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1 **Epigallocatechin-3-gallate and postprandial fat oxidation**
2 **in overweight / obese male volunteers – a pilot study**

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25 Short running head: Effect of EGCG on fat oxidation

1 **ABSTRACT**

2 **Objective:** Drinking green tea is associated with many health benefits, including
3 increased fat oxidation. We tested the hypothesis that Epigallocatechin-3-gallate
4 (EGCG), the main green tea catechin, increases fat oxidation in obese men.

5 **Methods:** Ten healthy overweight/obese males (BMI $31.3 \pm 0.8 \text{ kg/m}^2$) were studied in
6 a randomized, placebo-controlled, double-blind crossover trial. Study supplements
7 were low EGCG (300 mg), high EGCG (600 mg), caffeine (200 mg), EGCG/caffeine
8 (300 mg/200 mg) or placebo and were taken orally for 3 days. At the third day of
9 supplementation, O_2 consumption and CO_2 production was measured by indirect
10 calorimetry in order to assess energy expenditure and fat oxidation over 4 hours each
11 after overnight fasting and after a standardized test meal.

12 **Results:** Energy expenditure was not affected by any supplementation, neither after
13 overnight fasting nor after the test meal. During the first 2h after overnight fasting, fat
14 oxidation increased by 7.7 (n.s.), 15.2 (n.s.), 26.3 ($p < 0.05$ vs. placebo), and 35.4%
15 ($p < 0.01$ vs. placebo and low EGCG), for low EGCG, high EGCG, caffeine, and
16 EGCG/caffeine, respectively. During the first 2h after the meal, the mean increase in
17 fat oxidation was 33.3 ($p < 0.05$ vs. placebo), 20.2 (n.s.), 34.5 ($p < 0.05$ vs. placebo), and
18 49.4% ($p < 0.05$ vs. placebo) for low EGCG, high EGCG, caffeine, and EGCG/caffeine,
19 respectively.

20 **Conclusion:** Low EGCG increases postprandial fat oxidation in obese men and this to
21 the same extent as 200 mg caffeine, while high EGCG does not exert this effect.

22 **Fasting fat oxidation is increased only by caffeine (with or without EGCG).** There is no
23 synergism of low EGCG and 200 mg caffeine. Energy expenditure is not affected by
24 EGCG.

25 **Keywords:** EGCG, caffeine, energy expenditure, fat oxidation, calorimetry

1 INTRODUCTION

2 Obesity is recognized as an increasing health burden. In 1999-2000 the age-adjusted
3 prevalences of overweight and obese individuals in the US was 64.5% and 30.5%,
4 respectively (Flegal *et al.* 2002). There is growing interest in alternate strategies for
5 weight management. Green tea catechins (flavan-3-oles) have received particular
6 attention, specifically epigallocatechin gallate (EGCG), which represents about 43% of
7 green tea catechin content (Scholz E. *et al.* 1995, Yang and Landau 2000). Several
8 studies have shown that green tea and green tea extracts (GTEs) high in EGCG
9 reduce body weight and body fat tissue in humans when administered over 12 weeks
10 (Chan *et al.* 2006, Chantre and Lairon 2002, Hase *et al.* 2001, Kajimoto *et al.* 2006,
11 Nagao *et al.* 2005, Nagao *et al.* 2007, Tsuchida *et al.* 2002). However, the major mode
12 of action is still unknown. Several mechanisms have been suggested including (a)
13 inhibition of adipocyte differentiation and proliferation (Hung *et al.* 2005, Wolfram *et al.*
14 2005), (b) reduction of fat absorption (Juhel *et al.* 2000, Raederstorff *et al.* 2003, Yang
15 *et al.* 2001) and (c) inhibition of catechol-O-methyl-transferase, an enzyme that
16 catalyzes noradrenalin degradation in the central nervous system, thereby prolonging
17 the effects of noradrenalin (Borchardt and Huber 1975, Rhodes 1996). Recent human
18 studies suggest that the anti-obesity properties of green tea extract or oolong tea (high
19 in EGCG) may partly be attributable to increased fat oxidation (Dulloo *et al.* 1999,
20 Rumpler *et al.* 2001). In a randomized, double-blind, placebo-controlled trial, fat
21 oxidation increased by 35% after supplementation with encapsulated GTE (Dulloo *et al.*
22 *et al.* 1999). In this trial, male volunteers consumed either placebo, 150 mg caffeine or a
23 GTE containing 270 mg EGCG and 150 mg caffeine within 24h. Caffeine at the utilized
24 dose did not increase 24h energy expenditure or fat oxidation. However, the increase
25 in fat oxidation with the GTE exceeded the one observed with caffeine alone,

1 suggesting components other than caffeine contributing to the increase in fat
2 oxidation. In another randomized cross-over trial, 12 healthy volunteers consumed
3 oolong tea, resulting in daily consumptions of 244 mg EGCG and 270 mg caffeine, or
4 a comparable volume of water (control) within 24h (Rumpler *et al.* 2001). As a result,
5 24h fat oxidation increased by 12% with oolong tea vs. control. However, these two
6 trials used mixtures of catechins and caffeine; it is therefore not possible to deduce the
7 individual contribution of the main active ingredients in green tea, i.e., EGCG or
8 caffeine, to increased fat utilization. However, a long-term investigation observed
9 increases in fat oxidation of 37% and 32% for both sedentary and exercising healthy
10 subjects when consuming daily a beverage containing 570 mg green tea catechins
11 (218 mg EGCG) for 8 weeks (Ota *et al.* 2005). Interestingly, the beverage contained
12 <40 mg caffeine, suggesting that EGCG and/or other catechins are mainly responsible
13 for this observation.

14 Yet, the effect of pure EGCG on energy metabolism and fat oxidation in humans has
15 not been studied in detail. Therefore, in the present study, we wanted to test the
16 hypothesis that EGCG per se contributes to increased fasting and postprandial fat
17 oxidation in overweight / obese volunteers.

18

1 **METHODS**

2 ***Subjects.***

3 Twelve male volunteers (BMI 27 - 35 kg/m²) were included in the study. All volunteers
4 were healthy, non-smoking and drug-free as determined by medical history, physical
5 examination, and routine blood and urine tests. Specific exclusion criteria were: intake
6 of dietary supplements within the week prior to the study or during the study itself and
7 habitual consumption of caffeine \geq 300 mg per day (\geq 3-6 cups of coffee per day)
8 and/or of green tea \geq 5 cups per day. During the study, no caffeine and catechin
9 containing drinks and food were allowed. Volunteers were studied at the Franz Volhard
10 Clinical Research Centre (CRC), Charité Universitary Medicine Berlin, Germany. The
11 study protocol was approved by the Institutional Review Board of the Charité and all
12 participants gave written informed consent before study entry.

13 ***Study design.***

14 In this double-blind cross-over study, volunteers received encapsulated supplements
15 over 3 days in a randomized manner. Daily supplement doses were 300 mg (low)
16 EGCG, 600 mg (high) EGCG, 200 mg caffeine, 300 mg EGCG / 200 mg caffeine or
17 placebo (lactose). To reach these dose levels, volunteers took 2 capsules per day with
18 50 ml water, i.e., one capsule 1h prior to breakfast and dinner, respectively. Teavigo[®],
19 a highly purified extract from green tea leaves (*Camilla sinensis*) containing min. 94%
20 EGCG and max. 0.1% caffeine (DSM Nutritional Products, Basel, Switzerland), and
21 caffeine (Sigma-Aldrich, Taufkirchen, Germany) were used for preparing the
22 supplements. On the second day of supplementation, volunteers were hospitalized in
23 our CRC under controlled food intake and physical activity. In the morning of the third
24 day at 8:00 AM, metabolic measurements were started (**Fig. 1**). There was a wash-out
25 period of at least 7 days between the different treatments.

1 **Study protocol.**

2 Subjects stayed in the supine position throughout the whole test period of 8 hours with
3 the exceptions described below. During the test, subjects were allowed to watch TV.
4 Before starting the test, baseline heart rate, beat-by-beat blood pressure (Finapres,
5 Ohmeda, Louisville, CO) and brachial arterial blood pressure (Dinamap, Critikon,
6 Newport, UK) were measured. Then, one catheter was placed into a large antecubital
7 vein for blood sampling at baseline and every 30 min during the test. Metabolic
8 measurements by indirect calorimetry were carried out over 4h each in the fasting and
9 the postprandial state. There was a 1h break in between the two blocks in which
10 subjects consumed the standardized test meal, as well as 30 minutes in the middle of
11 each block during which the subjects were allowed to visit the bathroom. Thus,
12 samples were obtained and analyzed in two 2 h-intervals for each block (**Fig. 1**).

13 The half life of EGCG at the doses applied in this study varies from 1.9 to 4.3 hours
14 (Ullmann *et al.* 2003). Therefore, half of the daily doses of EGCG and caffeine were
15 provided 1 hour before the postprandial measurement to keep the plasma levels of
16 EGCG and caffeine comparable with the fasting conditions.

17 The standardized test meal (bread, butter, cheese, ham, tomato and cucumber) was
18 prepared by a dietician considering the individual energy requirement of each
19 volunteer. The meal provided 5 kcal/kg body weight with 50, 35, and 15% of energy
20 from carbohydrates, lipids, and proteins, respectively.

21 **Measurements and calculations.**

22 Anthropometry. Body weight was measured with an electronic calibrated scale
23 (Soehnle, Murrhardt, Germany) and height with a GPM anthropometer (Siber &
24 Hegner, Zurich, Switzerland). Body mass index (BMI) was calculated as body weight
25 [kg] / (height [m])².

1 Calorimetry. Oxygen consumption (VO_2 , ml/min) and carbon dioxide production
2 (VCO_2 , ml/min) were measured by a ventilated hood system (Deltatrac™ II, GE
3 Healthcare, Freiburg, Germany). Sampling rate was 1/min. Means of 15 min sampling
4 intervals were used to calculate fasting and postprandial energy expenditure (EE),
5 respiratory quotient (RQ), and fat (FOX) and carbohydrate (COX) oxidation rates
6 according to the equations proposed by Ferrannini (Ferrannini 1988)]. The RQ, the
7 quotient of VCO_2 and VO_2 , can vary between 1.0 indicating 100% carbohydrate
8 oxidation and 0.7 indicating 100% fat oxidation and can be used for assessing
9 changes in substrate oxidation rates.

10 Blood samples. Plasma insulin and glucose were measured according to international
11 standards and non-esterified fatty acids (NEFA) by an automated colorimetric test
12 (ABX Pentra 400 Chemistry Analyser, Horiba ABX, Bedfordshire, U.K.).

13

14 **Statistics.**

15 For each subject, global fitting was used to compare the response curves for EE and
16 RQ after the different supplements with the response curve for placebo. This is a non-
17 linear regression method in which one curve (placebo) for each subject is used as
18 baseline, allowing evaluations of discrepancy of the other curve (test supplement).
19 This method works in analogy to the paired samples t-test, using a pair of curves
20 instead of a pair of values. Mean 2h values of EE, RQ, FOX and COX within the 4h
21 pre meal and 4h post meal between the different treatments were compared by a
22 paired *t*-test. P-values < 0.05 were considered statistically significant. All data are
23 given as means \pm SE if not stated otherwise. All statistical tests were performed with
24 InStat, Version 3.0, Graphpad Software Inc., San Diego, CA.

1 RESULTS

2 Twelve subjects were enrolled in the study, 10 subjects completed all five
3 supplementation periods. One subject dropped out of the study after the second
4 supplementation for reasons not related to the study. Another subject withdrew due to
5 a reluctance to use of the catheter. There were no adverse effects attributable to
6 EGCG, neither with 300 mg nor with 600 mg for 3 days, or caffeine.

7 Screening characteristics of the enrolled study population are given in **Table 1**.

8

9 Time courses of energy expenditure (EE)

10 On placebo, resting EE was 5.79 ± 0.26 kJ/min and it remained at that level over the
11 following 240 min. Shortly after the test meal, EE rose to 6.59 ± 0.36 kJ/min followed
12 by a slow but steady decline back to baseline levels at the end of testing (**Fig. 2**).

13 Within the other treatment groups, resting EE was 5.68 ± 0.26 , 5.76 ± 0.27 , $6.01 \pm$
14 0.22 and 5.98 ± 0.21 kJ/min on low EGCG, high EGCG, caffeine and EGCG/caffeine,
15 respectively. In all four groups, EE did not change significantly over the following 240
16 min before the intake of the test meal and showed an almost identical postprandial
17 time course vs. placebo (**Fig. 2**).

18

19 Time courses of respiratory quotient (RQ)

20 Fasting RQ were 0.85 ± 0.02 , 0.87 ± 0.02 , 0.84 ± 0.02 , 0.84 ± 0.02 , and 0.82 ± 0.01
21 for placebo, low EGCG, high EGCG, caffeine, and EGCG/caffeine, respectively.

22 On placebo, RQ oscillated around 0.86 within the first pre-meal block, decreased then
23 to 0.79 ± 0.01 at $t = 280$ min, but returned to fasting values at the end of the second
24 pre-meal block (**Fig. 3**). After the test meal, RQ increased to 0.89 ± 0.02 within the first
25 post-meal block, but returned to the fasting value within the second post-meal block.

1 On low EGCG, time course of fasting RQ did not differ significantly from placebo. After
2 the meal, RQ increased only slightly and remained at lower values during the first
3 post-meal block ($p < 0.05$, low EGCG vs. placebo), but followed the time course under
4 placebo in the second post-meal phase.

5 On high EGCG, time courses of fasting and postprandial RQ did not differ significantly
6 from placebo values.

7 On caffeine, fasting RQ was significantly lower during the first ($p < 0.01$ vs. placebo) but
8 did not differ significantly from placebo during the second pre-meal period. After the
9 meal, RQ increased comparable to placebo during the first, but decreased to $0.81 \pm$
10 0.01 and hence below the values on placebo during the second post-meal period
11 ($p < 0.01$ vs. placebo).

12 On EGCG / caffeine, RQ was significantly lower during the 2 pre-meal periods
13 ($p < 0.001$ vs. placebo). After meal intake, RQ increased but remained below the time
14 course on placebo ($p < 0.001$, EGCG / caffeine vs. placebo).

15

16 **Mean fat oxidation rates (FOX)**

17 During the first pre-meal block, 300 and 600 mg EGCG tended to increase FOX
18 slightly by 0.29 g/h and 0.57 g/h compared to placebo; however the changes did not
19 reach statistical significance (**Table 2**). Caffeine and caffeine/EGCG increased FOX by
20 0.99 g/h ($p < 0.05$) and 1.33 g/h ($p < 0.01$), respectively, during that block. Although
21 blunted for all supplementations, the findings for the second pre-meal block showed
22 similar trends with significant increases of FOX only after caffeine and caffeine/EGCG.
23 During the first post-meal block, only low EGCG increased FOX significantly ($p < 0.05$
24 vs. placebo), not high EGCG. Caffeine and caffeine/EGCG resulted in an increase of
25 1.18 and 1.69 g/h, respectively (both $p < 0.05$ vs. placebo). During the second post-

1 meal block, the delta in FOX was only slightly, but not significantly higher with 300
2 (0.44 g/h) and 600 mg EGCG (0.26 g/h) vs. placebo. However, the change in FOX
3 after caffeine and caffeine/EGCG was with 1.16 g/h (both $p < 0.05$) still significantly
4 higher vs. placebo.

5

6 **Mean carbohydrate oxidation rates (COX)**

7 During the first pre-meal block, 300 and 600 mg EGCG tended to reduce COX slightly
8 by 1.21 g/h and 1.42 g/h, respectively, vs. placebo; however the changes were not
9 statistically significant (**Table 2**). Caffeine and caffeine/EGCG reduced COX by 1.78
10 and 2.70 g/h (both $p < 0.05$), respectively. Similar findings were recorded for the second
11 pre-meal block, with significantly lower COX only after caffeine/EGCG. During the first
12 post-meal block, only low EGCG reduced COX significantly ($p < 0.05$ vs. placebo), not
13 high EGCG. Whereas caffeine alone did not affect COX significantly, caffeine/EGCG
14 reduced it ($p < 0.05$ vs. placebo). During the second post-meal block, the delta in COX
15 was only slightly, but not significantly lower with 300 (-0.86 g/h) and 600 mg EGCG (-
16 0.32 g/h) vs. placebo. However, the delta in COX after caffeine and caffeine/EGCG
17 was 2.54 g/h and 2.50 g/h (both $p < 0.05$), respectively, vs. placebo. With caffeine, COX
18 was also significantly lower vs. low and high EGCG ($p < 0.05$).

19

20 **Blood Glucose, insulin and non-esterified fatty acids (NEFA)**

21 Fasting blood glucose and insulin did not differ significantly between the different
22 supplementations. Within 90 min after meal intake, glucose and insulin increased
23 about 1.25- and 4-fold, respectively, and returned to baseline until the end of the test,
24 regardless of supplementation.

25 Fasting NEFA under placebo were 0.29 ± 0.06 mmol/l and did not change significantly

1 over the entire pre-meal period (**Fig. 4**). During the first post-meal block, NEFA
2 decreased to 0.07 ± 0.01 mmol/l, but returned to baseline levels within the second
3 post-meal block. NEFA profiles did not differ significantly under low and high EGCG
4 supplementation when compared to placebo. In contrast, under caffeine or
5 caffeine/EGCG, pre-meal NEFA levels were higher when compared to placebo, but
6 the difference was not statistically significant. However, under the low EGCG dose,
7 pre-meal NEFA were significantly lower vs. caffeine ($p < 0.0001$) and EGCG/caffeine
8 ($p < 0.0001$).

9

10 **Resting blood pressure and heart rate**

11 Heart rate (HR) and systolic (SBP) and diastolic blood pressure (DBP) was measured
12 only in the morning of the third study day before supplementation. SBP was slightly
13 higher after caffeine (129 mmHg) vs. placebo (125 mmHg), whereas DBP was slightly
14 higher after EGCG/caffeine (76 mmHg) vs. placebo (72 mmHg). EGCG alone did not
15 affect either SBP or DBP. HR was not affected by any of the supplements (**Table 3**).

16

17

18 **DISCUSSION**

19 While a mixture of green tea catechins and caffeine increases fat oxidation in humans,
20 the individual contributions of the major catechin EGCG and caffeine to this effect
21 have not been studied, yet. This study shows that EGCG can increase fat oxidation in
22 overweight/obese males, particularly during postprandial conditions. As expected,
23 caffeine had a pronounced effect on fat oxidation. However, combining the two
24 ingredients did not result in a synergistic effect.

25

1 Impairments in sympathetic nervous system (SNS) mediated lipid mobilization and
2 oxidation may be important in the age-related increase in adiposity and insulin
3 resistance (Blaak 2000). SNS activity can be increased either directly by β -adrenergic
4 agonists or indirectly by norepinephrine releasers and reuptake inhibitors (Arch and
5 Wilson 1996, Dulloo 1993b, Landsberg and Young 1993). Interestingly, a number of
6 plant-derived compounds such as caffeine from coffee and tea, ephedrine from
7 ephedra and capsaicin from pungent spices or a green tea extract low in caffeine (<40
8 mg/day) can modulate diet-induced thermogenesis and / or fat oxidation (Dulloo
9 1993a, Dulloo 1998, Henry and Emery 1986, Ota *et al.* 2005, Yoshioka *et al.* 1998).
10 EGCG inhibits catechol-O-methyltransferase, an enzyme involved in the degradation
11 of norepinephrine (Borchardt and Huber 1975). As a consequence, norepinephrine
12 can longer stimulate adrenoreceptors. Caffeine inhibits the phosphodiesterase-induced
13 degradation of intracellular cAMP (Dulloo *et al.* 1992). A prolonged stimulation of β -
14 adrenoreceptors and increased intracellular cAMP levels lead to an increase in energy
15 expenditure and fat oxidation. An overview of these sympathomimetic mechanisms is
16 given in **Fig. 5**.

17

18 The maximum increase in both fasting (+35.4%) and postprandial (+49.4%) fat
19 oxidation was achieved with 300 mg EGCG/200 mg caffeine which is similar to the
20 results reported by Dulloo *et al.* who used an encapsulated green tea extract with 270
21 mg EGCG/150 mg caffeine per day which resulted in an increase in 24-h fat oxidation
22 of 35.2% (Dulloo *et al.* 1999). Due to the experimental set-up in the study by Dulloo *et*
23 *al.* it is not possible to distinguish between fasting and postprandial conditions. The
24 same authors report that the increase in fat oxidation was greater with the green tea
25 extract vs. caffeine alone, suggesting a strong contribution of EGCG since no

1 significant changes in energy expenditure and lipid oxidation were observed with 150
2 mg caffeine alone. The slightly higher increase in fat oxidation in our study might be
3 attributable to the higher EGCG/caffeine content of our capsule preparation. In a
4 similar trial, normal and overweight men received three times daily for three days
5 either water, water + 270 mg caffeine, half-strength oolong tea (122 mg EGCG/135 mg
6 caffeine), or full-strength oolong tea (244 mg EGCG/270 mg caffeine) (Rumpler *et al.*
7 2001). Relative to water, 24-h energy expenditure increased significantly by 2.9%
8 (+281 kJ/d) with the full-strength tea and by 3.4% (+331 kJ/d) with caffeinated water.
9 Fat oxidation increased significantly by 12% and 8% for the full-strength tea and the
10 caffeinated water, respectively, while there was no significant difference in fat
11 oxidation between the full-strength tea and caffeinated water.

12 The effects of acute ingestion of caffeine-free green tea extract was also tested during
13 moderate intensity exercise (Venables *et al.* 2008), where 890 mg polyphenols and
14 366 mg EGCG increased fat oxidation by 17% compared to exercise alone.

15

16 In contrast to Dulloo *et al.*, we did not observe an increase in energy expenditure at
17 the utilized doses (Dulloo *et al.* 1999). In mice, EGCG increased fat oxidation without
18 significantly affecting total energy expenditure (Klaus *et al.* 2005), suggesting that an
19 increase in energy expenditure might be dependent on the caffeine content present in
20 tea beverages. It is well documented that caffeine increases energy expenditure
21 (Acheson *et al.* 1980, Arciero *et al.* 2000, Astrup *et al.* 1990, Belza *et al.* 2009, Bracco
22 *et al.* 1995, Dulloo *et al.* 1989, Horton and Geissler 1996). Energy expenditure
23 increased also after ingestion of capsules containing 600 mg caffeine and varying
24 amounts of EGCG (Berube-Parent *et al.* 2005). According to a more recent study, 24-
25 h energy expenditure increased significantly by 4.6% and fat oxidation non-significantly

1 by 3.2 g/24h after consumption of a thermogenic beverage, based on a green tea
2 extraxt containing inter alia 282 mg EGCG, 300 mg caffeine and 633 mg calcium
3 (Rudelle *et al.* 2007). Based on literature analysis, the authors hypothesized that the
4 principle active component in the tested beverage is caffeine, accounting for 75% of
5 the observed response.

6

7 Another potential explanation for observed differences in energy expenditure and fat
8 oxidation might be the form of application – capsules vs. beverages. An increase in
9 energy expenditure was found following daily consumption of 1500 ml oolong tea
10 preparations (Rumpler *et al.* 2001) or 750 ml of a thermogenic beverage based on a
11 green tea extraxt (see above) (Rudelle *et al.* 2007). These findings are in contrast to
12 our observations where capsules were taken with a rather small volume of water.

13 Possibly, water intake in a sufficient high volume can affect the magnitude of the
14 thermogenic effect of caffeine. The large volumes of the beverage in the studies
15 (Rumpler *et al.* 2001) and (Rudelle *et al.* 2007) would support this hypothesis. Water
16 can also potentiate the pressor effect of ephedra alkaloids (Jordan *et al.* 2004).

17 Recently, we found a prompt increase in energy expenditure by about 30% in men and
18 women after drinking 500 ml water and this effect was mediated by an increased SNS
19 activity, obviously secondary to stimulation of osmosensitive afferent neurons
20 (Boschmann *et al.* 2003, Boschmann *et al.* 2007). However, these results could not be
21 confirmed by another group (Brown *et al.* 2006).

22

23 In our study, 300 mg EGCG seemed to be optimal to increase fat oxidation, since
24 doubling the daily EGCG dose to 600 mg did not result in a further increased fat
25 oxidation. Our finding is supported by others (Berube-Parent *et al.* 2005), who showed

1 that 270 mg EGCG, when given along with 600 mg caffeine, is the optimal dose for
2 affecting energy expenditure, since increasing EGCG to 600, 900 or even 1200 mg
3 with a constant intake of 600 mg caffeine did not exert a greater response. The
4 increase in 24-h energy expenditure was about 750 kJ after 270 mg EGCG/600 mg
5 caffeine, whereas fat oxidation was not altered and increasing EGCG did not exert
6 additional effects on fat oxidation. Interestingly, green tea extract has beneficial effects
7 on body weight regain in low, but not in high caffeine users (Westerterp-Plantenga *et*
8 *al.* 2005). The cut-off level was 300 mg caffeine per day. Therefore, it might be
9 speculated that the rather high caffeine doses of 600 mg (Berube-Parent *et al.* 2005)
10 and 300 mg (Rudelle *et al.* 2007) masked the effects of EGCG. Similar phenomena
11 have been reported for ephedrine (Liles *et al.* 2007). A supra-additive synergistic effect
12 of EGCG and caffeine (Dulloo *et al.* 1999) could not be confirmed by our data.
13 However, within the early post-meal period, low EGCG was equipotent with caffeine,
14 whereas within the late post-meal period, caffeine was equipotent with EGCG/caffeine
15 regarding the increase in fat oxidation. EGCG alone did not affect fat oxidation within
16 the late post-meal period. This outcome could originate from the different half-life of
17 EGCG (about 2h) and caffeine (about 4h). Therefore, EGCG affects obviously the
18 early, whereas caffeine affects both early and late postprandial fat oxidation.
19
20 Although within the physiological range, fasting plasma NEFA were significantly higher
21 with 200 mg caffeine and the combination of 300 mg EGCG/200 mg caffeine when
22 compared to 300 mg EGCG alone, suggesting that lipid mobilization slightly exceeded
23 the capacity of lipid oxidation when caffeine is consumed either alone or with EGCG.
24

1 **CONCLUSION**

2 This **pilot** study provides for the first time evidence that a single green tea catechin,
3 EGCG, can increase fat oxidation in obese men, at least within two hours after meal
4 intake. Within this postprandial phase, EGCG is equipotent with caffeine regarding fat
5 oxidation.

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9

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11 J.J. designed research; G.F., J.B., A.B., F.A., and M.B. performed research; F.T., and
12 M.B. analyzed data; and F.T., and M.B. wrote the paper.

13

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17

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1

2 **Table 1. Screening characteristics of the study population (all male, n=10)**

	Mean	SD	Range
Age (y)	34	7	23 - 40
Weight (kg)	101.3	9.7	81.4 - 117.7
Height (cm)	180	6	167 - 192
BMI (kg/m ²)	31.3	3.5	27.3 - 35.5
Triglycerides (mmol/l)	1.59	0.74	0.64 – 2.49
NEFA (mmol/l)	0.27	0.11	0.07 - 0.47
Total Cholesterol (mmol/l)	5.17	1.22	3.22 – 7.31
Glucose (mmol/l)	4.8	0.4	3.9 - 5.3
Insulin (μU/ml)	9.9	3.4	6.2 - 16.6
Systolic blood pressure (mmHg)	130	12	111 - 149
Diastolic blood pressure (mmHg)	73	12	53 - 90
Heart rate (beats/min)	63	9	50 - 84

3 Data are given as mean ± SD, n=10.

1 **Table 2. Effects of supplements on mean fat (FOX) and carbohydrate (LOX)**
 2 **oxidation rates (g/h) at pre and post meal conditions**

	Placebo	EGCG low	EGCG high	Caffeine	EGCG/Caffeine
FOX					
<i>pre meal</i>					
60 – 180 min	3.76 ± 0.53	4.05 ± 0.46	4.33 ± 0.51	4.75 ± 0.49*	5.09 ± 0.44**,\$
210 – 330 min	4.63 ± 0.51	4.79 ± 0.60	4.58 ± 0.49	5.23 ± 0.55*	5.84 ± 0.62*
<i>post meal</i>					
390 – 510 min	3.42 ± 0.66	4.56 ± 0.71*	4.11 ± 0.62	4.60 ± 0.70*	5.11 ± 0.75*
540 – 660 min	3.62 ± 0.60	4.06 ± 0.68	3.88 ± 0.61	4.78 ± 0.69*	4.78 ± 0.87*
COX					
<i>pre meal</i>					
60 – 180 min	9.10 ± 1.00	7.89 ± 0.86	7.68 ± 0.99	7.32 ± 0.94*	6.40 ± 0.74*
210 – 330 min	7.14 ± 1.03	6.66 ± 1.09	7.02 ± 0.76	6.22 ± 1.04	4.91 ± 1.10*
<i>post meal</i>					
390 – 510 min	11.40 ± 1.36	8.86 ± 1.14*	9.74 ± 1.32	9.40 ± 1.33	7.58 ± 1.31*
540 – 660 min	10.26 ± 1.36	9.40 ± 1.38	9.94 ± 1.37	7.54 ± 1.21*,§,‡	7.76 ± 1.64*

3 *Note.* Detailed doses are given in the Method section. Data are given as mean ± SE,
 4 n=10. * p<0.05, ** p<0.01 vs. placebo, § p<0.05 vs. EGCG low, ‡ p<0.05 vs. EGCG
 5 high
 6

1

2 **Table 3. Resting blood pressure (BP) and heart rate (HR) after two days of**

3 **supplementation with either placebo, EGCG low, EGCG high, caffeine or**

4 **EGCG/caffeine**

	Placebo	EGCG low	EGCG high	Caffeine	EGCG/Caffeine
Systolic BP					
(mmHg)	125 ± 5	122 ± 3	124 ± 4	129 ± 5	123 ± 4
Diastolic BP					
(mmHg)	72 ± 3	70 ± 2	71 ± 3	73 ± 4	76 ± 2
HR					
(beats/min)	64 ± 3	66 ± 2	65 ± 3	62 ± 3	64 ± 3

5 *Note.* Detailed doses are given in the Method section. Data are given as mean ± SE,

6 n=10

1 **Legends for figures**

2 **Figure 1** Study design. Volunteers took the supplements (300 mg EGCG, 600 mg
3 EGCG, 200 mg caffeine, 300 mg EGCG / 200 mg caffeine, or placebo given in two
4 doses/capsules per day) over 3 days in total. On the second day they arrived at the
5 clinical where a standardized dinner was provided. On the following day, after a 12-
6 hour fast the daily dose was administrated. One hour after capsule intake, fasting
7 metabolic rate was measured for a total of 4 hours. At the end of this period half of
8 the daily dose was provided to keep the plasma levels from fasting to postprandial
9 comparable. Then, monitoring was interrupted for 60 min. A standardized test meal
10 was provided and postprandial metabolic rate was measured for 4 hours.

11

12 **Figure 2** Time course of energy expenditure (EE) before and after intake of a test
13 meal under low EGCG (upper panel, left), high EGCG (upper panel, right), caffeine
14 (lower panel, left), and caffeine/EGCG (lower panel, right), respectively (filled circles),
15 vs. placebo (open circles). For details of supplementation and meal composition cf.
16 Methods section. Data are given as mean \pm SE, n=10

17

18 **Figure 3** Time course of respiratory quotient (RQ) before and after intake of a test
19 meal under low EGCG (upper panel, left), high EGCG (upper panel, right), caffeine
20 (lower panel, left), and caffeine/EGCG (lower panel, right), respectively (filled circles),
21 vs. placebo (open circles). For details of supplementation and meal composition cf.
22 Methods section. Data are given as mean \pm SE, n=10. * p<0.05, ** p<0.01, ***
23 p<0.001, test drug vs. placebo. Differences between the curves in the indicated
24 sections were tested by global fitting.

25

1 **Figure 4** Plasma non-esterified fatty acids (NEFA) before and after intake of a test
2 meal under low EGCG (upper panel, left), high EGCG (upper panel, right), caffeine
3 (lower panel, left), and caffeine/EGCG (lower panel, right), respectively (filled circles),
4 vs. placebo (open circles). For details of supplementation and meal composition cf.
5 Methods section. Arrow indicates intake of the test meal. Data are given as mean \pm
6 SE, n=10. Fasting plasma NEFA concentrations were significantly lower for low
7 EGCG vs. caffeine and the combination EGCG/caffeine ($p < 0.001$).

8

9 **Figure 5** Targets of different drugs within the sympathetic neuronal transmission in
10 order to increase the availability of norepinephrine (NE) within the synaptic cleft
11 (EGCG, ephedrine, amphetamine, sibutramine) or to prolong NE-induced increases
12 in lipid mobilization and oxidation (caffeine).

13 COMT: catechol-O-methyl transferase, VMA: vanillyl-mandelic acid, β -AR: β -
14 adrenoreceptor, Gs: stimulatory G-protein, PDE: phosphodiesterase.

15

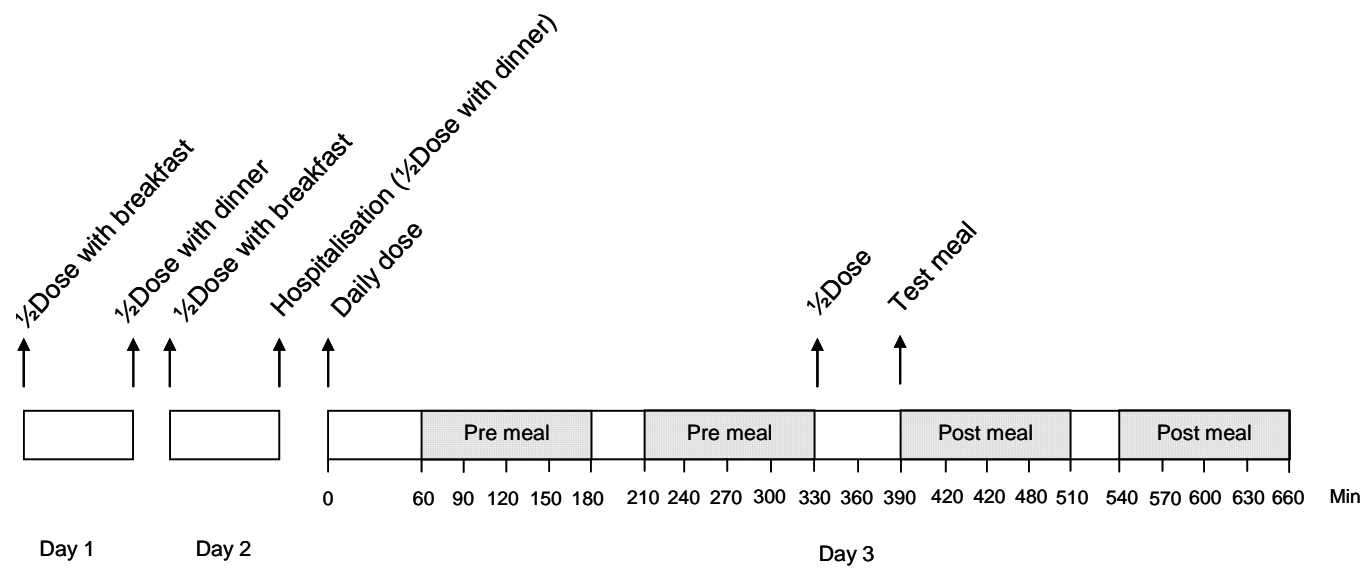


Fig. 1

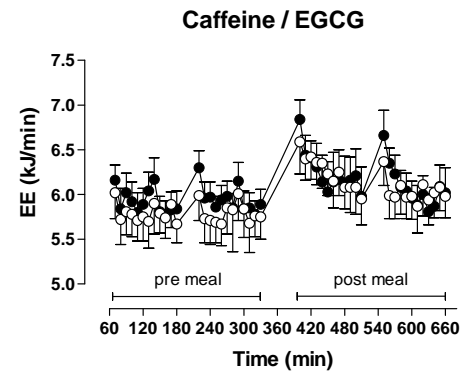
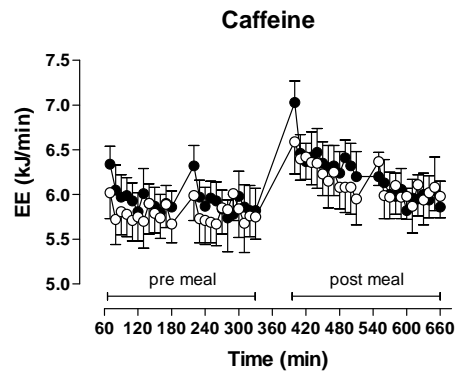
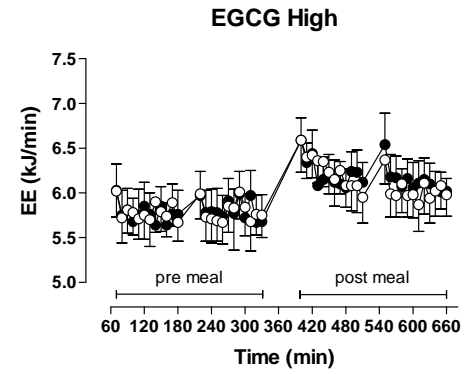
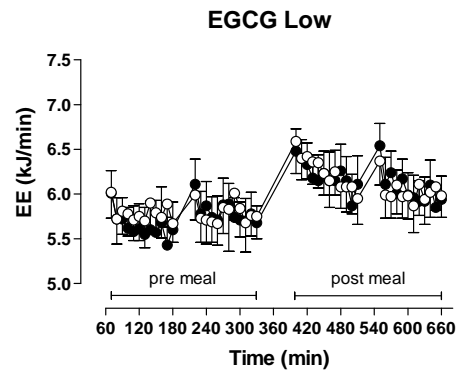


Fig. 2

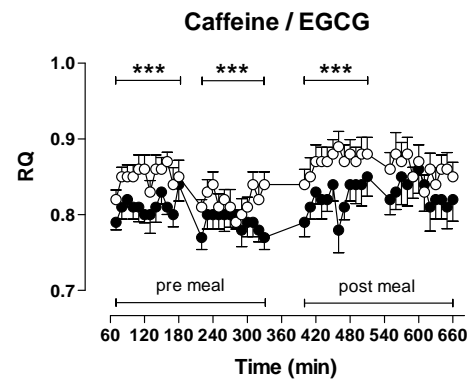
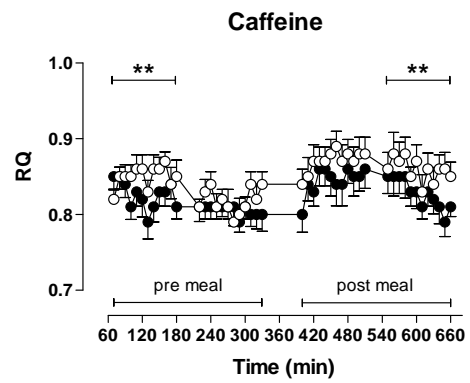
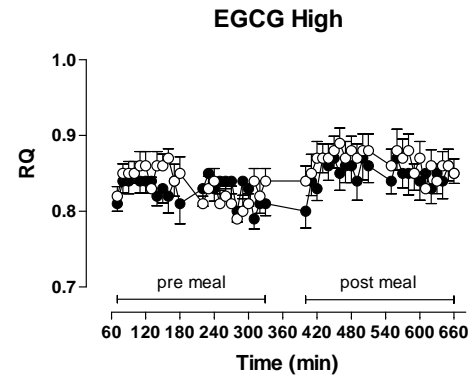
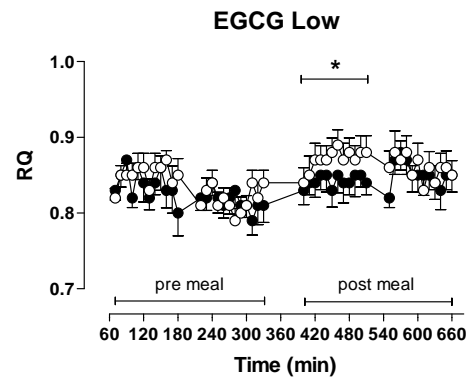


Fig. 3

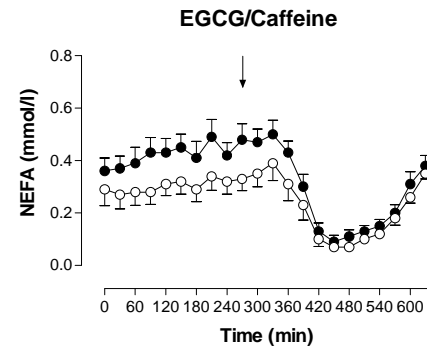
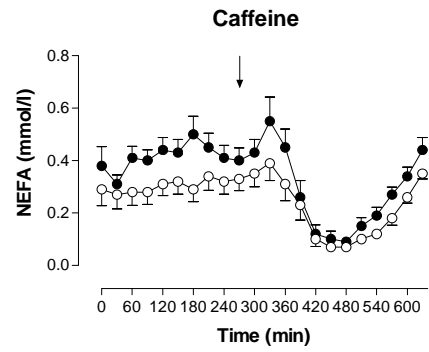
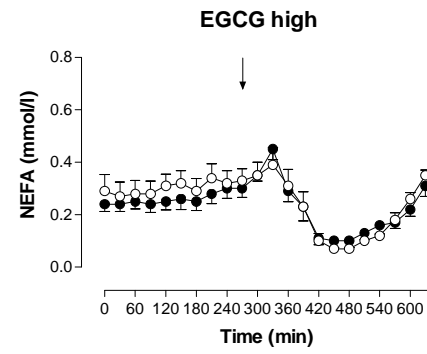
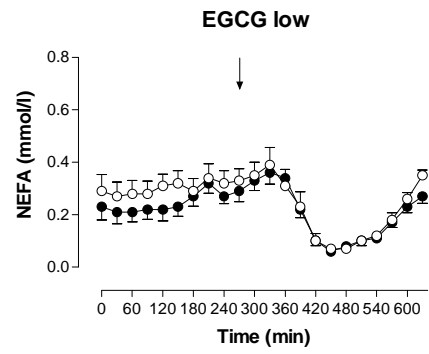


Fig. 4

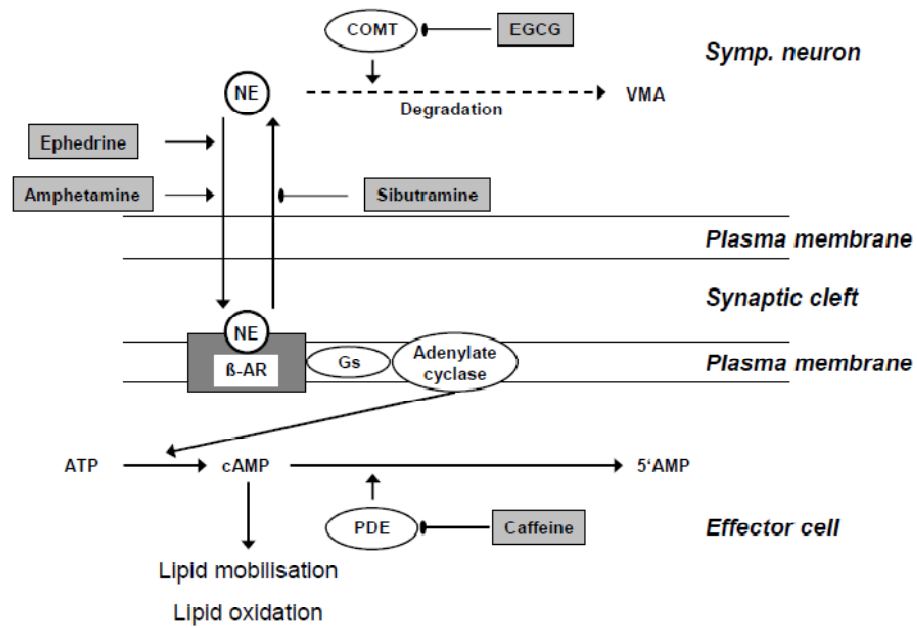


Fig. 5