulloo *et al*. (1999) are consistent with the inhibitory effect of EGCG on COMPT. The higher urinary excretion of NA is consequential to the reduction of its degradation, and hence, its spillover of into the circulation. Bérubé-Parent *et al.* (2005), showed similar results. They demonstrated that an EGCG + caffeine extract, compared to placebo, significantly increased 24 h energy expediture by 8% (750 kJ/d). In both studies, EGCG was given in a sufficient amount and in combination with caffeine. Together, these compounds interact synergistically to increase thermogenesis.

Bioavailability of catechins and caffeine

In order to exert its full effect, green tea extracts given to individuals must be composed of the main catechins EGCG and, most importantly, in combination with caffeine. The bioavailability of catechins is known to vary, particularly between the gallated (ECG & EGCG) and de-gallated (EC & EGC) compounds. The former are known to remain longer in plasma circulation in the non-conjugated form and to be excreted in the bile. The de-gallated compounds are eliminated faster in circulation in the conjugated form, since they are hydrophilic and they are excreted rapidly in urine (Yang *et al.* 1998; Van Amelsvoort *et al.* 2001). The non-conjugated form means that the catechins have not gone through any transformation process (e.g. methylation with COMT). When a catechin is conjugated, their phenolic hydroxyl groups are being replaced which results in quicker urine excretion (Chen *et al*. 1997).

Interestingly, a study by Nakagawa *et al.* (2009) showed that caffeine suppresses conjugation reactions resulting in increased plasma catechins levels. When caffeine is ingested in combination with EGCG, their action is not only increased, but the degradation of EGCG is slowed down. Additionally, EGCG has been shown to have a 60-fold higher activity than non-gallated catechins (EGC & EC) in inhibiting COMT (Lu, Meng & Yang 2003). It is proposed that the inhibition of COMT would reduce the degradation of NA and therefore prolonging its action in the synaptic cleft and on target tissues such as BAT (Dulloo *et al.* 2000; Shixian *et al*. 2006) or on other mechanisms activated by cold release NA. The increased action of NA by EGCG and caffeine on UCP-1 or substrate cycling would lead to a greater stimulation of NST.

Selection of appropriate dosage of EGCG and caffeine

In order to obtain an increase in NST, appropriate selection of EGCG and caffeine doses is mandatory. Pharmacological parameters of the two compounds are important characteristics that need to be identified and taken into account. It has been shown that EGCG in plasma can reach its highest level about 2 h after oral ingestion (Unno *et al*. 1996) and levels of EGCG (pmol/ml) are detectable in plasma after 1 h of ingestion (Nakagawa *et al.* 1999). Therefore, desired effects should be observed after about 1 h and the peak at about 2 h. On the other hand, caffeine is absorbed faster and its peak plasma concentration can be reached within 30 minutes (Bchir *et al.* 2006). Consequently, the EGCG and caffeine extracts should be given at the onset of cold exposure, early enough to see the effects of caffeine. Additionally, cold exposure should be of 3 h in order to see any changes resulting from the action of EGCG.

Moreover, Chow *et al.* (2001) demonstrated that the availability of EGCG is greatest at a higher dose (800 mg), than lower doses (200 mg, 400 mg, 600 mg). With a higher availability, and therefore with a higher dosage, EGCG concentrations in plasma would be sufficient to induce its action on COMT. For that reason, the maximal quantity of EGCG should be given to assure the desired effect. A study by Ullmann *et al.* (2003) gave up to 1600 mg of EGCG (without caffeine) in a single oral dose to 8 individuals without any detectable adverse effects. Caffeine must also be taken in a sufficient quantity with EGCG in order to get the desired response. In a study by Gregersen *et al.* (2009), green tea extract that was given with a limited amount of caffeine (25 mg) did not have a significant effect on thermogenesis. Therefore, the maximal amount of caffeine should be given with EGCG. A study by Bérubé-Parents *et al.* (2005) safely administered 600 mg of caffeine in combination

with 1600 mg of EGCG without any effects on heart rate or systolic blood pressure. Consequently, an extract containing 1600 mg of EGCG and 600 mg of caffeine should be sufficient to obtain the desired thermogenic response while being safe for the participants. Furthermore, the extract should be given right before the 3 h exposure to mild cold in order to see the desired effect on NST.

EPIGALLOCATECHIN-3-GALLATE, CAFFEINE AND COLD INDUCED NST

Mechanisms of actions of EGCG and caffeine on cold induced NST

Now that targeted NST mechanisms are identified and that the characteristics of green tea have been discussed, it is essential to understand by which means EGCG and caffeine present in green tea can interact with these mechanisms and increase NST in the cold. All the presented NST mechanisms share two things in common. The first, is that they are activated by cold-released NA. The second, is that they use cAMP dependent pathways to trigger the thermogenic response. If these two mechanisms can be increased, the NST response would be amplified. As it was discussed previously, EGCG has the potential of inhibiting COMT, the enzyme that degrades NA, and consequently is able of maintaining high levels of synaptic and circulating NA. As for caffeine, it has the power of inhibiting phosphodiesterase, resulting in an increased level of intracellular cAMP. Since cAMP is mediated through adrenergic receptors, hence NA, EGCG and caffeine are capable of a synergic action on NA-cAMP dependent mechanisms. EGCG and caffeine can therefore affect BAT, Ca^{2+} cycling in skeletal muscles and FFA/TG cycling by acting on their NA-cAMP dependent pathways.

For all three mechanisms, cold released NA would remain longer in the synaptic cleft through EGCG (inhibition of COMT) and its action on adrenergic receptors would be amplified, resulting in the activation of cAMP. Caffeine would then permit the increase of cAMP (inhibition of phosphodiesterase) and affect all cAMP dependent mechanisms. In BAT, this would create an activation of UCP-1 through released FFA (Cannon & Nedergaard, 2004) or possibly through the activation of TD2 as previously explained. In skeletal muscle, TD2 would be responsible for the elevation in Ca^{2+} cycling and increase in thermogenesis. Since TD2 needs the substrate T4 to convert it into T3, the production of T4 by the thyroid gland is necessary. EGCG still comes into play since the possibility arise that thyroid gland is sympathetically innervated and stimulated by NA (Spratt, Pont & Miller 1982; Sundler *et al.* 1989). Again, by inhibiting COMT, EGCG would allow NA to stay longer in the synaptic cleft and increase T4 release through the activated thyroid gland. As for FFA/TG cycling, it has the potential of being activated in white adipose tissue through NA (Garofalo *et al.* 1996) and possibly in the liver (Rolf & Brown 1997; Kim *et al*. 2008). By giving EGCG and caffeine in the cold it is expected that the stimulation of NST would increase its contribution in total heat production. Figure 1.1 (p.19) illustrates the possible NST mechanisms in BAT and skeletal muscles stimulated by EGCG and caffeine.

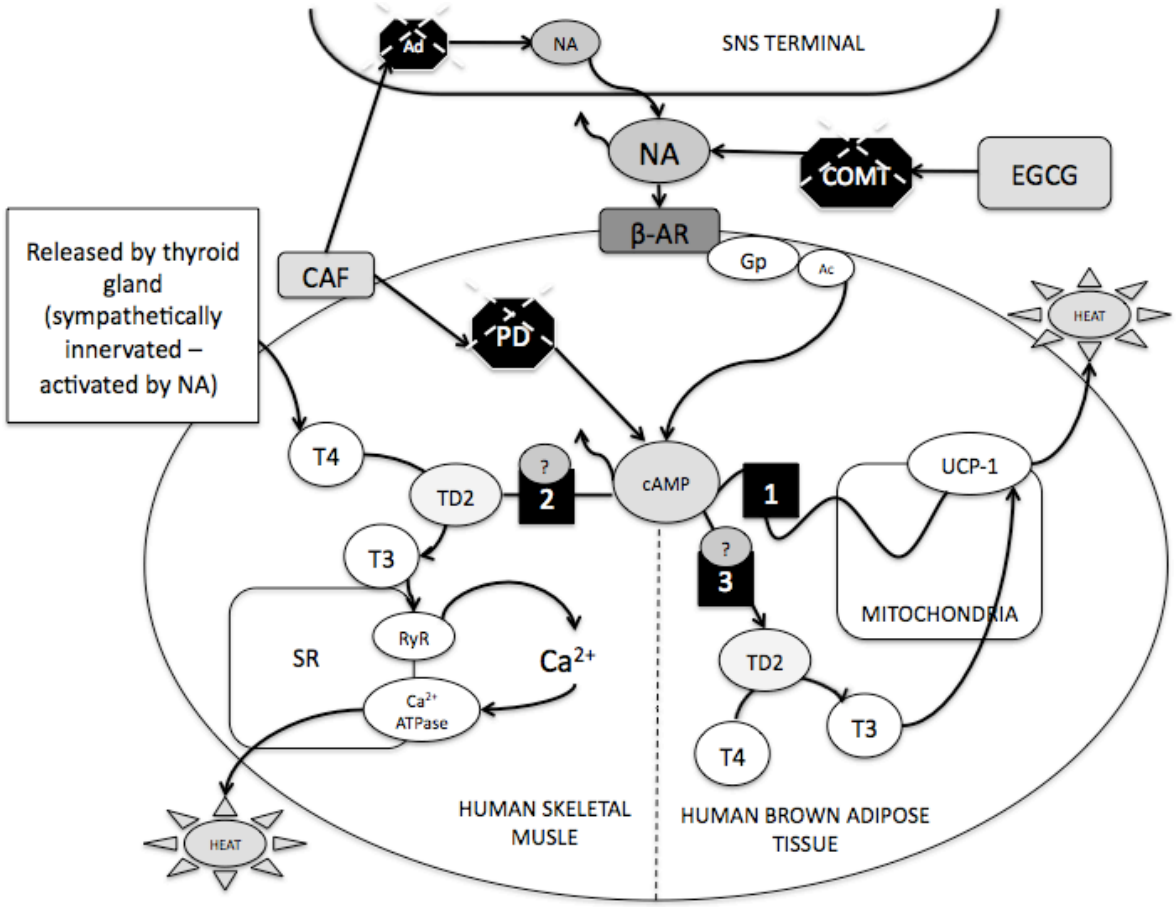

Figure 1.1. Potential mechanisms of induced NST by EGCG and caffeine: **1-** Noradrenaline (NA) released in the cold by the sympathetic nervous system (SNS) stimulates β-adrenergic receptors (β-AR), increasing cyclic adenosine monophosphate (cAMP) production via G proteine (Gp) regulated adenylyl cyclase (Ac) activity. The cAMP will then activate lypolisis and the release of fatty acids will open the mitochondrial channel protein UCP-1in the brown adipose tissue. **2-** Hypothetically, in the skeletal muscle, thyroxin 5'deiodenase (TD2) will be activated, through the same cAMP activation pathway than BAT, and convert thyroxin (T4) into triiodothyronin (T3). The release of Ca^{2+} in the cytosol could be the result of either muscle contraction or Ca^{2+} leakage from ryanodine receptor (RyR) activated by T3. This release in Ca^{2+} will activate Ca^{2+} ATPase to maintain ion gradient, resulting in heat production. EGCG by inhibiting COMT, will create an increase in extracellular NA while caffeine (CAF) by inhibiting phosphodiesterase (PD), will increase intracellular cAMP levels. Caffeine will also act as an antagonist on adenosine (Ad) receptors causing the increase in the release of NA. Synergistic interaction between EGCG and CAF will increase the previous mechanisms (1 & 2) leading to a greater heat production from NST. **3**-The expression of UCP-1 in BAT is activated by T3. This might be another way to optimize NST from BAT in humans, since it has been demonstrated that the presence of TD2 in rodents converts T4 in T3 (to be confirmed in humans).

Measuring NST in the cold

It has been reported that under severe cold, both shivering and NST are activated simultaneously (van Marken Lichtenbelt & Daanen 2003; Jansky 1973). However, different opinions have emerged concerning the onset of NST under mild cold conditions. A study by van Ooijen *et al.* (2005) refers to NST only when EMG is absent, suggesting that NST is activated before ST. On the other hand, Jansky (1973) reported that shivering during cold exposure usually starts before the capacity for NST is exhausted. This means that there seems to be no distinct transition between the onset of shivering and the end of NST. Although NST is sometimes not accompanied by an increase in muscle activity, muscle activation in the cold does not exclude the possibility of a concurrent activation of NST. Therefore, NST could still be present with shivering. An increase in NST could also result in a decrease in shivering as was observed in cold adapted rodents (reviewed in Cannon & Nedergaard 2011).

Unfortunately NST is difficult to quantify, since no direct method is available. Jansky (1973) proposed that NST can be determined indirectly by measuring whole-body heat production with simultaneous recordings of muscle shivering activity. Whole body heat production can be determined by measuring oxygen consumption (VO_2) via indirect calorimetry. By knowing the amount of oxygen consumed, it is possible to quantify the heat that is produced through substrate utilization and oxidation. As for muscle shivering activity, this can be measured through EMG. When comparing both measurements (VO_2 and shivering) between a condition where there is no treatment involved and a condition where EGCG and caffeine is ingested, it would possible to detect if EGCG and caffeine have an effect on cold induced NST. This increase in NST could be translated by either an increase

in VO_2 for a similar shivering intensity, or by a decrease in shivering for a similar VO_2. In both cases, this would indicate a stimulation of NST mechanisms by EGCG and caffeine.

It cannot be confirmed which mechanisms would be involved and their exact contribution in NST. Thus, we can only speculate that the previously mentioned cold stimulated NST pathways could be increased by ECGC and caffeine in the cold. Still, both EGCG and caffeine interact synergistically on NA-cAMP dependent mechanisms and UCP-1, Ca^{2+} and FFA/TG cycling are a result of their activation. Therefore, it would be logical to stipulate that these NST pathways can be increased by EGCG and caffeine in the cold.

Summary

Even if ST is the main contributor to heat production in humans, the NST component still plays an important role in maintaining temperature homeostasis in the cold. Its exact contribution from various tissues like BAT, skeletal muscles, liver or white adipose tissue is still unknown and remains unquantified. Nevertheless, there is evidence of their occurrence and activation in the cold. Also, there is a strong possibility that they can be increased by a thermogenic aid such as green tea. It has been demonstrated many times that EGCG and caffeine in green tea are capable of interacting and increasing energy expenditure in thermoneutral environments. The ability of EGCG to inhibit COMT, therefore increasing NA in the synaptic cleft and in circulation, and of caffeine to inhibit phosphodiesterase, therefore increasing intracellular levels of cAMP, can result in an increase of their actions on NA-cAMP dependent mechanisms. Since NA is the major hormone released in the cold, its action on NST contributors like white adipose tissue, BAT and skeletal muscle could be

increased by ECGC. Additionally, cAMP being a key element in many intracellular mechanisms such as lypolisis, activation of UCP-1 and TD2, is being increased indirectly by caffeine. Together, these compounds could interact synergistically to increase cold induced NST mechanisms and their contribution towards whole-body heat production.

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GOAL OF THE INVESTIGATION

The purpose of this thesis is to investigate the effect of green tea extracts on thermogenesis. More precisely, to quantify the effect of EGCG and caffeine on the stimulation of cold induced NST and its impact on ST. Previous studies have demonstrated the synergic effect of these two compounds in stimulating thermogenesis in a thermoneutral environment (Dulloo *et al.* 1999; Bérubé-Parent *et al.* 2005). Moreover, caffeine has also been shown to induce a thermogenic effect in the cold, by increasing oxygen consumption (Mac Naughton *et al.* 1990). Despite these findings, green tea or caffeine in the combination of EGCG have never been tested in the cold.

To achieve the thesis objective, a study was designed to quantify the effects of EGCG and caffeine on the processes of thermogenesis in the cold. CHAPTER 2 of this thesis will consist of an experiment that shows that the ingestion of EGCG and caffeine in men exposed to mild cold has the potential of increasing NST. In this study NST was determined by simultaneously measuring the metabolic response and the shivering intensity in the cold (Jansky 1973). It his hypothesized that the stimulation of cold-induced NST by EGCG and caffeine will increase NST. This will be assumed by a decrease in shivering intensity for a higher oxygen consumption. Interpretation of the results and the possible NST pathways stimulated by EGCG and caffeine will be addressed. Finally, a general conclusion will be presented in CHAPTER 3 where the limits of the study will be discussed as well as the importance of these findings.

CHAPTER 2: EFFECTS OF INGESTING EPIGALLOCATECHIN-3-GALLATE AND CAFFEINE ON COLD-INDUCED THERMOGENIC PROCESSES IN MEN

Based in part on

Chantal Gosselin & François Haman

ABSTRACT

It has been demonstrated that epigallocatechin-3-gallate (EGCG) and caffeine, naturally present in green tea, have thermogenic properties in thermoneutral conditions. The purpose of this study was to quantify the effects of simultaneously ingesting EGCG and caffeine on the thermogenic responses of a 3h mild cold exposure. Eight healthy males were exposed to a mild cold, using a liquid conditioned suit perfused with 15°C water, on two occasions and consumed a placebo (CON) or an extract of 1600mg of EGCG and 600mg of caffeine (EXP). The order of the trials was randomly assigned following a single-blind, balanced, crossover design. Thermic, metabolic and electromyographic measurements were monitored at baseline and during cold exposure. After 180min of cold exposure, shivering intensity was ~32% lower in the EXP condition compared to CON (p=0.01). The area under the curve of shivering intensity over the cold exposure period was reduced by ~21% in EXP (457±99 %MVC•min) compared to CON (361±81 %MVC•min; p=0.007). In contrast, the total area under curve of VO_2 was ~25% higher in EXP (33.3±5.5 L O_2) compared to CON (25.3±5.1 L O_2; p=0.03). Total H_{prod} also increased by about 11% in the EXP condition (1535±112 kJ) compared to control (1372 ±106 kJ; p=0.002). The decrease in shivering activity combined with an increase in VO_2 and H_{prod}, following the ingestion of EGCG and caffeine in the cold, indicates that NST pathways can be significantly stimulated in adult humans. The potential of stimulating NST and decreasing the contribution of ST would play a significant role during prolonged cold exposures, especially when fine motor control is required.

Keywords: epigallocatechin-3-gallate, caffeine, nonshivering thermogenesis

Abbreviations: BAT, brown adipose tissue; COMT, cathecol-*O*-methyltransferase; EGCG, epigallocatechin-3-gallate; %MVC, percent maximal voluntary contraction; NA, noradrenaline; NST, nonshivering thermogenesis; ST, shivering thermogenesis, TG/FFA, triglyceride/free fatty acid; TD2, thyroxine 5'-deiodinase type 2; T3, triiodothyronine; T4, thyroxine; UCP-1, uncoupling protein 1.

INTRODUCTION

Humans exposed to a compensable cold condition can maintain their core temperature by reducing heat loss through peripheral vasoconstriction and by increasing metabolic heat production through the stimulation of ST and NST. ST is composed of involuntary, asynchronous skeletal muscle contractions resulting in producing the largest amount of heat in cold exposed adult humans (Haman *et al.* 2010). In contrast, NST includes heat-production mechanisms, which liberate chemical energy without the involvement of muscle contractions (Jansky, 1973). Hormones play a central role in regulating the NST responses. A review by Leppäluoto *et al*. (2005) reported that NA is the main stress hormone released and plays a central role in maintaining temperature homeostasis during cold exposure. For example, NA stimulates cold-induced NST through the activation of UCP-1 in BAT (Cannon & Nedergaard, 2004; Richard *et al*. 2010; Richard & Picard, 2011) and through TG/FFA cycling in adipose tissue in rodents (Garofalo *et al.* 1996). NA could also induce NST by increasing TD2 activity in human skeletal muscle via cAMP (Hosoi *et al*. 1999), therefore activating Ca^{2+} cycling (Himms-Hagen 2004). The exact contribution towards NST for each of these processes in adult humans is still unknown. However, recent evidence demonstrating a greater than anticipated presence of BAT in adult humans, suggests that there is significant potential for this tissue to be a considerable contributor to cold-induced thermogenesis (van Marken Lichtenbelt *et al*. 2009; Virtanen *et al*. 2009; Ouellett *et al*. 2011). In view of this evidence, new work is needed to determine whether cold-induced NST can be potentiated to increase overall thermogenesis and/or reduce ST. In this context, could such an increase in NST be accomplished through the ingestion of specific compounds?

Several studies have been conducted using green tea and its components to increase energy expenditure in thermoneutral conditions (Dulloo *et al.* 1999; Rudelle *et al*. 2007; Bérubé-Parents *et al*. 2009). In one such study, Dulloo *et al.* (1999) showed that green tea (270mg of EGCG and 150 mg of caffeine) increased thermogenesis in thermoneutral conditions over a 24h 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. The most abundant and active form of catechin in green tea is EGCG (Chantre & Lairon 2002; Kovacs & Mela, 2006). The thermogenic effect of EGCG is associated with the inhibition of COMT, an enzyme that degrades NA in the synaptic clef (Chinet & Durand 1979). As a result, this inhibition increases the time of action of NA on target tissues such as BAT (Dulloo *et al.* 2000; Dulloo 2002; Shixian *et al*. 2006). On the other hand, the thermogenic action of caffeine resides in the inhibition of phosphodiesterase activity, an enzyme that breaks down NA-stimulated cAMP (Wolfram *et al.,* 2006). cAMP is a critical intracellular mediator (secondary messenger) in the action of catecholamines in the regulation of thermogenesis (Dulloo, Seydoux & Girardier, 1992). When ingested together in thermoneutral conditions, EGCG and caffeine synergistically stimulate thermogenesis (Dulloo *et al*. 1999; Rudelle *et al*. 2007). Much less is known about the effects of these compounds when ingested during cold exposure. For example, MacNaughton *et al.* (1990) reported a 30% increasing in oxygen consumption when cold-exposed men (2hrs in air at 5°C) ingested a gel capsule containing 385mg (5mg/kg) of caffeine. While this effect alone is significant, to date, the combined effects of both compounds on thermogenic processes have never been verified during cold exposure.

Therefore the purpose of this 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 heat production and muscle shivering intensity were quantified in non cold-acclimatized young men ingesting 1600mg of EGCG and 600mg of caffeine. It was hypothesized that, when given at the onset of cold exposure, these compounds would increase NST thus reducing overall shivering activity in 4 large muscles known to contribute significantly to ST during cold exposure (Haman *et al*. 2004a; 2004b; Haman *et al.* 2011). An increase in NST may be expressed differently between individuals due to dissimilarities in the metabolism and absorption of EGCG and caffeine as well as to variations in the prevalence of BAT and the cold response. The stimulation of NST will be assumed if, when compared to a placebo: 1) oxygen consumption increases when given EGCG + caffeine despite a similar shivering intensity or 2) shivering intensity decreases when given EGCG + caffeine for a similar oxygen consumption.

METHODS AND MATERIAL

Subjects

Eight healthy, non-cold acclimatized men volunteered for this study conformed to the standards set by the latest revision of the *Declaration of Helsinki* and approved by the Health Sciences Ethics Committee of the University of Ottawa with the written informed consent of all participants. Exclusion criteria were the following: cold acclimated (e.g. outdoor workers), percent body fat >15%, smokers, consumption of > 200mg/d of caffeine and use of dietary supplements or stimulants. Anthropometric measurements (height, weight, percent body fat) and maximal oxygen consumption (CSEP treadmill protocol) were taken prior to the first experimental session (Table 2.1).

Selection of appropriate dosage of EGCG and caffeine

Based on results from previous studies (Dulloo *et al.* 1999; Chow *et al*. 2001; Ullmann *et al*. 2003; Gregersen *et al.* 2009) an extract containing 1600mg of EGCG combined with 600mg of caffeine was ingested. This is considered the maximum amount that can be given without detectable adverse effects (Ullmann *et al*. 2003; Bérubé-Parent *et al*. 2005). This was ingested from the onset of the 3h cold exposure, given that the desired effects of these compounds peaks at about 2h (Unno *et al*. 1996).

Experimental protocol

Each subject participated in two experimental trials, separated by at least seven days. The order of the trials was randomly assigned and followed a single-blind, balanced, cross-over design. Participants ingested EGCG and caffeine (EXP) or a placebo (CON). 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 7h30 to 14h30. Participants refrained from consuming caffeine, alcohol and avoided physical activity 24h prior to the trials. The last evening meal, ingested between 18h00 h and 20h00 , was standardized (3220 kJ or 770 kcal, 42% CHO, 28% fat and 30% protein) and subjects were asked to report to the laboratory at 7h30 the next morning after a 12-14 h fast.

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 (LCS, three-piece high density, Allen-Vanguard Inc., Ottawa, ON). They were instrumented with thermal probes, a heart rate monitor and electromyography (EMG) electrodes. Maximal voluntary contraction (MVC) measurements were done for each recorded muscle. Afterwards they remained seated for 60 min at ambient conditions (22.7± 0.4 °C) under a canopy. Following this baseline period, subjects were asked to empty their bladder for a second time and ingested either the placebo or EGCG and caffeine treatment (with 250mL of water). The LCS was then perfused with 15°C water using a temperature-controlled circulation bath (Thermo Neslab Refrigirated RTE- 7 Bath Circulator, Ottawa, Canada). Thermal response, metabolic rate and muscle activity were measured continuously during baseline and during the 180 min cold exposure. Heart rate and thermal comfort were measured every 15 minutes throughout the whole trial.

Thermal response

Rectal (T_{rec}) and mean skin temperature ($\overline{T}_{skin}$) were monitored continuously prior to and during cold exposure using a peadiatric rectal (Mon-a-therm general purpose,

Mallinckrodt Medical Inc, St Louis, MO, USA) and heat flux transducers (area-weighed equation from 12 sites: forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings and hand (Hardy & Dubois, 1938), respectively).

Metabolic measurements

The composition of inspired (F_i) and expired air (F_e), as well as barometric pressure (BP) and temperature, were measured using a circuit respiratory system (FoxBox Field Gas Analysis System, Sable systems, Las Vegas, USA). Water vapor partial pressure of inspired (WVP_i) and expired (WVP_e) air were measured using a flow-through water vapor analyser (RH-300 Water Vapor Analyser, Sable Systems, Las Vegas, USA). Flow rate of expired gases (FR_e) was controlled using a mass flow controller and pump (Flow Generator/Controler, Sable System, Las Vegas, USA). Relative Humidity of inspired (F_iH_2O) and expired (F_eH_2O) air were calculated with the following equation respectively:

$F_iH_2O = BP \times WVP_i$	(1)

$F_eH_2O = BP \times WVP_e$	(2)

Inspired Flow rate was calculated using the following equation:

$FR_i = FR_e\ (1 - F_eO_2 - F_eCO_2 - F_eH_2O) / (1 - F_iO_2 - F_iCO_2 - F_iH_2O)$	(3)

Oxygen consumption rate ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were calculated using the following equations respectively:

$\dot{V}O_2 = (FR_i \times F_iO_2) - (FR_e \times FeO_2) \times 1000$	(4)

$\dot{V}CO_2 = (FR_e \times F_eCO_2) - (FR_i \times F_iCO_2) \times 1000$	(5)

Area under curve (AUC) was used to represent total oxygen consumption (VO_2) using middle Riemann sum.

$AUC\ VO_2 = \sum \dot{V}O_2\ [(y_1 - y_i)/2 \times Dt]$	(6)

Where *y* represents the $\dot{V}O_2$ value in $ml \bullet kg^{-1} \bullet min^{-1}$ and Dt is the time interval of 30 min.

Heat production

Heat production (H_{prod}) was calculated by indirect respiratory calorimetry corrected for protein oxidation. Total protein (RP_{ox}), carbohydrate (RG_{ox}) and lipid (RF_{ox}) oxidation rates (in $g \bullet min^{-1}$) were calculated as previously described by Haman *et al.* 2002 :

$RP_{ox}\ (g \bullet min^{-1}) = 2.9 \times UREA_{urine}\ (g \bullet min^{-1})$	(7)

$RG_{ox}\ (g \bullet min^{-1}) = 4.59 \times \dot{V}CO_2\ (l \bullet min^{-1}) - 3.23 \times \dot{V}O_2\ (l \bullet min^{-1})$	(8)

$RF_{ox}\ (g \bullet min^{-1}) = -1.70 \times \dot{V}CO_2\ (l \bullet min^{-1}) + 1.70 \times \dot{V}O_2\ (l \bullet min^{-1})$	(9)

where $\dot{V}CO_2\ (l \bullet min^{-1})$ and $\dot{V}O_2\ (l \bullet min^{-1})$ were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l \bullet g^{-1}$, respectively). RP_{ox} was estimated

from urinary urea excretion ($UREA_{urine}$) in urine samples collected after 60 min of baseline measurements, and at 180 min in the cold. Urinary concentrations were determined using a QuantiChrom™ Urea Assay Kit, BioAssay System (Hayward, CA, USA). Energy potentials of 16.3 $kJ \bullet g^{-1}$ (carbohydrates), 40.8 $kJ \bullet g^{-1}$ (lipids), and 19.7 $kJ \bullet g^{-1}$ (proteins) were used to calculate the relative contributions of each fuel to total heat production (Elia 1991; Péronnet & Massicotte 1991).

Determination of shivering intensity

Shivering intensity was measured using surface EMG. EMG sites were located on the right side of the body on the following muscles: *trapezius* (TR), *pectoralis major* (PE), *Rectus abdominis* (RA) and *rectus femoris* (RF). Raw EMG signals were analyzed with the use of custom-designed MATLAB algorithms (Mathworks, Natick, MA). 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) subtracted from RMS shivering (RMS_{shiv}) as well as RMS_{mvc} values. EMG_{shiv} was then normalized to RMS_{mvc} by using the following equation:

$$EMG_{shiv}\ (\%MVC) = \frac{RMS_{shiv} - RMS_{baseline}}{RMS_{mvc} - RMS_{baseline}} \quad (10)$$

AUC was used to represent total shivering intensity using middle Riemann sum.

AUC shivering intensity = $\sum EMG_{shiv}\ [(y_1-y_i)/2\ \times Dt]$	(11)

Where *y* represents the EMG_{shiv} value in %MVC and Dt is the time interval of 30 min. Area under curve was calculated for all muscles separately and the sum of their mean was used to represent AUC for total shivering intensity. Burst rate (burst/min) was also measured as described previously by Haman *et al.* (2004a).

Thermal comfort and heart rate

Thermal comfort of participants was monitored using a thermal comfort scale, ranging from 5 (being the hottest) to -5 (being the coldest). Heart rate was measured using a Polar heart rate monitor (Polar FS2C Fitness Heart Rate Monitor System, USA). Both measurements were taken every 15 min for the complete duration of the trial.

Interindividual differences

The VO_2 and shivering intensity values for CON were compared to those of the EXP condition in order to determine if all subjects responded the same way to the treatment during the cold exposure. After calculating AUC for both VO_2 and shivering intensity, the CON values were compared to the EXP values. The difference between the two conditions are expressed as percent change (%), relative to CON. A positive value means that the variable increased in the EXP condition compared to CON. These changes (%) between both conditions will be determinants of the interindividual differences observed when EGCG and caffeine were given during cold.

Statistical Analysis

Changes in T_{rec}, $\overline{T}_{skin}$, HR, $\dot{V}O_2$ and shivering intensity were assessed by a two-way ANOVA for repeated measures to study the main effect of time and condition, as well as their interaction (SPSS for Mac version 18.0, Chicago, IL). Significant interaction of time and condition for T_{rec} was followed up with a 2 (time) X 2 (condition) repeated measure ANOVA. Significant differences in time were followed up by a Bonferoni *Post-Hoc* test. Student paired T-tests were done to compare AUC between the two conditions for shivering intensity and total VO_2. Statistical differences were considered significant when $p < 0.05$. All values given are mean ± standard error of mean (s.e.m).

RESULTS

Thermal response

Changes in T_{rec} and $\overline{T}_{skin}$ are presented in Fig. 2.1. T_{rec} remained constant at 37.1±0.1°C during the first 120 min for both conditions, with a slight significant decrease from baseline and the last 30 min of cold exposure for CON (36.7±0.1°C) and EXP (36.8±0.2°C; $p<0.001$). No significant difference was found between the two conditions. $\overline{T}_{skin}$ decreased by ~19% in both conditions during cold exposure (from 34.1± 0.2°C to 28.5 ± 0.5°C in CON and from 34.1 ± 0.2°C to 28.7± 0.4°C in EXP; $p<0.001$).

Metabolic response

Changes in the whole body metabolic rate shown in Fig.2.2 are expressed as $\dot{V}O_2$ in $L \cdot min^{-1}$. $\dot{V}O_2$ increased 1.8-fold from baseline values (from 0.220± 0.01 $L \cdot min^{-1}$ to 0.407± 0.04 $L \cdot min^{-1}$) in CON and by 1.9-fold (from 0.220± 0.01 $L \cdot min^{-1}$ to 0.428± 0.03 $L \cdot min^{-1}$) in EXP after 180 min of cold ($p=0.001$). A difference was found for $\dot{V}O_2$ between both conditions ($p=0.001$). Area under curve (Fig. 2.4) for total oxygen consumption (VO_2) was significantly higher in EXP (33.3±5.5 L O_2) compared to CON (25.3±5.1 L O_2; $p=0.03$). Area under curve for total H_{prod} (Fig. 2.5) was also found significantly higher (10.7%) in EXP (1535±112 kJ) compared to control (1372 ±106 kJ; $p=0.002$).

Shivering intensity

TRA and PEC muscles were selected to represent shivering intensity, since minimal shivering occurred in RA and RF. Mean shivering intensity, expressed as %MVC, is presented in Fig.2.3. During cold exposure, shivering intensity increased over time but was

31.7% greater in CON compared to EXP (p=0.01). The area under curve shown in Fig.2.4 for total shivering activity was also significantly lower (20.9%) in EXP (361.3±80.6 %MVC•min) compared to CON (457.1±99.4 %MVC•min; p= 0.007). No significant differences were found in burst rate (data not presented).

Thermal comfort and heart rate

No significant difference was found for thermal comfort between both conditions. (EXP 2.1±0.3 compared to CON 2.1±0.3). Mean heart rate was slightly higher in EXP (64±4bpm) compared to CON (62±3bpm), but again with no significant difference between conditions.

Interindividual differences

The interindividual differences for VO_2 and shivering intensity are represented in Figure 2.6. The difference between the two conditions are expressed as percent change (%), relative to CON for each subject individually (n=8) and their mean ($\overline{x}$). A positive value means that the variable increased in EXP condition compared to CON. All values for shivering decreased in the EXP condition compared to CON, except for subject 5, where shivering increased 0.2 fold, but VO_2 increased 1.7 fold. In most subjects VO_2 increased in EXP compared to CON, except for subject 2 and 3, and shivering decreased. The greatest decrease in shivering was observed in subject 3 (0.4 fold).

Table 2.1. Characteristics of subjects (n=8).

Age (years)	**23 ± 1**
Mass (kg)	**75 ± 4**
Height (cm)	**177 ± 4**
Percent Body Fat (%)*	**13.4 ± 0.9**
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)†	**56.9 ± 1.8**

* Underwater weighing; Brosek *et al.* (1963).

† CSEP incremental treadmill exercise test.

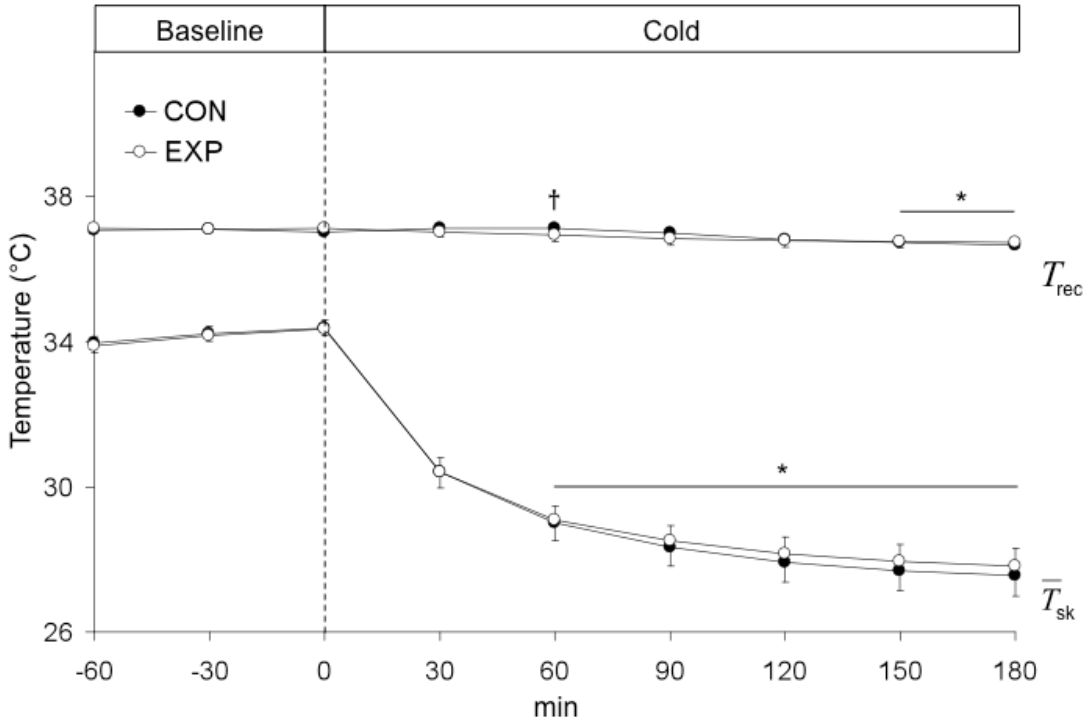

Figure 2.1. Change in rectal (T_{rec}) and mean skin ($\overline{T}_{skin}$) temperature before (Baseline) and following the ingestion of a placebo (CON) and 1600mg EGCG + caffeine (EXP), in men during mild cold exposure using a liquid condition suit (water at 15°C).

*Significantly different from baseline.

† Effect of interaction of time and condition.

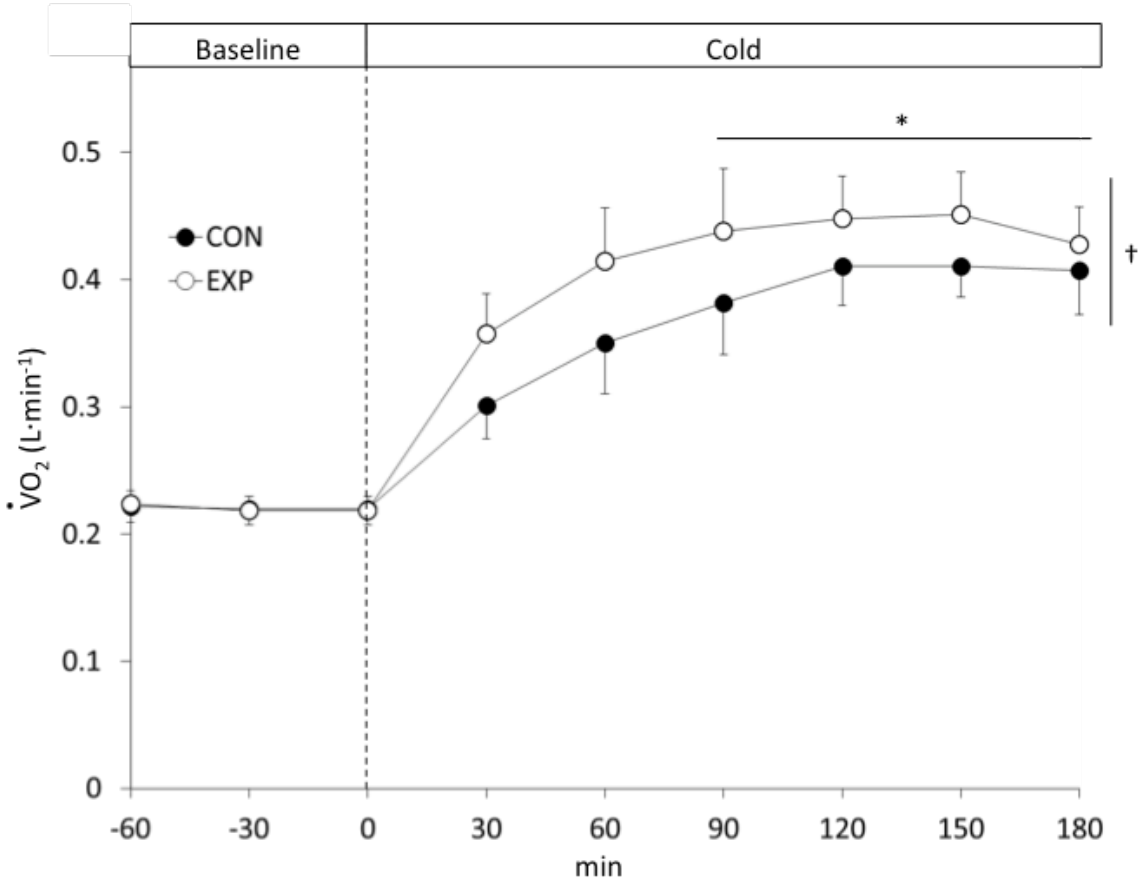

Figure 2.2. Oxygen consumption ($\dot{V}O_2$) before (Baseline) and following the ingestion of a placebo (CON) and 1600mg EGCG + caffeine (EXP), in men during mild cold exposure using a liquid condition suit (water at 15°C).

*Significantly different from baseline.

† Significantly different between conditions.

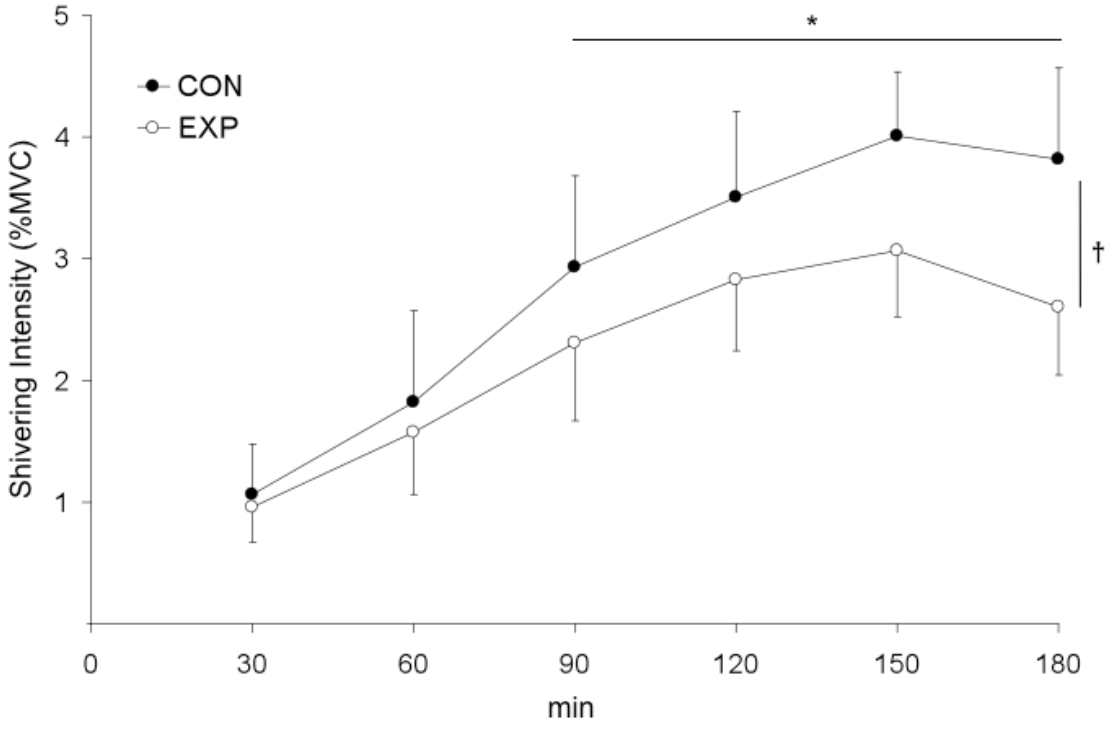

Figure 2.3. Mean shivering intensity (TRA & PEC) before (Baseline) and following the ingestion of a placebo (CON) and 1600mg EGCG + caffeine (EXP), in men during a mild cold exposure using a liquid condition suit (water at 15°C).

*Significantly different from baseline.

† Significantly different between conditions.

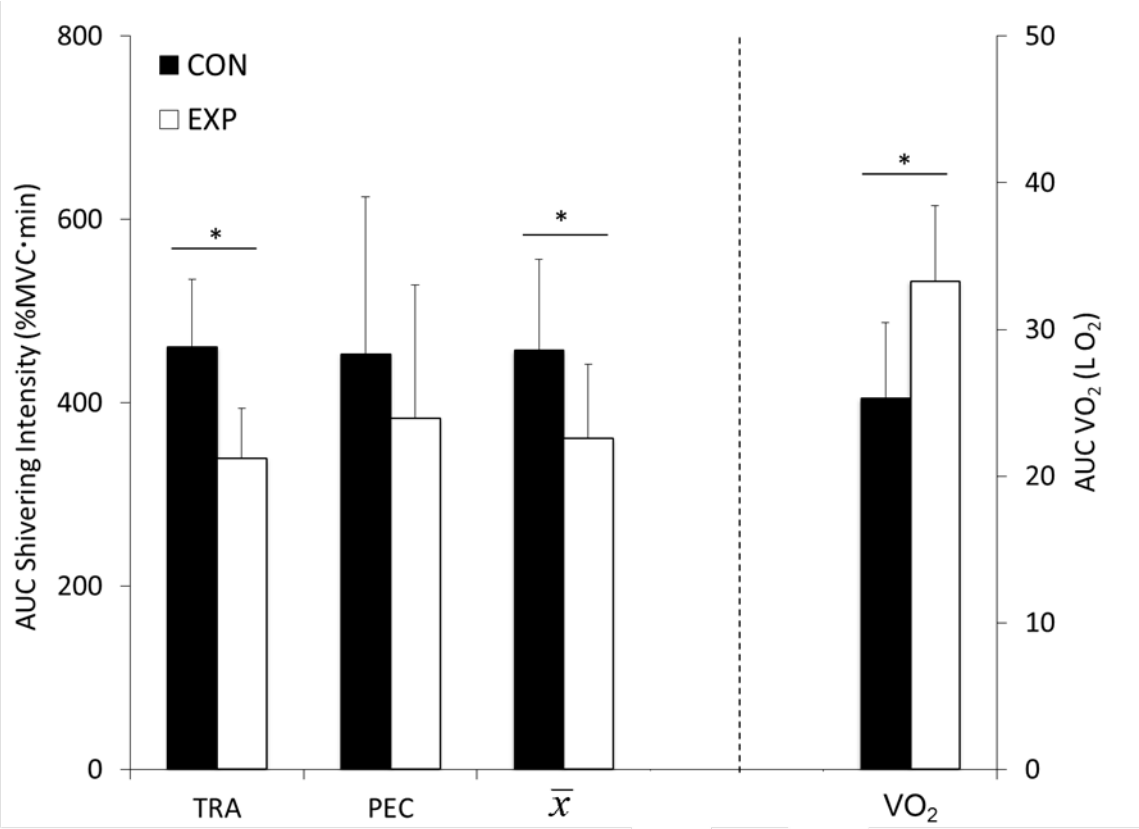

Figure 2.4. Area under curve (AUC) for shivering intensity and oxygen consumption (VO_2) during a 3 hour mild cold exposure using a liquid condition suit (water at 15°C), when ingesting a placebo (CON) and 1600mg EGCG + caffeine (EXP). AUC for shivering intensity is represented for (TRA & PEC) muscles individually, and their mean ($\bar{x}$), and AUC VO_2 is represented for whole body.

*Significantly different between the two conditions.

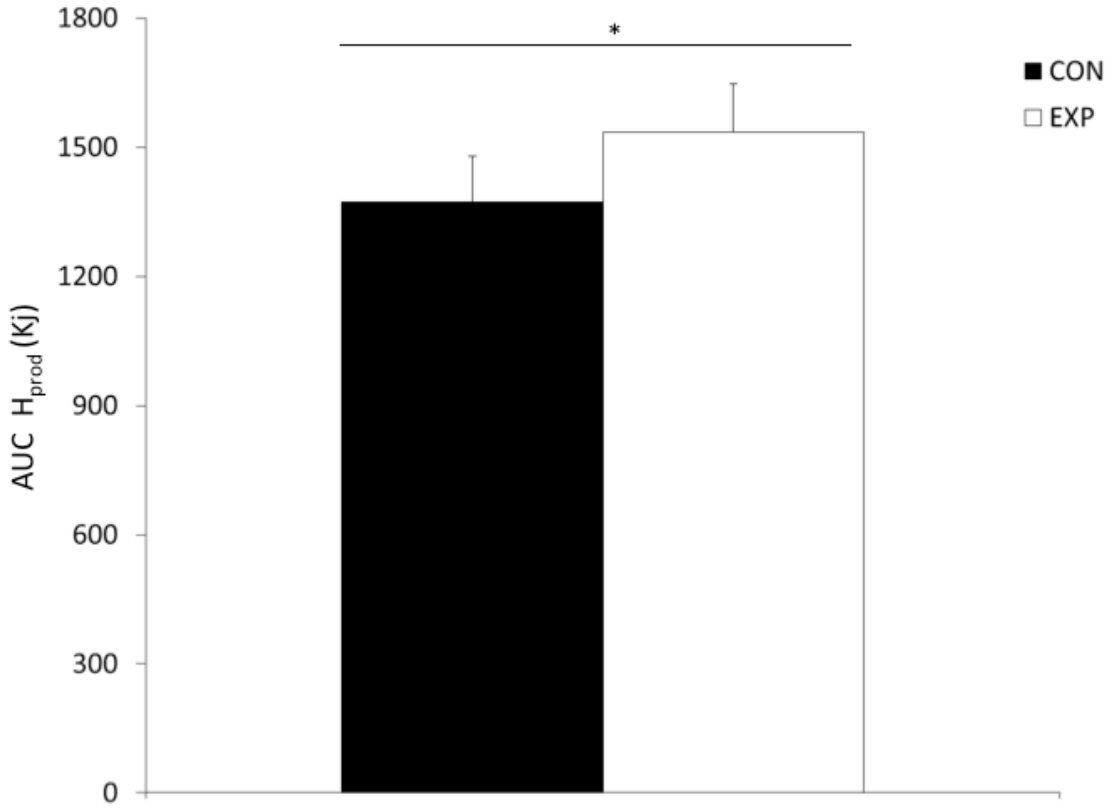

Figure 2.5. Area under curve (AUC) for total heat production (H_{prod}) during a 3 hour mild cold exposure using a liquid condition suit (water at 15°C), when ingesting a placebo (CON) and 1600mg EGCG + caffeine (EXP).

*Significantly different between the two conditions.

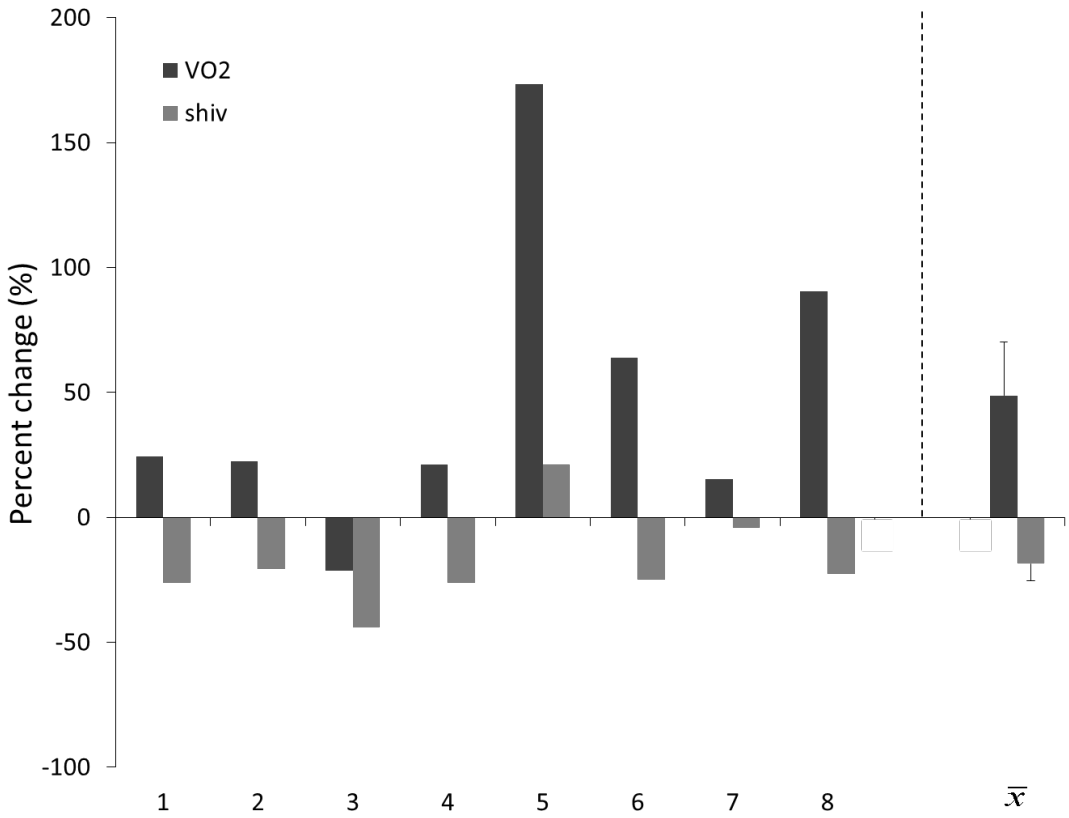

Figure 2.6. Interindividual variability to treatment (1600mg EGCG and 600mg caffeine) and cold response for VO_2 and shivering intensity (shiv). The differences between the two conditions are expressed as percent change (%), relative to CON for each subject individually and their mean ($\bar{x}$). A positive value means that the variable increased in EXP condition compared to CON.

DISCUSSION

The contribution of NST in cold exposed adult humans has long thought to be negligible. This study shows that the stimulation of NST with the combined ingestion of EGCG and caffeine is sufficient to reduce shivering EMG activity after a 3hr cold exposure by ~32% in young men (Figure 2.3). Results showed that total oxygen consumption was ~25% greater under the experimental condition compared to placebo (Fig. 2.4). Heat production over the 3hrs cold exposure was also found to be ~11% greater in the experimental condition (Fig. 2.5). This increase in total oxygen consumption and heat production was accompanied by a ~21% decrease in total shivering EMG intensity in active muscles (Fig. 2.4). These combined effects of EGCG and caffeine ingestion support both predictions that the stimulation of NST would increase thermogenic rate and reduce the contribution of ST. However, it is important to note that while a similar trend was observed in all subjects, the extent of this response was variable between individuals (Figure 2.6).

Changes in thermogenic rate

In cold exposed humans, total thermogenic rate and associated $\dot{V}O_2$ is accounted for by the combined activation of NST and ST. In the present study, the stimulation of NST could not be measured directly but could be assumed from the increase in total oxygen consumption accompanied by the decrease in shivering activity (Jansky 1973; Cannon & Nedergaard 2011). Using indirect calorimetry combined with electrophysiological measurements of skeletal muscle shivering activity, changes in VO_2 and shivering EMG intensity were compared in young non-cold acclimatized young men. These men were exposed to a 3h of cold stress at 15°C, following the ingestion of 1600mg of EGCG and

600mg of caffeine or placebo (blinded, crossover design). In these circumstances, by comparing VO_2 and shivering intensity, we can assume that any differences found of these variables between the two treatments can be attributed to the stimulation of NST by EGCG and caffeine.

In both conditions, the cold exposure elicited a predictable increase in metabolic rate accompanied by an increase in shivering (Figure 2.2 & 2.3). Figure 2.2. shows a 1.9 fold increase in $\dot{V}O_2$ for EXP compared to a 1.8 fold increase in CON. A greater increase in $\dot{V}O_2$ for EXP compared to CON can be observed during the first 90 min of cold. This might be explained by the rapid absorption and action of caffeine followed by that of EGCG. Indeed, caffeine is absorbed faster and its peak plasma concentration can be reached within 30 min compared to 60 min for EGCG (Nakagawa *et al*. 1999; Bchir *et al*. 2006).
Total VO_2 for the EXP condition was ~25% higher than in CON (Figure 2.4.). This is in agreement with the findings of Vallerand *et al.* (1989), where total VO_2 for 180 min of cold exposure was 17.6% greater than placebo, when an ephedrine-caffeine mixture (2.5 and 1.0mg/kg body mass respectively) was ingested. In their study, total energy expenditure in kJ/3hr cold exposure increased by 19% in the ephedrine-caffeine condition compared to control. Our study showed similar results with an 11% increase in total H_{prod} in the EXP condition (1535±112 kJ) compared to CON (1372±106 kJ). Therefore, the increase in metabolic rate can be attributed to the effect of the treatment. Unfortunately no EMG recordings were available in their study, thus they were not able to conclude if this increase in VO_2 was associated with a greater muscle contraction or not. In contrast, shivering activity was measured in the present study.

Changes in EMG shivering activity

EMG was performed on 4 muscles and shivering intensity was measured throughout the 3h cold exposure. The increase in VO_2 and H_{prod} for the EXP condition was accompanied by a decrease in EMG activity compared to CON. Indeed, shivering intensity was reduced by ~32% in EXP compared to CON by the end of the cold exposure (Figure 2.3). AUC for total shivering intensity also decreased significantly of ~21% in EXP compared to CON (Figure 2.4). Together, these results show that for a similar and even higher metabolic response in EXP compared to CON, shivering intensity decreased with the ingestion of EGCG and caffeine. The exact mechanisms behind this reduction in shivering by EGCG and caffeine is unknown. The stimulation of shivering is regulated by feedfoward mechanisms activated by peripheral thermoreceptors (Tanaka *et al*. 2006; Nakamura & Morrisson 2008; McAllen *et al*. 2009). Thermosensory pathways from skin thermoreceptors to the preoptic area, mediating feedforward signaling, are required to elicit rapid thermoregulatory responses (e.g.. shivering) to changes in environmental temperature (Nakamura & Morrison 2008; 2010). In our study, the decrease in $\overline{T}_{skin}$ for the EXP condition (5.4°C) is similar to CON (5.6°C), as well as the decrease in T_{rec} for EXP (0.3°C) compared to CON (0.4°C). These slight differences in temperature were not enough to be responsible for the differences in shivering intensity between conditions. Yet there is a possibility that the stimulated NST response (e.g. from BAT) induced a feedback response leading to a reduction of shivering, but this is speculative and remains unknown. What can be confirmed, however, is that mechanisms other than involuntary muscle contractions must occur in order to maintain or even increase the metabolic response when shivering is decreased. Therefore ST is being partly replaced by NST with the EXP treatment.

Estimation and stimulation of NST

The present thesis focused on measuring whole-body changes in NST and ST. Changes in NST were estimated by measuring total oxygen consumption and shivering EMG activity simultaneously. The cold-induced NST mechanisms stimulated by EGCG and caffeine in this study can only be speculated based on previous studies. Nevertheless, the suggested NST pathways all share one common thing; their activation by cold-induced sympathetic stimulation leading to a release in NA subsequently triggering the activation of cAMP-responsive pathways.

It is known that polyphenols found in teas have the potential to inhibit COMPT, the enzyme that breaks down NA in the synaptic clef (Borchard & Huber 1975). This increase of synaptic NA creates an overflow into the circulation (Dulloo *et al*. 1999) and the action of NA on target tissues is therefore amplified. Additionally, intracellular cAMP is increased with the inhibition of phosphodiesterase by caffeine (Dulloo, Seydoux & Girardier 1992). As shown by Dulloo *et al.* 2000, when NA was released (by ephedrine), EGCG and caffeine increased respiratory rate of *in vitro* intra-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* (Dulloo *et al.* 1999). Conversely, EGCG must be given with a minimal amount of caffeine in order to get the desired thermogenic effect (Gregersen *et al*. 2009). Together EGCG and caffeine exert a synergic effect on tissues, such as BAT, contributing to NST.

In the present study, BAT respiratory rate could not be measured, but based on Dulloo *et al*. (2000), we can speculate that together EGCG and caffeine have the potential to stimulate cold-induced NST by increasing the contribution of this tissue. Since BAT is

sympathetically innervated (Bartness & Song 2005; Bartness, Vaughan & Song, 2010), the inhibition of COMT by EGCG would increase the action of cold-released NA on adrenergic receptors of BAT. Simultaneously, caffeine would increase intracellular cAMP, therefore activating the release of fatty acid and inducing heat production from activated UCP-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 equilibrium. The pumping of ions through the Ca^{2+}-ATPase requires the hydrolysis of ATP and results in heat dissipation (Block 1994; van Marken Lichtenbelt & Daanen 2003). This mechanism could greatly contribute to NST, since skeletal muscle represents ~ 42% of body mass in adult humans, (Rolf & Brown 1994). Indeed, a study by Astrup *et al.* (1985) showed a greater thermogenic contribution from ephedrine stimulated skeletal muscle compared to BAT. In their study, ephedrine increased oxygen consumption of $40ml \bullet min^{-1}$ in skeletal muscle compared to 10 $ml \bullet min^{-1}$ in perirenal BAT. Just like cold exposure, ephedrine stimulates the release of NA (Dulloo & Miller, 1984). This thermogenic response could be increased with EGCG by maintaining a higher level of NA in circulation or by prolonging its action in the synaptic cleft.

Another heat producing mechanism that could be stimulated by EGCG and caffeine is triglyceride and free fatty acid (TG/FFA) cycling. The release of FFA is also stimulated by NA and activated through cAMP. Vallerand *et al*. (1989) speculated that the ingestion of a caffeine-ephedrine mixture in the cold (air at 10°C) had an effect on increasing TG/FFA cycling. Their results showed an increase in plasma glycerol and TG in the caffeine-ephedrine condition, with practically unchanged FFA levels and no increase in lipid

oxidation compared to placebo. This goes along the lypolisis-enhancing effect of caffeine in thermoneutral condition (Hetzler *et al*., 1990). Caffeine enhances lypolisis of TG and the release of FFA, but not necessarily their oxidation (Asheson *et al*. 2004). Later, Vallerand *et al*. (1999) showed that a mild cold exposure increased TG/FFA cycling through the activation of the sympathetic nervous system and release of NA. Caffeine given with EGCG has the potential of increasing TG/FFA cycling in the cold through the release of NA, thus increasing NST. Possible sites for this to occur would be white adipose tissue (Garofalo *et al*. 1996) and liver (Rolf & Brown, 1997; Kim, Saidel & Kalhan 2008).

Based on the results obtained in this 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 are contributing to this increase in NST.

Interindividual responses

Despite standardizing as much as possible for morphology, percent body fat, diet, and level of cold acclimation among the participants, large interindividual differences were observed in the total VO_2 and shivering response (Fig. 2.6). In general, the EGCG and caffeine treatment resulted in a decrease in shivering for most subjects except one (subject 5). In this case, shivering was slightly increased with the treatment, but total VO_2 almost doubled in EXP compared to CON. These differences among subjects are not surprising and may be either a result of the rate of absorption and elimination of EGCG (Chow *et al.* 2001;

Lee *et al*. 2002) and caffeine (Cornelis *et al.* 2006; Graham *et al.* 2008), of differences in the cold response (Haman *et al*. 2004b) and of differences in BAT distribution, volume and its activity (Heaton *et al*. 1972; Ouellette *et al*. 2011).

In conclusion, this study shows that the contribution of NST toward total heat production can be stimulated by the combined ingestion of EGCG and caffeine in cold exposed humans. When ingested before a mild cold exposure, EGCG and caffeine increased total oxygen consumption, as well as total heat production, and decreased shivering intensity compared to control. This increase in oxygen consumption accompanied by a similar decrease in EMG activity demonstrates the replacement of ST by NST. Possible contributing mechanisms to NST could be UCP-1 in BAT, Ca^{2+} cycling in skeletal muscle and TG/FFA cycling in white adipose tissue and liver. The ability of EGCG to inhibit COMT and of caffeine to inhibit phosphodiesterase, increases the noradrenaline and cAMP-dependent mechanisms responsible for NST. Although some interindividual differences were observed, EGCG and caffeine seems to stimulate NST either by a notable increase in VO_2 or a decrease in shivering intensity.

Given the high energetic costs of ST as well its influence on motor control, stimulating NST pathways may prove important for cold exposures and under conditions requiring fine motor control (e.g. cold survival). Additionally, increase evidence of BAT in adult human and its contribution to cold-induced NST has created a growing interest in potential ways for its stimulation.

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CHAPTER 3: GENERAL CONCLUSION

The general objective of this thesis was to demonstrate that green tea extracts have thermogenic properties in cold conditions. More precisely, that the combined ingestion of EGCG and caffeine can stimulate cold-induced NST in men exposed to a mild cold.

CHAPTER 1 reviews cold-induced NST pathways and shows how they can be stimulated through the release of NA *via* the sympathetic nervous system and through the activation of cAMP –dependent mechanisms. It also explains in which way these pathways can contribute to the increase of NST by EGCG and caffeine.

The experiments in CHAPTER 2 confirmed the hypothesis that the ingestion of EGCG and caffeine can stimulate cold-induced NST in men exposed to a mild cold. Evidence showed an increase in oxygen consumption and heat production accompanied by a decrease in shivering intensity when EGCG and caffeine were ingested compared to placebo. UCP-1 in BAT, Ca2+ cycling in skeletal muscles and TG/FFA cycling in white adipose tissue and liver are potential NST mechanisms that could have been stimulated by EGCG and caffeine. Due to certain limitations, the activation of these pathways by EGCG/caffeine could only be assumed based on previous findings. Although the activation of BAT in the cold is now well known, it would have been interesting to measure its activation using PET/CT and compare it between both conditions. For the measurement of TG/FFA cycling, isotopic enrichment would have been useful to determine its activation by the treatment. As for Ca^{2+} cycling in skeletal muscle, there is no direct evidence that this mechanisms contributes to cold-induced NST in humans and it requires more research. Nonetheless, it has been well documented to occur in birds and fish and certain findings like the presence of TD2 in human skeletal muscles, suggest the possibility of Ca^{2+} cycling as a NST mechanism.

Although it is possible to speculate which NST mechanism were stimulated by

EGCG and caffeine, the means by which shivering decreased remains uncertain. Skin and rectal temperature were not different between the two treatments, thus skin and visceral thermoreceptors were not responsible for this decrease. The possibility arises that other feedback mechanisms would have occasioned this change in shivering intensity.

In view of these findings, it is possible to say that there is evidence of the presence of NST in adult humans exposed to cold. Additionally, NST can be stimulated in the cold by EGCG and caffeine. The quantity of heat that was produced was not affected by the decrease in shivering. This could have potential implications in optimizing the accomplishment of a task in cold conditions. Shivering can alter manual dexterity, therefore increasing the difficulty of a task. The ingestion of EGCG and caffeine could potentially increase the performance in the execution of a task in the cold, by reducing the need to shiver while maintaining heat production. Since this experiment was conducted for a 3hr cold exposure, the long-term effects of the combined ingestion of EGCG and caffeine for a prolonged cold period remain unknown.