THESIS

EFFECT OF SHORT-TERM EPIGALLOCATECHIN-3-GALLATE SUPPLEMENTATION ON THE THERMIC EFFECT OF FEEDING AND RESTING METABOLIC RATE

Submitted by

Mark Charles Lonac

Department of Health and Exercise Science

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MARK CHARLES LONAC ENTITLED: EFFECT OF SHORT-TERM EPICALCATECHIN-3-GALLATE SUPPLEMENTATION ON THE THERMIC EFFECT OF FEEDING AND RESTING METABOLIC RATE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate work

Matthew S. Hickey

Tracy L. Nelson-Ceschin

Christopher L. Melby

Advisor: Christopher Bell

Department Head: Richard G. Israel
ABSTRACT OF THESIS

EFFECT OF SHORT-TERM EPICALCATECHIN-3-GALLATE SUPPLEMENTATION ON THE THERMIC EFFECT OF FEEDING AND RESTING METABOLIC RATE

The sympathetic nervous system (SNS) both provides tonic support of resting metabolic rate (RMR) and mediates approximately one-third of the thermic effect of feeding (TEF), the latter accounting for ~10% of daily energy expenditure. Epigallocatechin-3-gallate (EGCG), the most abundant and bioactive catechin in green tea, inhibits the catecholamine-degrading action of the enzyme catechol-O-methyltransferase, thereby prolonging SNS activation. Accordingly, we hypothesized that short-term EGCG supplementation would augment RMR and TEF in healthy adult humans. METHODS: Sixteen subjects reported to our lab on two mornings (≥ 10 days apart) after 12 hours without food, caffeine, and alcohol and 24 hours without exercise. Each visit followed double-blinded, randomized EGCG or cornmeal placebo supplementation (seven pills over 48 hours, 135 mg EGCG/pill). RMR and respiratory exchange ratio (RER) were determined using the ventilated hood technique for 45 minutes prior to consumption of a liquid mixed-composition meal providing calories equivalent to 40% of RMR. Energy expenditure (EE) and RER were then measured for the first 20 of every 30 minutes for five hours. RESULTS: All subjects (n = 9 males, n = 7 females; age 25 ± 2 yrs, mean ± s.e.; 22.6 ± 1.8% body fat) were weight stable and were washed out from prior green tea or EGCG use for ≥ one month. Results are reported as placebo vs. EGCG. EGCG did not affect RMR or baseline RER (RMR: 1665 ± 84 vs. 1610 ± 89 kcal/day; p = 0.10; RER: 0.82 ± 0.01 vs. 0.83 ± 0.01; p = 0.29). EGCG did not affect the
area under the TEF response curve, expressed as absolute postprandial EE (441,083 ± 22,436 vs. 429,350 ± 24,209 arbitrary units, A.U.; p = 0.22) or the absolute (58,104 ± 5,297 vs. 58,949 ± 5,672 A.U.; p = 0.88) or percentage (3,508 ± 263 vs. 3,647 ± 285 A.U.; p = 0.67) difference between absolute postprandial EE and baseline RMR. No differences between placebo and EGCG were observed for RER during TEF. CONCLUSION: These results indicate that, contrary to our hypothesis, short-term EGCG supplementation does not influence RMR or TEF in healthy adults. This suggests that reported positive benefits of green tea on energy balance are the result of increased physical activity EE and/or decreased energy intake.
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CHAPTER I: REVIEW OF THE LITERATURE

INTRODUCTION

Perturbations to either side of the classic energy balance equation (energy balance (EB) = energy intake (EI) – energy expenditure (EE), where EE is composed of resting metabolic rate (RMR), thermic effect of feeding (TEF), and physical activity EE) may unfavorably influence EB and increase propensity for weight gain, particularly as fat mass. In the United States, an increasing percentage of both children and adults are being classified as overweight or obese (126). While an abundance of research has focused on issues related to EI such as control of appetite, there is good evidence to suggest that aberrations of EE are at least as influential as abnormalities of EI with regard to weight gain (73, 139, 145, 174).

Efforts to favorably alter EB have been directed at both decreasing EI via dietary modifications, behavioral control, or pharmaceutical interventions, and increasing EE through increased physical activity or pharmaceutical means.

Over the past decade, an abundance of research in both animal models and humans utilizing both green tea (Camellia sinensis) and selected green tea extracts has demonstrated biological activities ranging from anti-oxidant (166) and anti-carcinogenic properties (136), positive effects on the cardiovascular system (37, 77, 107, 135), improved glucose tolerance (43, 87), and favorable alterations of substrate utilization (i.e. increased fat oxidation) and EE (54, 190), leading to weight loss (34).

This review of the literature will discuss the potential of the green tea extract epigallocatechin-3-gallate (EGCG) to increase EE by modulating RMR and TEF. We operationally
define RMR as EE measured in a quiet, dimly-lit, thermoneutral environment following either subject self-transport to the laboratory or in-patient housing (i.e. For the purposes of this review, we are not distinguishing, as some authors do, between basal and resting metabolic rate). We define TEF as the absolute or percentage increment in EE above basal RMR. RMR and TEF compose approximately 75% and 10% of total daily EE, respectively (44). It is likely that the sympathetic nervous system (SNS) is the modulator of a considerable percentage of the TEF response, and as such, its mechanistic role in EE, with particular reference to how EGCG may augment TEF, will be discussed following background information on the TEF response.

THERMIC EFFECT OF FEEDING

History and Physiology of the TEF Response

In 1902, Rubner noted a greater increase in EE following ingestion of protein versus sugar or fats, and he termed this the “specific dynamic action of food” (198). Around the same time R.O. Neumann described a process of metabolic wastage that buffered against weight gain during times of excess EI, which he termed “luxuskonsumption” (96). While the meaning behind these terms is still relevant and important, the terms ‘obligatory’ and ‘facultative/regulatory’ thermogenesis put forth in the 1980’s are now favored when describing the mechanism of the TEF response. Additionally, the terms TEF and diet-induced thermogenesis (DIT) are commonly used to describe the increase in EE above baseline, while the terms postprandial EE and postprandial thermogenesis are used to indicate the total amount of thermogenesis following food ingestion, including baseline RMR. Other lesser used terms include heat increment of feeding (HIF), post-meal total energy oxidation (PMTEO), and thermic effect of a meal (TEM).

Obligatory thermogenesis refers to the portion of TEF related to the digestion,
absorption, transport, and storage of nutrients, while facultative or regulatory thermogenesis refers to the excess EE beyond the obligatory component. Obligatory thermogenesis can be theoretically calculated as the summation of the metabolic processes required to handle ingested nutrients. However, in practice, this can be challenging due to difficulty in quantifying the energy cost of, for example, the synthesis of enzymes required for the digestion of nutrients. Another problem arises when considering macronutrient partitioning—different individuals with differing antecedent diets and a different complement of various proteins and their polymorphisms/isoforms may engage in variable macronutrient partitioning. Studies empirically quantifying the energetic value of obligatory thermogenesis have noted that propranolol (a β1- and β2-adrenergic receptor antagonist) infusion during glucose and insulin infusions reduces the increment in metabolic rate to a level on par with that of the theoretical cost of glucose storage as glycogen, or 0.20 kcal/g (1, 4, 62). This value is termed the obligatory component of thermogenesis for an intravenous glucose and insulin infusion.

Additionally, TEF can be broken into ‘cephalic’ and ‘digestive’ phases, with the cephalic phase response occurring during the first 30-40 minutes following meal ingestion, and the digestive (also confusingly called obligatory (97) or gastrointestinal (78)) phase lasting until EE returns to post-absorptive levels. Studies in dogs by Diamond and LeBlanc (50, 51) clearly indicate that both the cephalic and digestive phases contain obligatory and facultative (sympathetic nervous system (SNS)-regulated) components, as the facultative component is abolished following propranolol administration, whereas the obligatory component is unaltered (106).

TEF is a subtype of adaptive thermogenesis, which is “operationally defined as heat production in response to environmental temperature or diet, and serves the purpose of
protecting the organism from cold exposure or regulating EB after changes in diet” (110), and also includes non-shivering thermogenesis in response to cold (167). While some have assumed the facultative TEF response, as a form of adaptive thermogenesis, has the primary purpose of regulating EB, Stock has hypothesized that TEF evolved as a means of regulating an animal’s supply of essential nutrients such as protein and sodium, and that its secondary role in EB has assumed greater importance in recent years, with the advent of high energy- and low nutrient-density diets (174). However, reduced SNS activation (SNSA) likely drives potentially reduced EE in undernutrition (95, 104, 199), thus it is likely that the facultative component of TEF serves the dual function of regulating nutrient supply and EB. It has been noted that evidence is equivocal regarding potential energy savings due to reduced DIT in undernutrition (167).

The onset of the TEF response has been demonstrated to be independent of nutrient composition, and occurs within 5-20 minutes of meal ingestion (159). Depending on the macronutrient content and size of the meal, the response can last anywhere from < 2 hours (88) to > 6 hours (142), and both the magnitude and duration of the response can be potentiated by prior exercise, feeding, and ‘thermogenic status,’ related to conditions such as over- or under-nutrition that may alter SNSA.

Numerous afferent neural signals have been implicated in signaling the brain to increase SNS outflow following a meal. Additionally, different populations of neurons may be involved in the sympathetic cardiovascular versus the sympathetic metabolic/thermogenic response to a meal, and these do not necessarily all discharge simultaneously.

In the cephalic phase, sensory signals related to food ingestion (taste, olfaction, anticipation) can increase sympathetic outflow. Once food has entered the gastrointestinal tract, chemosensitive vagal afferents process information about the luminal contents, and these
afferents synapse in the nucleus of the solitary tract in the brainstem. Following processing in
the brainstem and hypothalamus, SNSA is elicited from these regions of the brain (117, 185).
Spinal nerve afferents also are involved, but are thought to be more responsive to noxious or
injurious stimuli, such as tissue ischemia or inflammation (67). The composition of the luminal
contents also influences the hormonal milieu in the gut, which includes peptides such as
cholecystokinin (CCK), gastric inhibitory peptide, glucagon-like peptide 1, serotonin, and others.
CCK and serotonin in particular may have a role in activating vagal afferents (141).

Gastric distention activates mechanoreceptors in the wall of the stomach, which
connect to vagal afferents, although the role of these receptors in activating TEF is uncertain
(23, 122, 148, 150). Gut and portal vein, but not central, osmoreceptors can also trigger SNS
outflow in response to either hypertonicity or hypotonicity, whereas isotonic saline evokes little
or no response (137). Cardiopulmonary (low-pressure) baroreceptors that respond to changes in
central blood volume following postprandial splanchnic pooling are also activated following a
meal, primarily to counteract the food-induced vasodilation and prevent a fall in blood pressure
(82).

Studies demonstrate that glucose and fatty acid sensors likely lie in the portal vein bed
and stimulate vagal afferents to the nucleus of the solitary tract. Further, there may be
differences between elevated fatty acid concentrations in the portal vs. systemic blood. A role
for amino acids in stimulating afferent signals leading to SNS outflow is unclear (185). Individual
nutrients also act directly on the hypothalamus after crossing the blood brain barrier.

Finally, other hormones investigated for a role in signaling the brain following a meal
include insulin, leptin, and amylin. While plasma leptin concentrations in humans are not
changed following a meal (184), amylin may exert effects both peripherally and centrally (129),
and insulin may activate SNS outflow by acting both directly on the hypothalamus, and indirectly via vasodilation-induced baroreflex activation (185).

Evidence for and Nature of SNS Regulation of the Facultative Component of TEF

To ascertain SNS involvement in the TEF response, several investigators have studied the metabolic response to nutrients under conditions of β-adrenergic blockade, and these examinations have yielded conflicting results (10, 185). Methodological issues such as choice of nutrient (carbohydrate (CHO), fat, protein), route of administration (oral vs. intragastric vs. intravenous), type of CHO (glucose vs. fructose vs. combination), dosage of beta-blocker, and small sample sizes may have been responsible for the conflict.

Schwartz et al. took an alternative approach utilizing clonidine, a centrally acting α2-adrenergic receptor agonist that inhibits SNS outflow, to avoid the confounding influence of propranolol on catecholamine metabolism. Their results demonstrated a 33% reduction in the TEF response to an 800 kcal high-CHO meal, or approximately the entire calculated facultative component of the response (158), when administering clonidine transdermally for 48 hours prior to a TEF measurement.

Taken together, the literature seems to indicate that an SNS-mediated facultative component of CHO-induced thermogenesis is responsible for about one-third of the total thermogenic response. A similar role in protein- and fat-induced responses is not currently supported by definitive evidence (185), although one study using microneurography to assess muscle SNSA (MSNA) demonstrated increases in MSNA following consumption of both fat emulsions and beef fillet, both of which contained very little carbohydrate (59).

Following processing in the brainstem and hypothalamus, an increase in SNS outflow
occurs, but there have been discrepant findings regarding the locus of increased EE due to this increased SNSA following a meal. Discrepancies related to the methodology of measuring SNSA may contribute to the varied findings (66, 189). Also of note, experimentally-induced lesions to the ventromedial hypothalamus in rats reduce postprandial thermogenesis as a result of impaired SNS outflow (117).

It has been known for decades that the SNS modulates non-shivering thermogenesis in rodents via activation of brown adipose tissue (BAT) (149). And while there are many parallels between non-shivering thermogenesis (in response to cold) and TEF (201), for years it was thought that BAT thermogenesis was probably negligible in adult humans, despite its occasional anatomical presence (188). More recent evidence suggests that BAT is indeed active in a large percentage of adults (96% in one 2009 study) in response to cold (153, 188), and thus it may also play a role in TEF.

Other tissues investigated for a potential role in TEF include skeletal muscle, white adipose tissue (WAT), splanchnic tissues, kidneys, heart, and brain. One group attempted to relate the increments in SNSA (measured via MSNA and norepinephrine (NE) spillover) to the increases in metabolic rate in various tissues, but no correlations were found—there was mismatching “between the triggered thermogenic response and the extent of postprandial sympathetic activation” (42). This group did note large increases in SNSA of skeletal muscle and kidney, while they suggested significant increases in oxygen consumption in muscle and adipose tissue, as their observed increases in renal and hepatomesenteric oxygen consumption were insufficient to explain the whole-body increase. Problems associated with using plasma NE measurements to assess postprandial SNSA, such as the fact that alterations in plasma NE clearance can account for up to 50% of the variation in plasma NE levels, may have contributed to the observed mismatching (189).
Other authors concluded that half the increase in postprandial oxygen consumption occurs in splanchnic tissues, while 30-35% occurs in skeletal muscle (83). Astrup’s group demonstrated a primary role for skeletal muscle as opposed to BAT in the thermogenesis induced by ephedrine in humans (8). Furthermore, cardiac and skin sympathetic activation appear to be unchanged following a meal (18, 42), while a 25% increase in brain metabolism has been noted upon presentation of appetitive food stimuli (viewing, smelling, and cotton swab tasting) (192), and the contribution of adipose tissue is likely small (170).

Several possibilities exist for the mechanism of increased EE elicited by SNSA, including increased dissipation of the mitochondrial electrochemical proton gradient (i.e. uncoupling of substrate oxidation and \( \text{ADP} \rightarrow \text{ATP} \) phosphorylation), increased activity of futile metabolic cycles such as the alpha-glycerophosphate shuttle, the fructose-6-phosphate—fructose-1,6-bisphosphate cycle (124, 174), fatty acid cycling, and the glucose/glycogen cycle (202), increased ion shuttling across membranes via \( \text{Na}^+/\text{K}^+ \) or \( \text{Ca}^{2+} \) ATPases (21, 172, 185), and accelerated protein turnover (202). Which mechanism predominates may depend on the degree of SNSA to various tissues involved in the thermogenic response, the mass of each of those tissues, and the protein complement of each tissue (e.g. uncoupling protein-1 is primarily located in BAT).

Some authors have investigated potential parasympathetic nervous system (PNS) mediation of the TEF response. Studies involving PNS inhibition with atropine show reduced thermogenesis in response to oral (122) but not intravenous (49) glucose, demonstrating that an intact PNS affects the rate of digestion, absorption, and storage of ingested nutrients via its influence on gut motility and hormone and digestive enzyme secretion (49).

Thus it appears that the obligatory portion of TEF following normal meal ingestion is mediated via the PNS, while facultative thermogenesis is mediated by the SNS. Thus, these two
branches of the autonomic nervous system, classically thought to work in opposition, actually simultaneously contribute to the TEF (albeit on different facets of the response).

**Role of Catecholamines and Adrenergic Receptors in TEF**

NE is the primary neurotransmitter utilized by the peripheral SNS in relaying impulses between post-ganglionic sympathetic neurons and target end-tissues, and along with epinephrine, NE effects metabolic changes in these target tissues via signaling cascades involving the various adrenergic receptors and their associated G-protein second messengers, most of which affect cAMP concentrations further downstream, leading to a variety of metabolic effects including lipolysis, glycogenolysis, changes in heart rate, and regulation of blood flow.

Epinephrine (E) released from the adrenal medulla also has the potential to exert thermogenic effects via activation of adrenergic receptors. However, while it is clear that NE concentrations increase after a meal, peaking in the first 30-60 minutes, a role of E is less certain. Some studies demonstrate no rise or a fall in E while measuring the TEF response (83, 100), while other authors report that a late rise in E due to glucose concentrations returning to baseline activates thermogenesis via a β2-AR mediated mechanism (7, 9, 91). It is possible that some authors reporting no change in E have not measured TEF for a long enough duration to see the potential rise in E due to normalization of blood glucose levels. Further, late rises in E would not be seen with euglycemic clamp studies, which by design maintain tight regulation of blood glucose levels. E may also contribute to thermogenesis via vasodilation of peripheral arteries, thereby aiding nutrient delivery to peripheral tissues.

Utilizing various methodologies including infusion of adrenergic receptor agonists
and/or antagonists, in some cases along with oral or intravenous glucose, multiple authors have demonstrated a lack of a role for α-ARs in sympathetically-mediated thermogenesis (21, 48, 160), although there may be small indirect effects of α2-ARs on beta-adrenergic modulation of metabolism (21).

Thus one or more of the three β-AR variants are responsible for activation of thermogenesis in target tissues. As skeletal muscle represents approximately 40% of total body mass, its population of β-ARs is almost exclusively of the β2-subtype (105), and E has ten- to thirty-fold more potent action vs. NE on β2-ARs (94), it is likely that adrenally-released E mediates the skeletal muscle component of facultative thermogenesis rather than NE released from sympathetic nervous terminals (7, 9), especially given that SNS innervation to skeletal muscle is primarily to the blood vessels (64). More recent evidence indicates the presence of β3-ARs (33), in addition to the preponderance of β2-ARs, in skeletal muscle, although multiple authors have noted that β3-AR involvement in thermogenesis is still unclear (21, 186). β3-ARs are also present in BAT and WAT, and several other organs (often in smooth muscle) including the gallbladder, urinary bladder, stomach, small intestine, colon, and possibly the heart atria and the brain, but not in the liver, lung, kidney, and thyroid gland. Any potential role of β3-ARs aside, the remainder of facultative TEF not mediated by β2-ARs in skeletal muscle is likely regulated by β1-ARs in other tissues.

**The Sympathetic Nervous System and Resting Metabolic Rate**

Numerous studies utilizing propranolol infusions either support (41, 122, 195) or refute (4, 9, 48) a role of tonic SNS activity on RMR. However, it was shown in 2001 that two major shortcomings of prior studies included propranolol dosing insufficient to exert complete beta-
blockade and insufficient statistical power. Remedy of these issues led to the demonstration that tonic sympathetic support of RMR is on the order of 5% in healthy adults (118). In light of this evidence, it is clear that interventions that alter SNS stimulation of thermogenesis have the potential to increase EE via an increase in RMR as well as TEF.

**Aberrations of TEF**

While the influence of aging on TEF has been investigated and shown to either reduce TEF or have no effect, potential influences of obesity and insulin resistance have been very thoroughly investigated, yet a conclusive answer as to whether or not these conditions coincide with alterations of TEF is yet to be established (47, 65).

Additionally, whether potential aberrations of TEF coinciding with obese and insulin resistance states are causal to, or resultant from, these altered metabolic states is a topic of further controversy. It is possible that there is interplay among the conditions. For example, the development of obesity can lead to insulin resistance, which might then impair TEF, reducing EE, and further exacerbating the obese state. Alternatively, genetically susceptible individuals, or those with a reduced TEF (possibly as a consequence of differences in SNSA), might be predisposed to obesity. The subsequent development of an obese state might cause or exacerbate insulin resistance, further reducing the TEF, etc.

The idea that TEF might be altered with obesity is based on the premises that the facultative portion of the TEF response is mediated by SNSA, and that there are differences between obese and lean individuals with regard to SNSA (47). That insulin resistance may result in impairments of TEF is based on the ideas that insulin has a direct role in mediating TEF via central stimulation of the SNS, possibly on the order of 25% of the facultative component of TEF.
(40), that insulin is the key hormone responsible for glucose uptake into peripheral tissues, thus allowing for glucose metabolism, and possibly also that insulin may increase EE by directly activating membrane-bound Na⁺/K⁺-ATPase activity (32).

De Jonge and Bray (47) and Granata and Brandon (65) have provided useful reviews on the subject of TEF and obesity. Each notes the myriad methodological discrepancies and physiological considerations that have not been adequately controlled in many experiments in this area. Yet rather convincing evidence for independent effects of both obesity and insulin resistance on deficient postprandial thermogenesis in adult males is available. In 1985, Ravussin et al. reported, using the same glucose infusion rate while varying the rate of insulin infusion to maintain a similar level of glucose metabolism in lean and obese subjects, that the defect in the thermic effect of infused glucose in obese subjects is due to a greater degree of insulin resistance, resulting in lower glucose uptake, storage, and metabolism by insulin-dependent tissues (primarily skeletal muscle) (138). In 1992, Segal et al. took an additional step and utilized four experimental groups, matching lean and obese men at two different levels of insulin sensitivity/resistance. Each of the lean (~15% body fat) and obese (~33% body fat) groups were matched for body fat percentage, and level of aerobic fitness and antecedent diet were controlled. Indirect calorimetry data from the three hours following a liquid mixed-composition meal (55% CHO, 24% protein, 21% fat) demonstrated a significantly blunted TEF in both obese as compared to lean subjects and in insulin resistant as compared to insulin sensitive subjects (163). The authors noted that long-term follow-up of these subjects may provide further insight regarding these metabolic abnormalities and further weight gain.

Numerous prospective cohort studies have examined relationships between insulin sensitivity and future weight gain, but the results are mixed, and may be confounded by
baseline weight and other residual confounders (74). Additionally, use of differing methodologies (i.e. hyperinsulinemic-euglycemic clamp vs. homeostatic model assessment of insulin resistance vs. insulin tolerance tests vs. measurement of fasting plasma insulin) for quantification of insulin levels or insulin resistance or sensitivity complicates interpretation and comparison of these studies. Studies in reduced-obese patients demonstrating still-impaired glucose-induced thermogenesis provide evidence that impaired postprandial thermogenesis resulting from autonomic nervous system defects predisposes to future weight gain or regain (6). Further, a more recent study has shown that “insulin resistance causes a depression in energy expenditure by food intake, and that energy is stored following the hypo-reactivity of the autonomic nervous system” (194). However, it has also been hypothesized that insulin resistance, while metabolically deleterious, is an “adaptation” that prevents further weight gain (74).

Finally, it is important to note that while numerous studies attempting to relate blunted TEF to insulin resistance and obesity speak to alterations in SNSA, it is important to consider both the degree of SNS outflow, and the metabolic responsiveness of the innervated tissues to this nervous stimulation. At face value, a group of subjects with a high degree of sympathetic nervous system outflow (as measured by microneurography) may appear to be resistant to obesity due to potentially increased EE as a result of this high SNSA. But chronically high levels of SNSA may result in decreased levels of tissue responsiveness to this nervous stimulation, either at the level of the β-adrenergic receptor, or at the level of another signaling protein further downstream of the receptor. Similarly, a group displaying low levels of SNSA at rest might appear to be predisposed to obesity, yet an enhanced degree of tissue responsiveness might yield greater EE during times of supra-basal sympathetic outflow.
Measuring the TEF response: Methodological Considerations

In a 2002 review, Granata and Brandon discussed the numerous methodological variations and shortcomings that may have contributed to conflicting results in the TEF literature, with particular reference to the potential blunted TEF in obesity (65). Consideration of some of these discrepancies may be important with regard to improving consistency of future studies investigating the TEF response, particularly as the increments in EE following a meal are typically small, on the order of 50 to 100 kcal over several hours (47).

Regarding experimental protocol, a first important consideration is whether an inpatient or outpatient measurement protocol will be utilized. One group demonstrated that with a 12 hour fast, no significant differences in RMR or respiratory exchange ratio (RER) occur between in- and out-patient protocols, even lacking control of the supper meal the night before measurement (30). There is a possibility that the authors’ instruments were not sensitive enough to detect small differences between protocols, however the system utilized is accurate to ±0.05% for oxygen and ±0.03% for carbon dioxide. Thus it is likely more convenient to utilize an outpatient protocol, so long as subjects are dietarily compliant and are able to transport themselves to the laboratory in the morning with minimal exertion. However, the authors did recommend having an investigator transport subjects to the lab, as older subjects have been shown to have ~7% higher RMR when transporting themselves to the lab, versus spending the night in the lab (17).

Another consideration is whether a ventilated hood, mouthpiece, face mask, or whole-room indirect calorimetry system will be used for collection of expired gases. One author reported no significant differences in oxygen consumption, RER, and RMR between hood, face mask, and mouthpiece systems (162), while another group reported 5.6% lower RMR using
hood vs. mouthpiece systems, and lower RER (0.82 vs. 0.90) on mouthpiece vs. hood systems (146). Still another group showed systematically 13% lower resting EE on canopy vs. whole-room systems (89). It appears that any of these four systems is acceptable for clinical and research purposes, but mixing or switching between systems should be avoided. Habituation to the various techniques may also be important, and claustrophobia may be an issue with ventilated hood systems, while discomfort can be associated with prolonged use of a mouthpiece (162).

The use of continuous versus intermittent measurement protocols must also be determined alongside with the duration of TEF measurement that will be employed. Granata and Brandon reported on 26 of 49 studies utilizing continuous measurements, while the remaining 23 used intermittent protocols (65). Results of one study demonstrate a 50% lower TEF response over 6 hours of measurement using an intermittent vs. continuous protocol, possibly attributable to a greater degree of restlessness and fidgeting during the continuous protocol (133), which has been shown to confound TEF measurements, but may be able to be quantified and corrected by utilizing a radar system similar to that developed by Bessard et al (19). However, it is difficult in practice to convert an amount of fidgeting to a caloric value. The benefit of continuous measurement is that a larger percentage of the TEF response is monitored, thus reducing dependence upon extrapolation.

Duration of TEF measurement can be relatively short if only the cephalic phase response is being investigated, but studies of the entire TEF response typically last for 3-6 hours after the test meal, although both shorter and longer protocols have been employed (65). While Segal et al. demonstrated blunted TEF in obesity over both 3 and 6 hours (165), suggesting that three hours encompasses a sufficiently large (~70%) percentage of the response, Reed and Hill maintain that even 5 hours of measurement may miss 9% of the 6-h response, and that
important information is contained in the latter portion of the response (142). It is possible that differences between groups, or within groups given different treatments, may manifest toward the end of the postprandial response, which may last longer than 6 hours, depending on test meal size. A few studies have employed day-long respiratory chamber measurements to measure TEF following three meals (22, 156), and this methodology may help reconcile discrepancies, especially considering some authors have found no thermic response to a high CHO breakfast meal (84, 88), and high CHO test meals are commonly utilized.

**Measuring the TEF response: Dietary, Test Meal, and Other Considerations**

Intravenous infusions of nutrients or consumption of test meals have been used to examine the TEF response. While infusion studies have been useful for investigating SNS involvement in TEF (particularly under conditions of β-blockade) and for quantifying the energy cost of nutrient storage, the response to an orally-ingested test meal is more relevant to free-living conditions. Additionally, intragastric administration of nutrients has been used to separate the influence of cognitive factors related to meal consumption such as taste and smell. Comparisons (i.e. the difference) between intragastric and intravenous infusions can also be examined to quantify the energy needed for digestion and absorption of nutrients.

Investigators must decide whether a fixed-calorie meal, or a meal whose energy content is scaled to body mass, fat free mass (FFM), or RMR will be administered. While some studies suggest a standardized meal of a given calorie content is more appropriate (171), this represents a dramatically different energetic challenge for subjects whose daily energy requirements may vary by more than two-fold (167). Thus, a meal with caloric content scaled to RMR represents a comparable energetic challenge across subjects of widely varying sizes.
Composition of test meals is another important consideration which has varied widely among TEF studies. Again, while single-macronutrient test meals are useful for quantifying responses to that specific macronutrient, standard diets are composed of mixed-meals; thus a mixed-meal represents an energetic challenge more commonly encountered in free-living situations. Values for TEF in response to specific nutrients, expressed as a percentage of the calories in the test ‘meal,’ are typically 0-3% for fat, 5-10% for carbohydrates, 20-30% for proteins, and 10-30% for alcohol (177, 197). Overall TEF for a typical mixed-meal fed at EB is between 5-15% of the amount of calories ingested (197).

Studies have suggested that the subtype of each macronutrient also influences TEF. Isocaloric substitution of medium-chain for long-chain triglycerides augments TEF (125, 200), although this effect may be attenuated over time (200). Tappy et al. have demonstrated a higher TEF following ingestion of fructose versus glucose, attributable to the necessity to hydrolyze an extra ATP for the phosphorylation of glyceraldehyde (178). Variable handling of different amino acids may also result in varied responses to different proteins.

Beyond meal composition, investigators have examined other potential influences on the TEF response including meal size (72, 142, 210), regularity and frequency (60, 210), habitual exercise status, exercise training or aerobic capacity (31, 72, 100, 155, 173, 175, 183, 203), acute bouts of prior exercise (58, 108, 130, 210), gene variants, particularly of genes involved in thermogenesis such as those coding for β-adrenergic receptors (128, 140, 179, 191), phase of the menstrual cycle (46, 115, 176), and seasonality (134). Results from these studies demonstrate an increased TEF response with increased meal size (caloric content or food volume), regular meal pattern, fewer large meals versus more smaller meals, and acute bouts of prior exercise, whereas the influence of training status or aerobic capacity, phase of the
menstrual cycle, and gene variants of β2- and β3-adrenoceptors is controversial, and potential changes in TEF seasonally may be related to variations in EI.

Other studies have demonstrated that the form (homogenized > solid-liquid) (132), palatability (increased palatability = increased TEF) (28, 98), cognitive factors such as meal familiarity (unfamiliar meal = higher TEF) (196), and method of delivery (oral > intragastric) (99) of the test meal can substantially influence TEF. Additionally, the temperature of a liquid meal changes the rate of gastric emptying, which may influence TEF (13).

Given that the SNS-mediated portion of TEF probably contributes only about 3-5% to total daily EE (4), it may be appropriate for some study designs to choose test meals unbalanced with respect to total calories (i.e. overfeeding), protein content (both very high and very low protein diets have been shown to increase TEF in rats and humans), or content of other components such as sodium, as this may exaggerate the degree of SNSA following the test meal (174), which may help expose differences between groups, allowing for discrimination of those prone to weight gain. That is, studies wishing to investigate the SNS component of the TEF response should utilize a feeding challenge known to induce appreciable SNSA.

The influence of antecedent diet on TEF has also been considered, and Granata and Brandon also note a lack of sufficient control of this important variable (65). Antecedent diet has the potential to influence TEF in several ways. Varying degrees of SNSA due to prior overfeeding (increased SNSA) or underfeeding (decreased SNSA) may influence the magnitude of the facultative component of TEF following the trial-day test meal (95, 96). Varying macronutrient distributions of antecedent diets have the potential to influence subjects’ metabolic and hormonal status during the experimental trial. Variability in glycogen stores due to varying degrees of prior CHO intake may also influence the metabolic fate of the nutrients ingested
during the test meal—replete glycogen stores would result in preferential test meal CHO oxidation or possibly de novo lipogenesis and storage as triglyceride, whereas depleted glycogen stores would result in preferential glycogenesis utilizing test meal CHO (5). These variations in nutrient metabolism are associated with different ATP costs (177).

**Variability of RMR and TEF Measurements**

Several authors have reported a large degree of variability in the TEF measurement, while variability in RMR on a given day or between days is considerably smaller. Typical intra-individual coefficients of variation (CV) for RMR measured either morning vs. afternoon or on separate mornings are 2-5% (146). While one author determined that RMR is essentially constant throughout the day, eliminating the need for sham-feeding control experiments to verify RMR constancy during TEF studies (198), other authors have determined that the subtle diurnal variability of RMR contributes to the large reported CV’s of up to 16-31% for TEF measurements (45, 164), and that measuring RMR on single (164) or multiple (52) occasions separate from the TEF measurement (and using this as the baseline for TEF measurement) can significantly reduce TEF variability.

**GREEN TEA**

*Background, Composition, and Widespread Health Effects*

Tea is one of the most widely consumed beverages in the world, and knowledge of its potential health benefits can be traced back to 2,700 B.C (205). Traditionally, green tea in particular has been consumed in order to eliminate toxins such as alcohol, improve blood flow, relieve pain, and resist disease. In addition to traditional beliefs regarding tea’s benefits, since
1995 there has been an exponential increase in the number of scientific publications related to tea, catechins, and epigallocatechin-3-gallate (EGCG) consumption, with well over 1,000 papers in 2005 alone (87). These papers have investigated various potential health-promoting effects of green tea including its anti-cancer, anti-arthritic, anti-inflammatory, antiviral, antibacterial, and antioxidant properties, and its potential to prevent the various co-morbidities of the metabolic syndrome including cardiovascular disease, type 2 diabetes mellitus, obesity, and hypertension (205).

Polyphenols comprise approximately 30% of the dry weight of green tea leaves, and are thought to be primarily responsible for the variety of green tea’s health effects. The polyphenol content of green tea includes flavanols, of which catechins are the predominant subset. In green tea, catechin, epicatechin, epicatechin gallate, epigallocatechin, EGCG, and others are present, and EGCG is the predominant (~43% of catechins) and likely the most bioactive of these (56, 207). In contrast to green tea, the fermentation processes used to generate black tea result in the conversion of catechins to theaflavins and thearubigins, which also may have significant biologic activity (109, 208).

In addition to its polyphenol content, green tea contains caffeine in amounts that may be able to exert physiological effects, such as enhanced lipolysis driven by phosphodiesterase inhibition. Particularly with regard to modulation of fat oxidation and thermogenesis, many studies have examined EGCG or tea catechins alone or in combination with caffeine. Given the current obesity epidemic and the current abundance of interest in the health benefits of tea, the importance of studying the effects of individual components alone, particularly EGCG, has been noted (205). The remainder of this review will focus on research related to tea’s ability to affect fat oxidation and thermogenesis (with particular reference to EGCG), and it will also include a
discussion of mechanisms of action, potential toxicity, alterations of effective EI, and other botanical extracts with the potential to enhance lipolysis and induce thermogenesis.

*Mechanism of Action and Enzyme Kinetics*

Catechol-O-methyltransferase (COMT) is a ubiquitously-distributed enzyme found in both soluble and membrane-bound forms. Its distribution includes the membrane of post-synaptic nerve terminals. Utilizing a methyl group from S-Adenosylmethionine, it catalyzes the first step in one degradation pathway of NE and E, first converting these catecholamines to normetanephrine and metanephrine, respectively. Additionally, catecholamines can be inactivated by monoamine oxidases present at nerve terminals and in other tissues.

Any compound containing a catechol group (i.e. benzene with two hydroxyl groups in ortho configuration), including catecholamine neurotransmitters, catechol estrogens, and catechol flavonoids, has the potential for degradation by COMT. EGCG contains a catechol group, but molecular modeling studies indicate that its 3-galloyl moiety results in high-affinity binding leading to high-potency inhibition of COMT activity, while simultaneously rendering it a poor substrate for degradation by COMT due to the binding angle induced by the 3-galloyl group (169, 212). Additionally, modeling studies demonstrate that depending on the substrate, EGCG can act as either a competitive or non-competitive inhibitor.

By preventing the degradation of NE in the synaptic cleft (25, 36, 112), EGCG has the potential to provide prolonged stimulation of adrenergic receptors, both at rest owing to tonic sympathetic support of metabolism (118), and particularly during times of increased SNSA, such as following a meal or during exercise.

As caffeine, another important component of tea, is often studied along with EGCG, a
brief discussion of its mechanism of action is also relevant. While caffeine does cross the blood-brain barrier and exert a direct effect on the central nervous system via adenosine receptor antagonism, its effects on lipolysis at the level of the white adipocyte and thermogenesis may occur via one of several mechanisms. “Antagonism of the antilipolytic effects of adenosine” (3) owing to caffeine and one of its main metabolites, paraxanthine (70), blocking adenosine receptors on the surface of adipocytes (63) and potentiation of the effects of sympathetically-released NE and adrenally-released E via phosphodiesterase inhibition (55) acting through β-adrenergic receptor pathways may both occur to augment the degree of lipolysis. The cyclic nucleotide subtype of phosphodiesterase enzymes is responsible for the degradation of cyclic-AMP, and this degradation reduces lipolysis via decreased protein kinase A activation leading to reduced hormone-sensitive lipase (HSL) activation. Thus, inhibition of phosphodiesterase activity results in greater HSL activity.

While the mechanisms underlying caffeine-induced thermogenesis are uncertain, stimulation of futile cycling (triglyceride-fatty acid or glucose-lactate) or activation of uncoupling proteins may be involved. Non-adrenergic mechanisms, such as calcium cycling induced by caffeine binding ryanodine receptors (3), may also be involved.

It is important to note that there is not necessarily coupling between the degree of lipolysis and the degree of fat oxidation and thermogenesis, as hydrolyzed triglycerides may be reesterified if the energy requirements of the organism are relatively low at the time. Research shows that “stimulation of lipolysis per se is not a major factor in the regulation of lipid oxidation or energy expenditure and casts doubts on strategies aimed primarily at stimulating lipolysis to control body weight” (3).

It has been proposed that EGCG and caffeine have the ability to function synergistically
in increasing lipolysis and thermogenesis by relieving inhibition at different control points along the NE-cAMP axis—EGCG inhibits COMT-mediated NE degradation and caffeine reduces cAMP degradation by phosphodiesterase inhibition (56). These effects may also be more pronounced under conditions of enhanced NE release, either pharmacologically using agents such as ephedrine, or physiologically during cold exposure or exercise, or postprandially.

The widespread effects of EGCG have been noted, and different modes of action are likely responsible for its varied effects. For example, inhibition of fatty acid synthase (FAS), expressed at low levels in non-malignant cells and high levels in certain carcinomas, is likely responsible for some of EGCG’s anticancer activity (136), while its ability to induce eNOS activity in endothelial cells is responsible for its positive effects on endothelial function and blood flow (109). The 67-kDa laminin receptor also binds EGCG, and the ubiquitous distribution of the several isoforms of this receptor not only on cancer cells, but also on muscle cells, hepatocytes, endothelial cells, and others may also help explain EGCG’s numerous effects (87, 119).

**Effects of Green Tea Extract and EGCG on Body Weight, Fat Oxidation, and EE**

The 17th century pharmacist Wang Ang noted that green tea consumption can help eliminate body fat (205). More recently, investigations in cell cultures, animals, and humans have examined the ability of green tea to increase fat oxidation and EE and reduce body and fat mass either at rest or coupled with an endurance exercise training regimen. Results seem to indicate that green tea, and EGCG in particular, have modest roles in increasing daily EE on the order of 2-8% (12, 15), while lowering RER, indicating a greater relative proportion of fat oxidation. It is important to note that in some studies the degree of increase in thermogenesis may be influenced by the caffeine content of the green tea acting synergistically with EGCG.
In cell culture experiments on 3T3-L1 adipocytes, 24 hour incubation with 10 µM EGCG significantly reduced intracellular lipid accumulation, with simultaneous increases in HSL mRNA (101). This same group also noted the ability of EGCG to up-regulate uncoupling protein-2 (UCP-2) mRNA in a dose-dependent manner in this same cell line, and evidence suggests EGCG directly stimulated the proximal promoter in the UCP-2 gene (103). UCP-2 may have a role in uncoupling the mitochondrial electrochemical proton gradient, either as a contribution to RMR or in order to limit reactive oxygen species (ROS) formation. A potential role for UCP-2 in exporting fatty acid anions from the mitochondrial matrix to limit ROS formation is also likely (151).

Murase et al. demonstrated that mouse diets supplemented for 10 weeks with 0.2% or 0.5% wt/wt green tea extract (GTE) containing a mixture of catechins but no caffeine prolonged time to exhaustion by 8% and 24% in an endurance swimming test versus mice fed the same diet without GTE (121). Both groups of mice were swim-trained twice a week for the 10 weeks of the study. Measurements of resting energy metabolism 1 hour after a feeding period indicated a dose-dependent significant increase in fat oxidation with a non-significant increase in oxygen consumption (EE) in the GTE condition. Further study with EGCG alone determined that EGCG was at least partly responsible for these effects, and the potential mechanism may involve up-regulation of the genes for fatty acid transporter (CD36) and medium chain fatty acyl-CoA dehydrogenase. Peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator-1α mRNA was unaffected.

Also in mice, this same group showed that regular aerobic exercise in combination with GTE-supplemented diets stimulates both liver and skeletal muscle fat oxidation, and helps attenuate obesity caused by high-fat diets more effectively than either exercise or a GTE-supplemented diet alone (168).
Other recent results shed light on the potential mechanism of EGCG-induced reductions in body weight and fat depot mass. Lee et al. demonstrated that EGCG-supplemented diets reduced body mass and mass of several WAT depots in a dose-dependent manner in high-fat diet-induced obese mice, versus a control group fed the same diet without EGCG. Moreover, numerous genes related to adipogenesis were downregulated (PPAR-γ, CCAAT enhancer-binding protein-α, regulatory element-binding protein-1c, adipocyte fatty acid-binding protein, lipoprotein lipase, and FAS) while mRNA levels of genes related to lipolysis (carnitine palmitoyl transferase-1, HSL, adipose triglyceride lipase) and thermogenesis (UCP-2) were increased. Whether these changes in mRNA expression were a direct result of EGCG interactions with the promoters or other regions of the genes, or an indirect effect, was not elucidated, but support for a direct effect comes from another recent study demonstrating the ability of EGCG to directly stimulate the gene for cholesterol 7 α-hydroxylase (102). Furthermore, it is also unclear how changes in gene expression (mRNA levels) relate to changes in protein levels. Additionally, EGCG did not result in reduced EI in Lee’s study. Whether or not EGCG decreases food intake is still an unsettled issue (87).

Recent studies have yielded somewhat conflicting results regarding the ability of green tea catechins to reduce abdominal fat loss in obese human subjects. Epidemiological evidence suggests that habitual green tea consumption for several years is associated with a lower percent body fat and a more favorable body fat distribution (206). One group examined the influence of a daily beverage containing 625 mg catechins (214 mg EGCG) and 39 mg caffeine (vs. an iso-caffeinated control beverage) on exercise induced weight loss in obese men and women, with an exercise recommendation of ≥ 180 minutes per week. The catechin group tended to experience greater weight loss versus the control group, while changes in waist
circumference did not differ significantly. Total, but not percentage, abdominal fat area and abdominal subcutaneous fat area decreased more after 12 weeks in the catechin group (113). Another group found significant decreases, but no difference between intervention and control groups, in waist circumference, total body fat, abdominal fat, and intra-abdominal adipose tissue in obese women exercising three times per week for 45 minutes and consuming two 150 mg capsules of Teavigo® brand EGCG for 12 weeks (71). No significant changes in body mass were observed. Others noted greater fat oxidation without differences in EE during rest and during 30 minutes of treadmill exercise in a group drinking a daily beverage containing 570 mg total catechins (218 mg EGCG) vs. a group drinking a control beverage for two months (131). Each group engaged in 30 minutes of treadmill walking exercise three days per week.

Another recent study investigated the ability of a GTE supplement containing EGCG and caffeine (90 mg EGCG and 50 mg caffeine, 3x/day with meals) in combination with adequate- and high-protein (AP and HP) diets to maintain weight and body composition following weight loss (75). Versus the AP placebo group, the GTE supplement in combination with either diet and the HP placebo group had a similar capacity for weight and FFM maintenance; thus no synergism between the GTE supplement and HP diets was evident, although absorption issues related to protein-polyphenol complex formation were discussed.

In exercising humans, Venables et al. demonstrated a 17% higher fat oxidation rate during 30 minutes of acute cycle ergometry at 60% of VO₂ max following acute supplementation (four capsules of 340 mg polyphenols including 136 mg EGCG over 24 hours) with decaffeinated GTE, notable, the authors stated, because it demonstrated the ability of GTE to increase fat metabolism even under conditions of elevated lipolysis and fat oxidation (190). However, this result may not be so unexpected given that moderate exercise represents a condition of greatly
elevated SNSA versus the resting state. Thus, there is a greater pool of NE in the synaptic cleft for EGCG to influence during exercise than during resting conditions.

Multiple authors (53, 109) have demonstrated that tea consumption can improve blood flow (i.e. endothelial function). Thus, in addition to its modulation of adrenergic signaling, EGCG may also improve fat oxidation by improving blood flow to tissues such as skeletal muscle or adipose tissue (71).

Finally, as heat-processing of teas can epimerize up to half of the catechin content of teas, and since the consumption of heat-treated tea products is increasing, it is also necessary to elucidate the effects of these heat-altered catechins. Research has demonstrated that both the original and heat-treated products are effective in reducing visceral fat deposition, hepatic triglyceride content, and the activities of multiple enzymes of fatty acid synthesis in rats (76).

**Botanical Extracts with the Potential to Augment EE**

In the context of research relating to natural compounds with the potential to modulate energy balance, green tea is only one of numerous botanical products with the potential to favorably influence body energy status. Results from studies on oolong tea suggest that by virtue of caffeine and catechin polyphenol (including polymerized polyphenols) content similar to that of green tea, oolong tea increases EE and fat oxidation in adult humans (92, 152).

A placebo-controlled study of the influence of twelve potentially thermogenic plant preparations (guarana, ephedra, Malabar tamarind, green tea, artichoke, maté, *Iris versicolor*, hazel, sea fennel, *Fucus vesiculosus*, Virginian poke, and *Laminaria digitata*) demonstrated no increase in EE (3 hours of indirect calorimetry following consumption in the post-absorptive phase) for any of the plant preparations, which varied in form from powder to capsules to
tincture. Only the maté extract decreased RER (considered to be a favorable effect), indicating a greater proportion of fat oxidation at the same level of EE, while guarana and ephedra powders and hazel tincture increased RQ, indicating a greater proportion of CHO oxidation. However, the study examined the influence of an acute single dosage of each plant preparation, and both short-term (i.e. several day) and longer-term studies including varying dosages of each preparation could prove useful. Like Dulloo (56), these authors note that sufficient amounts of caffeine may be necessary to invoke a synergism between these compounds and the caffeine in order to elicit thermogenesis.

Other examples of plant preparations studied for their potentially thermogenic and/or lipolytic properties include *Salix matsudana*, *Nelumbo nucifera*, and *Cirsium oligophyllum*. *S. matsudana* is a Chinese tree which contains a mixed polyphenol fraction, as does green tea, and has been demonstrated to reduce body weight and adipose tissue weight and to lower hepatic cholesterol content in mice fed high-fat diets, likely via a combination of inhibition of α-amylase activity, a reduction of palmitic acid absorption into brush border membrane vesicles (but not via inhibition of pancreatic lipase), and an acceleration of NE-mediated lipolysis in adipocytes (68). *N. nucifera*, the lotus flower, has been shown to contain various flavonoids, including quercetin 3-O-α-arabinopyranosyl-(1→2)-β-galactopyranoside, (+)-catechin, hyperoside, isoquercitrin, and astragalin, that induce lipolysis via the β-adrenergic receptor pathway in both visceral (primarily) and subcutaneous white adipose tissue of mice (127). Finally, *C. oligophyllum*, a species of thistle, has very recently been shown to have efficacy in the reduction of subcutaneous adipose tissue mass via a β-adrenergically mediated mechanism, when applied perorally or topically to rats, with concomitant up-regulation of uncoupling protein-1 in subcutaneous brown adipose tissue and uncoupling protein-3 in skin (120). This is important
because subcutaneous adipose tissue depots are classically thought to be less responsive to catecholamine-mediated lipolytic action as compared to visceral adipose tissue (69).

Thus there is considerable interest in the search for natural remedies for obesity. Previous research has included investigations of the influence of whole plant preparations (e.g. drinkable green tea) or of singular components of these plants (e.g. EGCG from green or oolong tea, capsaicin from red peppers). Although the necessity for elucidating the specific mechanistic effects of singular compounds has been noted (205), the importance of studying the administration of whole preparations cannot be underestimated, as synergy between components may be key to the biological activity of these preparations, and this synergy often occurs at doses far lower than the toxic dose of individual components of the mixture. Moreover, this synergy may occur at doses lower than the dose required for single components to evoke thermogenesis, and utilizing this synergy may help prevent potential blunting of the response with long-term administration. The old adage “United they stand, divided they fall” has been used with specific reference to EGCG and green tea catechins and inhibition of carcinogenesis (24), but the principle may hold true with regard to other plant preparations and their effects on a variety of physiological functions. With reference to green tea in particular, while EGCG alone may induce a greater degree of lipid oxidation (vs. placebo), other components of green tea such as caffeine or other bioactive polyphenols (epicatechin-gallate, epigallocatechin, quercetin, etc.) may work additively or synergistically with EGCG to exert even more effective weight-suppressing effects with habitual consumption. In support of this notion, Dulloo et al. noted that “the efficacy of the green tea extract to potentiate brown adipose tissue 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
noradrenaline” (56). Synergism may occur on the energy intake side of the energy balance equation as well, as there is also evidence suggesting that when N-oleyl-phosphatidylethanolamine, a phospholipid plentiful in soy, eggs, and chocolate, is administered with EGCG it is more effective than either ingredient alone in reducing food intake and enhancing compliance with a hypocaloric diet (147).

Possible Effects of Tea on Macronutrient Digestibility and Absorption

While much of the research on tea focuses on modulation of EE, some studies suggest that green, black, and oolong teas, and EGCG, may reduce EI via reduced digestibility and absorption of macronutrients (34, 38, 57, 208, 211). Research on the influence of tea on protein digestibility dates to the 1960’s and 1970’s, at which time results were conflicting, with some showing no effects of tea or coffee on growth or feed utilization of rats (123), while others implied reduced vegetable protein digestibility and enhanced animal protein utilization in chickens. Eggum et al. demonstrated in 1983 that green tea, black tea, and coffee all reduce true protein digestibility, although the effect of green tea was smallest in magnitude (57). These results are in line with more recent findings that suggest oolong and black teas may reduce protein absorption (208).

Research on the influence of tea on gastric emptying rate and intestinal transit time is sparse, but data indicate that the thearubigin fraction of black tea can reduce (i.e. make faster) gastrointestinal transit time in mice (35), while certain saponins present in the tea plant can inhibit gastric emptying while accelerating gastrointestinal transit. No studies to our knowledge have investigated the potential influence of EGCG on gastrointestinal transit, although given that the parasympathetic nervous system is most responsible for digestion and that
acetylcholine is the primarily neurotransmitter of the parasympathetic nervous system, EGCG would not be expected to exert effects on gastrointestinal transit time, as COMT does not act on acetylcholine. However, as EGCG is potent in stimulating endothelial nitric oxide synthase activity, at least in the endothelium of bovine aortic endothelial cells and rat aortic rings (109), if EGCG affects nitric oxide production in the gut, it may alter gastrointestinal motility through interactions with non-adrenergic, non-cholinergic neurons (114). Any alterations of the rate of gastric emptying or the degree of intestinal motility have the potential to influence nutrient absorption during a test meal.

Tea extracts may inhibit a variety of enzymes involved in glucose and triglyceride digestion and absorption, including α-amylase, α-glucosidase, pancreatic lipase, sodium-dependent glucose transporters, gastric H⁺,K⁺-ATPase, and sucrase (87, 211). However, it is yet to be discerned how in vitro enzymatic studies translate to in vivo human scenarios (205), also considering that many studies on tea extracts and digestibility/absorption have been performed in rats. One study did recently show that a combined extract of black, green, and mulberry teas causes significant malabsorption of ~25% of ingested carbohydrate but not of fats in adult humans (211). In rats, GTE added to a high-fat diet slightly (~3%) reduced the digestibility of the diet, and other recent work suggests that “green tea catechins, particularly (-)-epigallocatechin gallate interfere with the emulsification, digestion, and micellar solubilization of lipids” and there is a “possibility that green tea or catechins may influence the uptake and intracellular processing of lipids and assembly and secretion of chylomicrons” (93). This represents a potential mechanism by which green tea extracts not only lower macronutrient digestibility and absorption (which may in fact decrease TEF if the effective EI is lessened), but also potentially improve plasma lipid profiles.
Finally, as tea is often consumed with milk, some authors have investigated this combination of drinks on absorption. In the 70’s it was demonstrated that tea polyphenols form a complex with casein protein present in milk, but protein digestibility is not limited. More recently, the opposite effect was studied—whether milk inhibits bioavailability of tea catechins. It was found that the amount of catechins from black tea measurable in the bloodstream are the same when black tea is drunk with or without milk (187). An abundance of further evidence is available related to consumption of milk with tea and is summarized by Hursel and Westerterp-Plantenga (75).

EGCG Metabolism and Toxicity

Experiments in animals have shown that EGCG is rapidly absorbed, and reported relative bioavailabilities range from 1.6% to 13.9% depending on dose. Similarly, in humans, both the rate and degree of absorption increase in a dose-dependent manner (182), although dosages relative to body weight were several fold higher in animal experiments. A significant degree of first-pass metabolism in the liver may contribute to the limited bioavailability noted even with fasted subjects.

EGCG is found in the plasma in both conjugated and free forms, with the free form predominating (in contrast to other catechins such epigallocatechin) (39). Maximum plasma concentrations are achieved between one and five hours following oral ingestion and range from 130 ng/mL to 3,400 ng/mL after single doses of 50 mg to 1,600 mg. Reduction in plasma concentrations following the peak occurs multiphasically, with distribution and elimination phases. Elimination half-lives are approximately 2-5 hours, depending on dose, in both humans and animals following oral ingestion. These short half-lives indicate no risk of accumulation with
multiple doses. Elimination of EGCG occurs via extensive degradation in the gut by microorganisms and by glucuronidation, sulphonation, and O-methylation by enzymes including COMT and UDP-glucuronosyltransferases (111), although COMT action on EGCG is limited (see above). Excretion is primarily through bile in the feces, although small (~3% of total) amounts are excreted as conjugates in urine (182).

There is a large degree of inter-individual variability in the EGCG pharmacokinetic data, even with fasted subjects. The presence of food in the gut represents a significant barrier to EGCG bioavailability, and food both reduces the degree of EGCG absorption and slows its timecourse. Studies on EGCG pharmacokinetics have been performed with coincident food consumption, and with food consumption hours following the EGCG dosage.

While it is not uncommon for people in some Asian cultures to consume nearly 10 cups of green tea per day without adverse events (180), there have been several studies on potential toxic effects of extracts of green tea. While one group reported good tolerance of single oral doses of up to 1,600 mg of 94% pure crystalline EGCG with fasted human subjects (182), others have reported cases of hepatotoxicity in the form of acute hepatitis in human subjects, albeit at a low rate of 1 adverse event per 100,000 boxes of the Exolise® brand supplement (154) (116). Toxicity typically occurs after several weeks or months of use, and typically resolves upon termination of supplementation, although there is a report of a case of fulminant hepatitis in 2001. These human reports are in contrast to studies in rats which indicate a protective effect of EGCG on hepatotoxicity induced by 2-nitropropane (43).

The mechanism of EGCG on liver toxicity is unclear, and there is also a possibility that individuals reporting adverse events had an allergic reaction to the green tea itself or another component of the extract, or that there was contamination during the growing or production process (116).
Other reports on potential EGCG toxicity indicate that Teavigo® brand is not genotoxic in rats, induces mild dermal irritation in some but not all animal species tested, produces strong eye irritation in rabbits, and reduces the growth rate of rat offspring at extremely high doses. Investigators established a ‘no-observed adverse effect level’ of 500 mg/kg/day (79-81) in rats, while doses of up to 800 mg/day for 10 days of Teavigo® brand in humans have not led to any adverse effects (181). However, after synthesizing information from multiple animal and human studies and utilizing a safety/uncertainty factor of 100, a conservative value for acceptable daily intake in humans of 5 mg/kg/day (300 mg EGCG/day for a 60 kg human) is indicated given that humans may partake in long-term consumption of EGCG (80). For comparison, the dose of Teavigo® brand EGCG supplement used for the present experiment was three 135 mg doses per day (independent of body weight). While this dosage is greater than the recommended acceptable daily intake just mentioned for long-term consumption, the present experiment consisted of less than three days supplementation, and thus appears quite conservative in light of an absence of negative effects in a study delivering 800 mg/day for 10 days.

*Lack of Negative Cardiovascular Effects Typical of Sympathomimetics*

A variety of sympathomimetic agents (e.g. β-adrenoceptor agonists) have the ability to increase EE and therefore, have the potential to reduce fat and body mass. However, many of these agents are associated with adverse cardiovascular events and outcomes, including elevated heart rate and blood pressure, which may be particularly dangerous for the subpopulation of obese also suffering from hypertension (169). Since EGCG has the capacity to prolong adrenergic stimulation via COMT inhibition, an important clinical concern is whether EGCG treatment is also associated with potentially hazardous cardiovascular outcomes.
However, this does not seem to be the case, as multiple authors have reported little or no increases in heart rate and blood pressure, or even decreases in heart rate, over a range of doses of EGCG or green tea extract, alone or in combination with other potentially thermogenic substances (15, 16, 34, 54, 56, 71). However, possible increases in both systolic and diastolic blood pressure have also been reported, but these may be attributable to the caffeine content of a combined EGCG-caffeine supplement (12).

A Pilot Study on EGCG and TEF

A 2007 pilot study on six otherwise-healthy obese men (BMI 29.9 ± 1.6 kg/m²) by Boschmann and Thielecke found no differences in fasted resting EE or postprandial EE between EGCG and placebo (27). The only statistically significant difference between treatments was a lower RER (~0.84 vs. ~0.91) during the first 2 hours of a 4 hour TEF measurement for EGCG vs. placebo. Dosage was two 150 mg pills (Teavigo® brand, minimum 94% EGCG) daily for two days prior to the test, with a final 150 mg capsule taken one hour prior to initiation of RMR measurement, at 07:00 hours. Following four hours of RMR measurement, a 5 kcal/kg body weight test meal with macronutrient composition 50% CHO, 35% lipid, and 15% protein was administered over 30 minutes, followed by four hours of TEF measurement. Details regarding the nature of the test meal were not published. The authors concluded that their data provides justification for further studies with a greater sample size utilizing subjects of different ages and body mass indices.

Justification and Purpose of the Current Experiment

Given, 1) the current obesity epidemic is reflective of aberrations of the classic EB
equation, with EI exceeding EE, 2) current interest in the health properties of various tea preparations and the need to discriminate the individual effects of various components of tea extracts, 3) the ability of EGCG to modulate SNSA via COMT inhibition, potentially leading to increased EE, 4) the modest potential of EGCG to reduce macronutrient digestibility and absorption, we sought to investigate more thoroughly the effects of EGCG on RMR and TEF. Our experiment utilized a larger and more heterogenous group of subjects than the pilot study of Boschmann and Thielecke, and we sought to verify and/or extend these preliminary findings with a placebo-controlled, double-blinded, randomized testing of the effects of EGCG on postprandial thermogenesis and substrate oxidation, while secondarily examining cardiovascular variables.

**HYPOTHESIS**

We hypothesize that short-term EGCG supplementation will augment RMR and TEF in healthy adult humans.

**SPECIFIC AIMS**

1) Given that the SNS supports RMR and that EGCG inhibits COMT-mediated catecholamine degradation, we sought to investigate whether short-term EGCG supplementation augments RMR.

2) Given that meal consumption elicits appreciable SNS activation and that EGCG inhibits COMT-mediated catecholamine degradation, we sought to investigate whether EGCG augments TEF.

3) Given that EGCG has been shown to promote lipid utilization, we sought to investigate
whether EGCG decreases RER during RMR and TEF.

4) Given that the SNS influences heart rate and blood pressure, we sought to investigate whether EGCG influences heart rate and blood pressure, in order to determine if EGCG may be a safer therapeutic than sympathomimetics, which can be contraindicated for certain populations.
CHAPTER II: INTRODUCTION

There is a need to develop effective solutions for the burgeoning obesity epidemic both in the United States and abroad (126). Given that obesity is caused by aberrations of energy balance (energy balance (EB) = energy intake (EI) minus energy expenditure (EE)), an effective modality targeting reduced body mass and fat mass will exert effects upon one or more components of the EB equation.

While pharmaceutical approaches aimed at reducing EI such as Orlistat® or Rimonabant® may be effective at reducing nutrient absorption or controlling appetite, side effects are numerous. Thus, recently there has been an abundance of interest in both the lay and scientific community in more natural remedies for weight control that act by reducing EI or increasing EE (87).

Green tea (Camellia sinensis) is one such potential remedy and has been consumed in Asian cultures for centuries for its purported weight-controlling effects. Additionally, green tea has also been reported to possess antioxidant, antiviral, anti-arthritis, anti-angiogenic, and numerous other beneficial properties (205).

The precise mechanisms by which green tea or its extracts exert their positive effects upon body composition are uncertain, but may include control of appetite, interference with nutrient absorption, and increased EE. Green tea contains a large fraction of polyphenols, of which catechins such as epicatechin, epigallocatechin, epicatechin-gallate, and epigallocatechin-3-gallate (EGCG) compose a large percentage (180). Research has demonstrated that EGCG is
the most abundant and probably the most bioactive catechin in green tea (207). Molecular modeling studies indicate that EGCG has a potent capacity to inhibit catechol-O-methyltransferase (212), a ubiquitously-distributed enzyme that represents one pathway for the degradation of catecholamines, which play an important role in substrate metabolism and EE via \(\beta\)-adrenergic receptor-mediated processes such as lipolysis and glycogenolysis.

It is unclear via which components of the EB equation green tea, and EGCG in particular, exert their effects. Likewise, it is unclear whether EGCG alone has the capacity to modulate any of the components of EB, or whether synergism between EGCG and caffeine, other polyphenols, or other components of green tea such as the flavanol quercetin is requisite (56). A pilot study investigating thermogenesis and fat oxidation in obese men at rest and postprandially following short-term EGCG supplementation indicated no difference in EE between the placebo and EGCG conditions, but a significantly increased percentage of fat oxidation during the first two hours postprandially (27). Other studies indicate that EGCG may augment the beneficial effects of an endurance exercise regime on body composition (113). Animal and human studies also suggest that teas have a modest capacity to cause malabsorption of one or more of the macronutrients (38, 211). Thus it is clear that there is potential for green tea and EGCG to favorably influence multiple components of EB.

Given this information, we sought to investigate the hypothesis that short-term EGCG supplementation augments resting metabolic rate (RMR) and the thermic effect of feeding (TEF) in a heterogeneous group of healthy adults. We also sought to confirm and extend the findings of the aforementioned pilot study, and to provide further detail regarding through which components of EB green tea and EGCG exert their favorable effects on body composition.
CHAPTER III: METHODOLOGY

Subjects

We studied nine men and eight women aged 18-52, recruited from the Colorado State University (CSU; Fort Collins, CO) student, faculty, and staff population. Subjects were healthy (no overt disease such as type 2 diabetes) nonsmokers and were not taking any medications that might confound the results (e.g. β-blockers or vasoactive drugs). Subjects were not taking antioxidant supplements, and were not currently consuming either green tea beverages or any variety of green tea extract (GTE). One male subject was a habitual consumer of an exercise nutrition supplement containing epigallocatechin-3-gallate, the main compound used in the present study. We instituted a wash-out period of one month before testing this subject. Table 1 provides further information regarding subject characteristics.

The nature, purpose, risks, and compensation/benefits of participation were explained verbally to each subject before written, informed consent was obtained. The experimental protocol was approved by the Institutional Review Board at CSU.

Measurement of Subject Characteristics

Following completion of an email pre-screening questionnaire, all subjects’ first visit to CSU’s Human Performance and Clinical Research Laboratory (HPCRL) included written, informed consent, body composition assessment via dual-energy x-ray absorptiometry (DEXA; Hologic Discovery-W™ with QDR™ for Windows software, Bedford, MA), measurement of VO₂ max,
defined as the maximal rate of oxygen utilization during incremental exercise to exhaustion, and other subject characteristics including height and weight (physician’s stadiometer and beam scale), waist and hip circumference (Gulick tape measure; average of three trials), resting heart rate (HR; Polar® heart rate monitor) and blood pressure (BP; standard sphygmomanometer). BMI (kg/m²) and waist-to-hip ratio were calculated.

For the VO₂ max test, a Hans-Rudolph® (St. Louis, MO) two-way non-rebreathing mouthpiece and valve apparatus was used to direct subjects’ expired gases through a heated, linear pneumotachometer (Hans Rudolph® Model 3813) which measured breath-by-breath ventilation, and subsequently into a gas analyzer (Parvo TrueOne 2400 Metabolic Measurement System, Sandy, UT; gas- and flow-calibrated daily) for determination of oxygen and carbon dioxide content of the expired gases. Following volitional fatigue during an incremental treadmill (Quinton Q65 Series 90) or cycle ergometer (Lode Excalibur, Groningen Netherlands) test, absolute (L/min) and relative (mL/kg/min) VO₂ peak and peak respiratory exchange ratio (RERpeak) were determined.

GTE and Placebo Supplementation

Teavigo™ (DSM Nutritional Products, Basel, Switzerland) brand GTE was purchased for use in our experiment. Each 150 mg capsule reportedly provides a minimum of 90% (135 mg) (-)-epigallocatechin-3-gallate (EGCG); the remainder of each capsule is composed of filler ingredients including starch, gelatin, magnesium stearate, and purified water, none of which would be expected to confound interpretation of our results. Capsules were stored at room temperature (~22°C) in a dark cabinet and were delivered to subjects in a plastic bottle covered in duct tape to prevent photochemical alterations.
Empty capsules were filled with organic whole grain yellow cornmeal (Arrowhead Mills, Melville, NY) by one of the experiment’s researchers and served as the placebo control. The capsules were similar in appearance to the GTE capsules, and both were odorless.

Double-blinding was achieved by delivering all capsules to an investigator with no relation to the present study. This investigator randomly coded the treatments ‘A’ and ‘B.’ The order of treatments was randomized.

The short-term supplementation (GTE and placebo) regime was as follows: one capsule three times per day at meal time for two days, then one capsule on the morning of the experiment taken with a minimal amount of water.

Resting Metabolic Rate Measurement

Subjects arrived at the CSU HPCRL between 0500 and 0900 hours after 24 hour abstention from structured exercise and alcohol, 12 hour abstention from food, all non-water beverages, and caffeine, and 2 hour abstention from water. After voiding, body weight (kg) was measured and the subject lay down in a semi- to full-recumbent position of their choosing. A 20- or 22-gauge anterograde venous catheter was placed in an antecubital vein for subsequent blood collection and was kept patent with a saline drip.

Subjects were outfitted with an automated 3-lead sphygmomanometer and electrocardiogram (GE Healthcare Datex-Ohmeda Cardiocap/5, Helsinki, Finland) for measurement of blood pressure and beat-by-beat heart rate. Heart rate and blood pressure were recorded at minutes 0, 15, 30, and 45 of the baseline measurement period.

RMR was measured for 45 minutes (15 minutes of habituation followed by 30 minutes for data analysis) in a quiet, dimly lit room at room temperature (19-23°C) using the ventilated
hood technique in combination with a custom built indirect calorimetry system (Nighthawk Design, Boulder, CO). The system was gas- and flow-calibrated daily with two primary standard gases (gas 1: 21% O₂, 0% CO₂; gas 2: 16% O₂, 4% CO₂; Airgas Intermountain, Denver, CO). For operation, briefly, a fan drew air through the hood and connecting tubes at approximately 40 L/min while a sampling line at the end of a 4-liter mixing chamber led into a respiratory mass spectrometer (MA Tech Services MGA 1100, St. Louis, MO) for analysis of oxygen and carbon dioxide content of expired gases. An ultrasonic flow sensor was located shortly past the end of the mixing chamber. RMR was calculated in kcal/day and kcal/min. RER was calculated as VCO₂/VO₂.

Feeding

Following measurement of RMR, subjects were allowed 15 minutes to consume an Ensure® brand (Abbott Nutrition, Columbus, OH) liquid meal with caloric value equivalent to 40% of their RMR measured just prior, equivalent to ~30% of the daily requirement for EB if RMR is 75% of total daily EE. The macronutrient distribution of the test meal was 64.0% carbohydrate, 21.6% fat, and 14.4% protein. Flavor was held constant between trials, although all flavors were nutritionally comparable. Complete nutritional information on the Ensure® products is located in Appendix A.

Thermic Effect of Feeding

Immediately following consumption of the liquid meal, subjects were studied for 5 hours of postprandial EE measurement. Periods of 20 minutes under the ventilated hood (5 minutes habituation, 15 minutes for data analysis) were alternated with 10 minute recovery
periods out of the hood. During the recovery periods, the lights were turned on and subjects were allowed to talk, adjust their position on the bed, and void if necessary.

This resulted in ten, thirty minute periods of TEF measurement with the first 20 minutes of each period under the hood (exception: the last 30 minute period was all under the hood and the last 25 minutes was used for data analysis). Heart rate and blood pressure were recorded at minutes 0, 10, and 20 of each TEF measurement period.

**Blood Sampling**

During the RMR measurement blood sampling was performed at 30 minutes. During the TEF measurement sampling was performed immediately following each twenty minute period under the hood. At each sampling time, one 10 mL sample for analysis of plasma insulin was placed in a test tube (Vacuette®, Greiner Bio-One NA, Inc., Monroe, NC) containing K$_3$-EDTA preservative. Samples were placed on ice, centrifuged (Sorvall® Biofuge Primo R, Thermo Fisher Scientific, Waltham, MA), and stored at -80° Celsius until analysis. Additionally, at each sampling time, a 2 mL sample was drawn for real-time determination of plasma glucose concentration (YSI 2300 Stat Plus, Yellow Springs, OH). Insulin was quantified by human insulin enzyme-linked immunosorbent assay (Millipore, Billerica, MA) from blood samples run in duplicate (TEF periods 8 and 10) or triplicate (all others) and corresponding to baseline RMR and to TEF monitoring periods 1, 2, 4, 6, 8, and 10.

**Statistical Analyses**

All statistical analyses were performed using Statistica for Mac (StatSoft Inc, Tulsa, OK). Baseline values for day-of-trial body mass, RMR, RER, HR, BP, fasting plasma glucose, and fasting
plasma insulin were compared on GTE vs. placebo using paired Student’s t-tests. The incremental area under the curve (AUC) was calculated using the trapezoidal method for the absolute value of postprandial EE and for both the absolute and percentage rise in EE above baseline RMR. It was assumed that baseline RMR held constant throughout the period of TEF monitoring. Student’s paired t-tests were used to compare the AUC’s.

Two-way analysis of variance (ANOVA) with repeated measures was used to detect differences in EE (expressed each of the three ways described above) during the postprandial monitoring periods. If differences were detected, Newman-Keuls post-hoc analysis determined the location (i.e. which TEF monitoring period) of the differences. Two-way ANOVA with repeated measures was also used to identify postprandial differences with regard to RER, HR, BP, plasma glucose, and plasma insulin.

Results are reported as mean ± se. Statistical significance was set at p < 0.05.
CHAPTER IV: RESULTS

Subject Testing

We tested 17 subjects, and one was excluded from analysis due to technical problems with measurement of EE. On average, subjects were young, of a healthy body fat percentage, lightly to moderately active, and normotensive. Detailed subject characteristics are shown in Table 1.

All subjects completed both the EGCG and placebo visits. No subjects reported failure to comply with the pill supplementation (all pill bottles were returned empty), fasting (12 hrs), water abstinence (2 hours), or exercise and alcohol abstinence (24 hrs) regimes.

During testing, all subjects were able to consume the required amount of Ensure® within the allotted 15 minutes.

Resting Comparisons

All results are reported in the order of placebo vs. EGCG. Short-term supplementation with EGCG did not significantly affect body mass (75.1 ± 5.4 vs. 74.8 ± 5.5 kg; p = 0.22), RMR (1665 ± 84 vs. 1610 ± 89 kcal/d; p = 0.10; Fig. 1), baseline RER (0.82 ± 0.01 vs. 0.83 ± 0.01; p = 0.29; Fig. 2) resting HR (59 ± 2 vs. 57± 3 bpm; p = 0.37), mean arterial BP (85 ± 2 vs. 84 ± 2 mmHg; p = 0.58), fasting plasma glucose (73.7 ± 2.0 vs. 75.7 ± 1.5 mg/dL; p = 0.30) or fasting plasma insulin (4.7 ± 1.0 vs. 4.1 ± 0.8 µU/mL; p = 0.41) on the mornings of the trials.
EGCG did not influence the area under the TEF response curve (Fig. 3) when expressed as absolute postprandial EE (441,083 ± 22,436 vs. 429,350 ± 24,209 arbitrary units (AU); p = 0.22; Fig. 4), or as either absolute (58,104 ± 5,297 vs. 58,949 ± 5,672 AU; p = 0.88; Fig. 5) or percentage (3508 ± 263 vs. 3647 ± 285 AU; p = 0.67; Fig. 6) change in EE above baseline RMR. Values are expressed as arbitrary units due to the limited physiological relevance or meaningfulness of group-summed TEF values. Expressing the AUCs as absolute or percentage changes above baseline is a way to examine the magnitude of the TEF response corrected for differences in baseline RMR, whereas absolute postprandial EE is a combination of the magnitudes of the RMR and the TEF responses.

Similarly, there were no differences in the AUCs between placebo and EGCG during the first two hours of TEF measurement, expressed any of the above three ways (Table 2). This period corresponds to the greatest degree of sympathetic nervous system (SNS) activation (SNSA) following a meal (185).

We also examined potential differences at specific time points during the postprandial measurement period, which could reflect a change in the shape of the TEF response curve with EGCG supplementation, indicative of a time-by-treatment interaction. As expected with a TEF measurement, there was a highly significant (p < 0.001) main effect of time (Fig. 3). Post-hoc analysis revealed that the EE at each postprandial measurement period was significantly greater (p < 0.05) than baseline RMR, indicating that at the end of our measurement period (5 hours) EE had not returned to pre-feeding levels. This is not unexpected, as data suggests that measuring TEF for five hours ‘misses’ 9% of the response recorded with 6 hour measurements following normal-sized meals (142).
There was not a significant main effect of treatment, and no time-by-treatment interactions were evident for the TEF response curves, measured as absolute postprandial EE (p = 0.72; p = 0.88; for treatment effects and time-by-treatment interactions, respectively; Fig. 3), absolute change in EE above RMR (p = 0.94; p = 0.80; data not shown), or percentage change in EE above RMR (p = 0.75; p = 0.83; data not shown).

ANOVA also revealed highly significant (p < 0.001) main effects of time for postprandial RER (peaked during the second postprandial period, 35-50 min. post-meal; Fig. 7), heart rate (all postprandial HRs greater than resting HR; Fig. 8), plasma glucose (first postprandial period higher than all others; Fig. 10), and plasma insulin (first postprandial period higher than all others; Fig. 11). However no significant (p > 0.05) main effect of treatment or time-by-treatment interaction was detected for any of these variables. Systolic and diastolic blood pressure are diagrammed in Figure 9.

Table 2 summarizes the main results.
CHAPTER V: DISCUSSION

Given that the SNS supports approximately 5% of RMR and one-third of TEF, that EGCG inhibits catecholamine degradation by COMT, and that EGCG exerts significant physiological effects in humans, we examined the influence of short-term EGCG supplementation on RMR and TEF in a heterogenous group of healthy adults. Contrary to our hypothesis, EGCG did not affect RMR, TEF, or RER during RMR and TEF.

Our results are partly in line with those of Boschmann and Thielecke (27), who also found no difference in postprandial EE following supplementation with Teavigo® brand EGCG. However, they did find a significantly lower RER value (EGCG ~0.84 vs. placebo ~0.91) in the two hours following the test meal. To some degree these results are not unexpected, as the potential of EGCG to increase fat oxidation (lower RER) without having significant effects upon total EE has been reported in mice (90). It is unclear why we found no significant differences in RER between treatments at any time point, as we utilized a larger dose of EGCG and had similar test meal composition to that of Boschmann’s study (50% CHO, 35% fat, 15% protein vs. 64% CHO, 22% fat, 14% protein in present study). However, their subjects were middle aged (40 ± 1 yrs), overweight and obese (BMI 29.9 ± 1.6 kg/m²) men, while our subjects were primarily normal- or slightly over-weight (BMI 24.6 ± 1.2 kg/m², although 4 subjects had BMI ≥ 29), younger (25 ± 2 yrs) men and women. Age, weight or body fatness, and sex all may influence TEF (14, 65, 85) via differences in SNSA or tissue responsiveness to a given level of SNSA; thus investigations of these different groups or populations might be expected to yield slightly different results. Although not the primary focus of the present study, there were no sex specific
(p = 0.21) or weight class specific (BMI < 24.9 vs. BMI > 25.0 kg/m²; p = 0.47) RMR and/or TEF interactions with EGCG.

The labeling accuracy for nineteen green tea dietary supplements, some containing other botanical extracts, has recently been tested using high performance liquid chromatography (HPLC) (161), a common method for determination of polyphenol content (61, 121). Teavigo® brand was not analyzed in this study, but unpublished data from the Colorado State University (CSU) Center for Environmental Medicine indicates that while Teavigo® brand claims at least 90% EGCG content by mass, actual values are closer to 67.4 ± 6.2% (mean ± sd) EGCG. Analyses in both the referenced study and the experiments done at CSU utilized the same methodology, which involved HPLC detection of caffeine and various polyphenols including EGCG at 278 nanometers and the use of linear regression analysis with standards run at 2.5, 5, 10, 20, and 40 µg/mL. In the referenced study, actual EGCG levels measured via HPLC ranged from 12% to 143% of label claims, while in the triplicate analysis performed at CSU, EGCG content was measured at 60.7% (18.2 µg/mL), 68.4% (20.5 µg/mL), and 73.1% (21.9 µg/mL) of total pill mass, translating to 67.4%, 76%, and 81.2% of that claimed on the label (90% of pill mass). However, despite the fact that these analyses demonstrate lower-than-claimed EGCG contents of Teavigo®, significant results in a variety of human and animal studies demonstrate that this extract is able to exert measurable physiological effects.

Further, as a point of reference for future studies, if we assume that our subjects actually consumed ~100 mg EGCG (based on CSU Center for Environmental Medicine data) on the morning of the trial, based on published pharmacokinetic data we would expect to find peak plasma EGCG concentrations (total EGCG = conjugated EGCG + free EGCG) on the order of 100-200 nanograms per milliliter approximately 1.5-2 hours post-ingestion (182). It may be
worthwhile in future investigations involving EGCG to utilize a larger, acute dose taken closer to the beginning of measurement. Given that the terminal elimination half-life of smaller (< 500 mg) doses of EGCG appears to be 2-3 hours, the time following ingestion to reach maximum plasma concentration is approximately 1.5-2 hours, and the fact that our subjects ingested their capsules approximately 1-2 hours before the commencement of data collection, we suspect maximum plasma concentrations were achieved during RMR measurement, with a slow decline throughout the TEF measurement. Not only have half-lives of 4-5 hours been noted with EGCG doses of ~1 g, but peak plasma concentrations of up to 3 µg/mL have been noted, or approximately 15-20 times those seen with a 100 mg dose. While dosing of this nature is supraphysiological (peak plasma concentrations several times higher than the highest noted with normal tea ingestion), it can be useful for identifying the potential of EGCG to influence sympathetic physiology without the simultaneous influence of caffeine or other agents affecting SNSA.

While we did not assay plasma levels of EGCG following supplementation to verify that a physiologically meaningful amount of EGCG entered the systemic circulation (i.e. that a significant amount was absorbed and made it past first-pass metabolism), numerous other studies using Teavigo® brand EGCG supplement in rodents have demonstrated reduced energy absorption, decreased lipogenesis with downregulation of adipogenic genes, increased fat oxidation with upregulation of lipolytic genes, attenuated body fat and body mass accumulation, inhibition of adipocyte differentiation, and improved endurance capacity and increased muscle lipid oxidation (90, 102, 121, 204). However, EGCG doses administered to rodents are often extremely supraphysiological and potentially unsafe for humans in terms of mg EGCG/kg body weight. Nevertheless, studies using Teavigo® in humans have demonstrated reduced RER.
postprandially (without increased energy expenditure (EE)) and reduced resting heart rate and plasma glucose in subjects with impaired glucose tolerance undergoing an exercise regime (27, 71). Additionally, our lab recently demonstrated that short-term ingestion of Teavigo® improves VO₂ max during incremental cycle ergometry to exhaustion ((143); in press). However, the stimulus of maximal exercise represents a condition of greater SNSA than the postprandial period; thus, it may be easier to detect effects of EGCG during periods of greater SNSA. It might be expected that the magnitude of influence of EGCG could follow a exercise > TEF > RMR hierarchy corresponding to the degree of SNSA.

Despite the fact that our supplementation regime should be sufficient to result in physiologically meaningful concentrations of plasma (and presumably synaptic cleft) EGCG, it could be argued that subject compliance may have been limiting. However, despite the fact that our only measure to verify proper supplementation was to obtain verbal confirmation from subjects and to collect their empty pill bottles, this same method was used during our study of Teavigo’s® influence on VO₂ max, and furthermore, our supplementation was brief, uninvasive, simple, and without side effects—it would not be considered a huge burden to ingest seven pills over the course of two days.

Measurements of TEF have been reported to have coefficients of variation of up to 30%; thus there is the possibility that a significant effect of EGCG was masked by the large degree of within-subject variability (45). Assuming no change in variability, however, if there was a significant effect of EGCG on TEF, power analysis revealed it would be necessary to test 631 subjects to demonstrate this effect. Regarding the heterogeneity of the TEF response in the present study, nine subjects displayed a greater TEF (measured as a percentage of baseline RMR) on placebo, while seven displayed a greater TEF on EGCG.
Some authors recommend the use of baseline RMR measured on a separate day from TEF to serve as a proxy for the RMR on the day of TEF measurement in order to account for subtle diurnal fluctuations in RMR (45, 164), as this may reduce intra-individual variability in TEF. However, an important methodological problem with one of these studies (45) is that the RMR measurement on the day of the TEF trial, taken from 1130-1200 hours, only required a 4-5 hour fast, whereas the RMR measurement on a separate day required a 12-14 hour fast. Others have determined that RMR measured immediately prior to test meal administration is sufficient (198), and several studies (9, 20, 27) including the present investigation utilized this protocol along with an overnight fast and RMR measurement first thing in the morning. This protocol can be considered superior because the TEF response is then measured relative to the RMR for that given day. With our protocol, the caloric value of the test meal is also calculated based on 40% of the RMR measured on that day.

The possibility exists that our EGCG supplementation regime was sufficient to cause malabsorption of a small fraction of the test meal (38, 211). Since absolute postprandial EE was not different between EGCG and placebo, it could be argued that EGCG resulted in a greater thermic response (if a degree of malabsorption occurred), especially given that RMR during EGCG supplementation was non-significantly lower (by 55 kcal/day; p = 0.10) than RMR during placebo supplementation, resulting in a slightly smaller test meal on EGCG. Speculation aside, to ascertain any degree of EGCG-induced malabsorption, quantification of the energy content of feces or utilization of tracer methodology would be necessary, and these procedures were not undertaken in the present study.

If the results of the present study and Boschmann’s are confirmed by future investigations, it suggests that the mechanism by which EGCG exerts body mass-lowering effects
is not through increased RMR or TEF, but via other components of the EB equation, namely reduced EI or increased EE due to physical activity. There has been suggestion that EGCG reduces food intake, at least in rats (86), and as mentioned above EGCG may reduce the digestibility or absorption of one or more of the macronutrients. There is also evidence that individuals (mice and humans) consuming EGCG while engaging in an endurance exercise regime lose more body mass or fat depot mass than individuals engaging in an exercise regime alone (113, 168). This could be due to EGCG-induced COMT inhibition interacting with the large degree of SNSA during exercise, enhancing lipolysis and fat oxidation. To decipher exactly through which components of EB EGCG is acting, more rigorous and thorough investigations looking at each component of EB simultaneously are necessary, especially given that our results are at odds with a hypothesis put forward by Dulloo et al (54). In a 24-h study of the effects of a GTE containing caffeine and EGCG, this group noted no elevation of nocturnal EE, and thus speculated that a 40% increase in total postprandial thermogenesis over the course of all meals consumed throughout the day could be responsible for their observed 4% increase in 24-h EE in their study. However, their subjects engaged in a sedentary lifestyle for the days monitored in the respiratory chamber. Our results in combination with Dulloo’s methodology provide further suggestion that increases in physical activity EE may result from EGCG and/or caffeine supplementation.

In 2001, Rumpler et al. noted the capacity of oolong tea (5 x 300 mL daily, each serving containing 54 mg caffeine, 185 mg total polyphenols, and 49 mg EGCG) to increase 24 hour EE by 2.9% and fat oxidation by 12% (with no change in CHO oxidation) versus an isovolumetric water control in adult males (152). A group drinking water + caffeine (also 5 x 300 mL daily, with 54 mg caffeine per serving) had 3.4% increased EE and 8% increased fat oxidation (also with no
change in CHO oxidation) over the 24 hours. Unfortunately this study did not include a non-drinking control group, which would be relevant given that consumption of both cold and body-temperature water may induce some degree of thermogenesis, although this observation is controversial (26, 29, 185). The authors noted that their dosage of oolong tea contained a similar amount of EGCG and nearly twice as much caffeine as a study by Dulloo et al. suggesting potential synergy between EGCG and caffeine in inducing fat oxidation and thermogenesis (54). Rumpler et al. also concluded that oolong tea (which at 15-75% oxidation lies midway between green tea—not oxidized to any significant degree, and black tea—fully oxidized) by virtue of its capacity to raise 24 hour EE and fat oxidation, may have beneficial effects on maintenance of a lower body fat content, but that this effect may only be realized if benefits of tea drinking are sustained with chronic consumption. They also note the importance of studying the effects of the noncaffeine components of tea (i.e. polyphenols) independent of the caffeine.

Evidence from a randomized, diet- and placebo-controlled trial in 2007 in obese Thais consuming 3 Herbal One® brand capsules (250 mg capsules: 0.24 mg gallic acid, 4.09 mg catechin, 28.86 mg caffeine, 33.58 mg EGCG, 9.28 mg epicatechin gallate, other components not specified) per day with meals indicates that there may be diminishing effects over time of a consistent dosage of green tea extract (11). After 8 weeks, the supplemented group had lost 4.44 kg body mass and 7.22% body fat while the placebo group had lost 1.93 kg body mass and 3.02% body fat. After 12 weeks, the magnitude of the differences between groups was reduced. Versus baseline, the supplemented group had lost 2.7 kg body mass and 3.8% body fat and the placebo group had lost 2.0 kg body mass and 2.77% body fat, indicating regain of some of the lost mass in the last four weeks of the trial. The fact that some of the fat mass lost by week 8 was regained by week 12 in the supplemented group indicates that the effects of the
supplement might have been diminishing, and that a ‘floor’ of body/fat mass was not experienced. Longer term trials investigating the influence of green tea on weight loss were recommended,

A mechanism for potentially attenuated body- and fat-mass loss with prolonged green tea supplementation may involve a diminished response to the prolonged β-adrenergic receptor cascade stimulation provided by EGCG-induced catechol-O-methyltransferase (COMT) inhibition and caffeine-mediated cyclic nucleotide phosphodiesterase inhibition. EGCG’s primary mechanism of action with regard to lipolysis and thermogenesis is thought to be COMT inhibition. COMT is one of the enzymes that degrades catecholamines. Inhibition of their degradation would potentially result in increased catecholamine residence time in the synaptic clefts of postganglionic sympathetic neurons and in the plasma, providing prolonged stimulation of β-adrenergic receptors. With chronic prolonged stimulation due to repetitive green tea supplementation, there could be downregulation of the β-adrenergic receptor population in various tissues including white adipose tissue and skeletal muscle. There is also the possibility of a blunted degree of signaling downstream of the beta-adrenergic receptor, 1) as a response to chronically elevated β-adrenergic receptor stimulation due to EGCG-induced catechol-O-methyltransferase inhibition of norepinephrine (NE) degradation or, 2) as a result of the chronic effects of caffeine on phosphodiesterase. Together, a down-regulated β-adrenergic receptor population and a reduced propagation of the signaling cascade initiated downstream of the receptor, in multiple tissues, could lead to a smaller degree of lipolysis and a reduced degree of thermogenesis in response to β-adrenergic stimulation. This could be responsible for the potentially attenuated effects of green tea supplementation in medium- or longer-term studies. However, an intermittent EGCG dosage, or combining an EGCG supplementation regime with
endurance exercise may help avoid potentially attenuated effects of EGCG on fat mass loss.

There remains the possibility that unidentified ingredients in green tea drank traditionally (i.e. not in processed, encapsulated form) could exert β-adrenergic receptor sensitizing effects and counter the potential blunted long-term response seen with encapsulated EGCG or caffeine supplements. More detailed biochemical analyses and long-term epidemiological and clinical trials are necessary in this area, as it is still unclear whether longer-term supplementation leads to attenuated effects versus those seen in the short-term. It could be useful to combine such a body mass and body fat loss trial with measurements of EE under conditions of β-adrenergic receptor stimulation (i.e. intravenous infusion of isoproterenol (isoprenaline)), as a blunted metabolic response to such stimulation (i.e. a lower response after months of green tea supplementation, versus a baseline measure) might indicate pharmacological tolerance to the supplement.

There is also a possibility that exercise, by virtue of its sensitizing effects on NE-induced lipolysis, exerts a permissive effect on the ability of EGCG, caffeine, etc. to increase thermogenesis. It has been suggested that the increased lipolytic response to catecholamines in exercise-trained versus sedentary women is due to increased efficiency of the β-adrenergic signaling cascade, specifically at a point in the cascade beyond adenylate cyclase (144), possibly at the level of protein kinase A, hormone-sensitive lipase, or adipose triglyceride lipase. If long-term supplementation with EGCG, caffeine, or both does indeed result in a diminished propagation of the signal at this point in the cascade, exercise may have the capacity to work synergistically with green tea supplements to increase thermogenesis both during the exercise itself and during rest, owing to its sensitization of the β-adrenergic signaling cascade.

Our RER data demonstrate that our moderately high-CHO test meal (64% CHO, 21.6%
fat, 14.4% protein) resulted in preferential CHO oxidation in the postprandial phase, consistent with the idea that ingested CHO, but not fat, has a strong capacity to stimulate its own oxidation (2, 157). Therefore this type of test meal may not provide the ideal conditions for evaluating the response of potentially thermogenic agents such as green tea extracts on the TEF response. Additionally, a high-CHO test meal results in a large insulin response, and insulin is known to inhibit lipolysis via phosphodiesterase-3B activation (193). Thus it may be desirable to study the influence of EGCG or other green tea extracts on TEF under conditions of relatively greater lipolysis. The addition of capsaicin, the pungent component of many varieties of peppers, to test meals has the potential to elevate fat oxidation and decrease CHO oxidation, while increasing total postprandial EE, when added to both high fat and high-CHO test meals (209). Further, given that a role for increased SNSA in protein- and fat-induced thermogenesis is unclear (185), and given that EGCG is purported to influence EE via an influence on the SNS, it may be advantageous to study the effects of EGCG on TEF during a high-CHO test meal supplemented with capsaicin, despite the aforementioned drawback of insulin-mediated lipolytic inhibition, as a role for CHO stimulation of SNSA is more definitive.

Many opportunities for future research are possible. As stated above, it is important to elucidate the specific effects of individual compounds such as EGCG and caffeine, and of mixtures of compounds and whole plant preparations, on all facets of the EB equation—RMR, physical activity EE, TEF, and EI. In doing so, proper experimental control and protocol are vital, as previous experiments have utilized a multitude of different protocols which may be flawed for several reasons, including insufficient duration of measurement, insufficient dosage of treatment compounds, etc. Further, due to the highly variable nature of the TEF measurement, future studies measuring this component of EE (simultaneously with green tea or other
pharmacological agents, or otherwise) may benefit from utilizing an average of multiple measurements on each treatment condition. Despite the tedious nature of measuring postprandial EE for five or more hours, this duration (used in the present study) of measurement appears necessary to sufficiently encompass the majority of the TEF response, and multiple measurements of this duration during each treatment may prove useful in circumventing the inherent variability of the TEF measurement.

It may also be useful to recruit and perform studies in groups varying in only one characteristic. For example, studying males and females matched for body fat percentage, aerobic exercise status, and basal SNSA could help elucidate sex differences in response to EGCG supplementation. As women have a greater percentage of type I fibers in skeletal muscle, and type I fibers have a $\beta$-adrenergic receptor density three times that of type II fibers in the same muscle (209), women might be expected to show a greater response to EGCG supplementation (although women may also have lower tonic SNSA or lower tissue responsiveness to a given level of SNSA (14)). Further control for fiber type distribution could help elucidate other effects of sex. Performing several experiments of this nature may prove key to reconciling previously discrepant results and discovering exactly the effects of these treatments.

Future experiments evaluating the efficacy of green tea compounds for weight loss, fat-mass loss (including specific depots), and numerous other effects during different phases of supplementation are also important. That is, more research is necessary to determine whether tolerance to the beneficial effects of green tea occurs in studies of several months’ or years’ duration.

In conclusion, we demonstrated with a well-controlled experimental protocol of sufficient measurement duration that short-term EGCG supplementation does not influence RMR or TEF in a heterogeneous population of healthy adult humans.
### Table 1: Subject Characteristics

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<th>Variable</th>
<th>Mean ± SE</th>
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<td>Sex (M/F)</td>
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</tr>
<tr>
<td>Age (years)</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173 ± 3</td>
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<tr>
<td>Body mass (kg)</td>
<td>74.9 ± 5.6</td>
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<td>BMI (kg/m²)</td>
<td>24.6 ± 1.2</td>
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<tr>
<td>Waist : Hip ratio</td>
<td>0.84 ± 0.02</td>
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<tr>
<td>% Body Fat</td>
<td>22.6 ± 1.8</td>
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<tr>
<td>VO₂peak (mL/kg/min)</td>
<td>38.1 ± 1.5</td>
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<tr>
<td>RERpeak</td>
<td>1.24 ± 0.03</td>
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<tr>
<td>Resting HR (bpm)</td>
<td>59 ± 2</td>
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<tr>
<td>Resting BP (mmHg)</td>
<td>117/69 ± 3/2</td>
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### Table 2: Primary Results

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<th>Variable (units)</th>
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<th>EGCG</th>
<th>p-value</th>
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<tr>
<td>Body mass, day of trial (kg)</td>
<td>75.1 ± 5.4</td>
<td>74.8 ± 5.5</td>
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<tr>
<td>RMR (kcal/d)</td>
<td>1665 ± 84</td>
<td>1610 ± 89</td>
<td>0.10</td>
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<td>RER – prefeeding</td>
<td>0.82 ± 0.01</td>
<td>0.83 ± 0.01</td>
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<td>Fasting plasma glucose (mg/dL)**</td>
<td>73.7 ± 2.0</td>
<td>75.7 ± 1.5</td>
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<td>Fasting plasma insulin (µU/mL)**</td>
<td>4.7 ± 1.0</td>
<td>4.1 ± 0.8</td>
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<tr>
<td>Abs. PP EE5 (AU)¹</td>
<td>441,083 ± 22,436</td>
<td>429,350 ± 24,209</td>
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<td>Abs. PP EE2 (AU)²</td>
<td>157,104 ± 7,891</td>
<td>154,025 ± 8,852</td>
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<td>Δ TEF EE5 (AU)³</td>
<td>58,104 ± 5,297</td>
<td>58,949 ± 5,672</td>
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<tr>
<td>Δ TEF EE2 (AU)⁴</td>
<td>31,168 ± 2,591</td>
<td>32,393 ± 2,917</td>
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<td>Δ% TEF EE5 (AU)⁵</td>
<td>3,508 ± 263</td>
<td>3,647 ± 285</td>
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<tr>
<td>Δ% TEF EE2 (AU)⁶</td>
<td>1,890 ± 134</td>
<td>1,998 ± 145</td>
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</table>

** n= 8 for fasting plasma glucose (technical problems with analyzer or blood flow for the other 8 subjects); n = 13 for fasting plasma insulin (technical problems with blood flow for other 3 subjects); n = 16 for all other results

1: Absolute postprandial energy expenditure over the whole 5 hour TEF measurement period
2: Absolute postprandial energy expenditure over the first 2 of 5 hours
3, 4: same as 1 and 2, but expressed as the absolute difference between total postprandial EE and baseline RMR
5, 6: same as 3 and 4, but expressed as the percentage difference rather than absolute difference
Figure 1: EGCG does not affect resting metabolic rate (p = 0.10). Each line represents the results for an individual subject. Each bar is the average for each treatment condition (placebo:1665 ± 84 vs. EGCG:1610 ± 89 kcal/day; data mean ± se)
Figure 2: EGCG does not affect baseline respiratory exchange ratio (data mean ± se).
Figure 3: EGCG does not affect ($p = 0.72$ for main effect of treatment; $p = 0.88$ for time by treatment interaction) absolute postprandial energy expenditure at any time point (data mean ± se).
Figure 4: EGCG does not influence area under the curve for absolute postprandial energy expenditure for the entire TEF monitoring period (data mean ± se).
Figure 5: EGCG does not affect area under the curve for absolute change in energy expenditure above RMR baseline for the entire TEF monitoring period (data mean ± se).
Figure 6: EGCG does not affect area under the curve for percentage change in energy expenditure above RMR baseline for the entire TEF monitoring period (data mean ± se).
Figure 7: EGCG does not affect respiratory exchange ratio during TEF measurement (p = 0.57 for main effect of treatment; p = 0.83 for time by treatment interaction; data mean ± se).
Figure 8: EGCG does not affect heart rate during TEF measurement (p = 0.73 for main effect of treatment; p = 0.54 for time by treatment interaction; data mean ± se).
Figure 9: EGCG does not affect systolic (p = 0.83 for main effect of treatment, p = 0.13 for time by treatment interaction) or diastolic (p = 0.60, p = 0.64) blood pressure during TEF measurement (data mean ± se).
Figure 10: EGCG does not affect blood glucose concentration during TEF measurement (p = 0.65 for main effect of treatment; p = 0.95 for time by treatment interaction; data mean ± se).
Figure 11: EGCG does not affect plasma insulin concentration during TEF measurement (p = 0.96 for main effect of treatment; p = 0.86 for time by treatment interaction; data mean ± se).
REFERENCES


19. Kristorp CN, Toubro Sr, Astrup A, and Svendsen OL. Comparison of resting energy expenditure measurements by ventilated canopy and by


195. Welle S, Schwartz RG, and Statt M. Reduced metabolic rate during beta-adrenergic
### Ensure® Nutrition Information

#### Product Data

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<th></th>
<th>Homemade Vanilla</th>
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#### Nutrient Data

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