

Metabolic response to epigallocatechin-3-gallate in relapsing-remitting multiple sclerosis: a randomized clinical trial^{1–5}

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ABSTRACT

Background: Muscle weakness and fatigue are common symptoms in multiple sclerosis (MS). Green tea catechins such as (–)epigallocatechin-3-gallate (EGCG) are known to improve energy metabolism at rest and during exercise.

Objective: We tested the hypothesis that EGCG improves energy metabolism and substrate utilization in patients with MS.

Design: Eighteen patients (8 men) with relapsing-remitting MS (expanded disability status scale score <4.5, all receiving glatiramer acetate) participated in this randomized, double-blind, placebo-controlled, crossover trial at a clinical research center. All patients received EGCG (600 mg/d) and placebo over 12 wk (4-wk washout in between). After each intervention, fasting and postprandial energy expenditure (EE), as well as fat oxidation (FAOx) and carbohydrate oxidation (CHOx) rates, were measured either at rest or during 40 min of exercise (0.5 W/kg). At rest, blood samples and microdialysates from adipose tissue and skeletal muscle were also taken.

Results: At rest, postprandial EE and CHOx, as well as adipose tissue perfusion and glucose supply, were significantly lower in men but higher in women receiving EGCG compared with placebo. During exercise, postprandial EE was lower after EGCG than after placebo, indicating an increased working efficiency (men > women). After placebo, exercise EE was mainly fueled by FAOx in both men and women. After EGCG, there was a shift to a higher and more stable CHOx during exercise in men but not in women.

Conclusions: Our data indicate that EGCG given to patients with MS over 12 wk improves muscle metabolism during moderate exercise to a greater extent in men than in women, possibly because of sex-specific effects on autonomic and endocrine control. This trial was registered at clinicaltrials.gov as NCT01417312. *Am J Clin Nutr* 2015;101:487–95.

Keywords energy metabolism, epigallocatechin-3-gallate, multiple sclerosis, sex difference, calorimetry, microdialysis

INTRODUCTION

Multiple sclerosis (MS)⁶ is an autoimmune inflammatory disorder of the central nervous system with focal lymphocytic infiltration leading to demyelination, axonal and neuronal damage (1, 2), and finally accrual of neurologic disability and atrophy of the brain, the spinal cord, and the retina (3, 4). Common symptoms of patients with MS are muscle weakness and excessive fatigue (5, 6), leading to reduced physical activity.

Drinking green tea is thought to have preventive and therapeutic effects on a number of neurodegenerative diseases. These effects are mainly attributed to the tea polyphenols/catechins such as (–)epigallocatechin-3-gallate (EGCG, 50–80% of total green tea catechins) (7). Studies on mice with experimental autoimmune encephalomyelitis showed beneficial effects of EGCG on disease progression, inflammatory infiltration, and demyelination of the central nervous system (8–10).

At rest, skeletal muscle energy metabolism is mainly fueled by fat oxidation. During low- to moderate-intensity (<65% maximal oxygen consumption) exercise, fat oxidation increases but decreases at higher intensities (11). Recently, we reported different kinetics of carbohydrate oxidation (CHOx) and fat oxidation (FAOx) rates in patients with MS compared with healthy controls during the early and late phase of moderate-intensity exercise (12). Green tea extract (GTE) also affects fat metabolism, but the effects are different under resting or exercise conditions and also after short- or long-term GTE intake (13). In

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³ Supplemental Figures 1 and 2 are available from the “Supplemental data” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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⁶ Abbreviations used: CHOx, carbohydrate oxidation rate; EE, energy expenditure; EGCG, epigallocatechin-3-gallate; FAOx, fat oxidation rate; GTE, green tea extract; MS, multiple sclerosis; RER, respiratory exchange ratio.

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healthy, physically active men, 7 days of GTE supplementation enhanced aerobic energy metabolism (increased fat mobilization and oxidation) at rest and anaerobic energy metabolism (increased glycolysis and carbohydrate oxidation) during moderate-intensity exercise. Under both conditions, GTE did not stimulate the adrenergic system because no increase in norepinephrine and related catecholamines was observed (14).

Animal studies suggest that longer term GTE intake results in an increased expression of genes and proteins/enzymes specific to lipid metabolism in liver, adipose tissue, and skeletal muscle, such as peroxisome proliferator-activated receptor γ coactivator 1- α (13). Targeting this regulator of mitochondrial biogenesis and function could be useful in MS because mitochondrial dysfunction might contribute to the pathogenesis of axonal damage (15). There is also some evidence that EGCG inhibits vascular endothelial growth factor and therefore tumor angiogenesis and proliferation in a number of human cancer cell models (e.g., gastric cancer) (16).

The aim of our study was to test the hypothesis that EGCG improves systemic and tissue energy metabolism and finally metabolic capacity at rest and during exercise in patients with MS.

METHODS

Study design

This randomized, double-blind, placebo-controlled, crossover study (registered at clinicaltrials.gov as NCT01417312; **Figure 1**) was conducted at the Experimental & Clinical Research Center of Charité University Medicine Berlin from July 2011 to November 2012. Patients were randomly allocated based on a computer-generated list for multiple treatments (balanced permutation for 30 subjects). An external person not involved in the study generated the randomization list and numbered capsule containers accordingly. All people involved in the study (i.e., patients, health care providers, data collectors, and outcome assessors) were blinded to the treatment sequence.

Patients

We screened 23 patients with relapsing-remitting MS from our outpatient department (July 2011–April 2012; last follow-up November 2012) according to the 2005 panel criteria (17). Key inclusion criteria were a stable immunomodulatory therapy with

glatiramer acetate for at least 6 mo, an expanded disability status scale score <4.5 (18), age between 20 and 60 y, and a BMI (in kg/m^2) between 18.5 and 30.0. Key exclusion criteria were primary or secondary progressive forms of MS; clinical relapses within 3 mo before or during the study; clinically relevant heart, lung, liver, and kidney diseases; habitual caffeine intake (>300 mg/d) and/or green tea consumption; and alcohol or drug abuse. The institutional review board of Charité University Medicine Berlin approved the study, and written informed consent was obtained from all participants prior to study entry.

Intervention

Patients were randomly allocated to one of 2 treatment sequences. They started with 600 mg EGCG/d (Teacare; Limmer Nutraceuticals BV) or placebo for 12 wk. This first period was followed by a 4-wk washout period. Then patients repeated the treatment period such that patients who were first on placebo received EGCG and vice versa. Identically appearing capsules contained either EGCG (2×150 mg) or starch (placebo) and were taken twice daily, 1 h before breakfast and dinner, respectively. An amount of 300 mg EGCG could also be consumed by drinking ~ 600 mL green tea (19). During the study, patients visited the study center every 4 wk for evaluation of possible adverse events, compliance, and liver enzymes (safety parameter).

Outcome measures

The primary outcome measure was the postprandial increase in fat oxidation (FAOx, %) assessed by indirect calorimetry (canopy system), and the secondary outcome measure was the improved efficiency of muscle work during moderate-intensity exercise assessed by indirect calorimetry (respiratory chamber) after a 12-wk intake of EGCG or placebo.

Clinical protocols

On the last 2 d of the treatment periods, patients came to our Clinical Research Center for metabolic evaluations after a 12-h overnight fast and intake of 300 mg EGCG in the morning. With protocol I, systemic, adipose tissue, and skeletal muscle metabolism was studied while resting in the supine position. With protocol II, systemic energy metabolism was studied during standardized exercise (12).

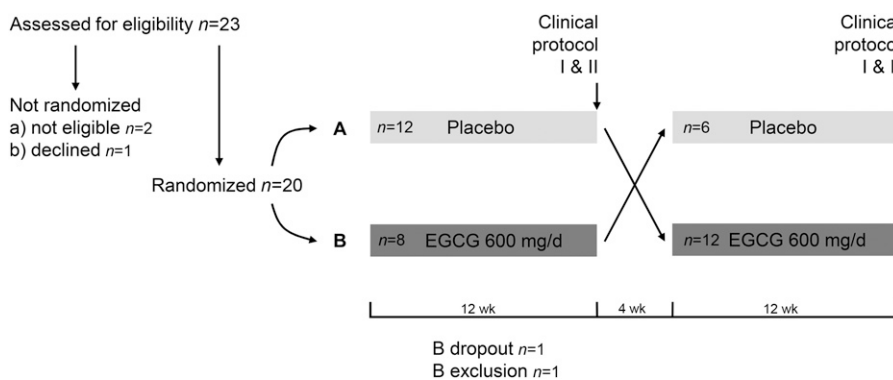


FIGURE 1 Study design of the 28-wk, double-blind, placebo-controlled crossover study. EGCG, epigallocatechin-3-gallate.

Protocol I

A catheter was placed in a large antecubital vein for blood sampling. One microdialysis probe (CMA/60; M Dialysis AB) was inserted into abdominal subcutaneous adipose tissue and vastus lateralis muscle. After probe insertion, tissue perfusion was started with lactate-free Ringer solution (+50 mmol/L ethanol) at a flow rate of 2 μ L/min by using CMA/102 microdialysis pumps. Ethanol was added to assess changes in tissue perfusion by using the ethanol dilution technique (12). After a recovery period of 75 min, patients received a standardized test meal (bread, butter, cream cheese, tomato, and cucumber) prepared by a dietitian that provided 5 kcal/kg body weight with 50%, 35%, and 15% of energy from carbohydrates, fats, and proteins, respectively. Blood and dialysis samples were taken at baseline and every 15 or 30 min after the meal. Carbon dioxide (CO₂) production and oxygen (O₂) consumption were measured by a canopy calorimeter (DeltatracII; Datex Ohmeda) to assess changes in energy expenditure (EE), respiratory exchange ratio [RER = VCO₂/VO₂ (where VCO₂ is CO₂ production and VO₂ is oxygen consumption)], and CHOx and FAOx rates according to Ferrannini (20).

Protocol II

Body composition was determined by Air-Displacement Plethysmography (Bod Pod; Life Measurements Inc.). CO₂ production and O₂ consumption were measured in a respiratory chamber—a comfortable, airtight room (width: 2.5 m; depth: 2.0 m; height: 2.2 m) that is constantly supplied with fresh air like an open-circuit indirect calorimeter. Calculations (EE, RER) were the same as in protocol I. While subjects were seated in a comfortable chair, their resting EE was measured over 40 min followed by consuming a test meal as described in protocol I. Then, patients started exercising on a bicycle ergometer (Viasprint 150 P; Ergoline) at a workload of 0.5 W/kg body weight over 40 min. During exercise, heart rate was monitored continuously and rates of perceived exertion were recorded on a 10-point scale (21) every 10 min. Exercise was followed by a 40-min recovery period.

Assays

Plasma glucose, insulin, triglyceride, norepinephrine, and leptin concentrations were measured according to international standards, and free fatty acids were determined by an automated colorimetric test (ABX Pentra 400 Chemistry Analyzer; Horiba ABX). EGCG plasma concentrations were measured as described previously (22). Perfusate (inflow) and dialysate (outflow) ethanol concentrations were measured with a standard spectrophotometric enzymatic assay. A decrease in the ethanol outflow/inflow ratio (ethanol ratio) is equivalent to an increase in tissue perfusion and vice versa (23). Dialysate glucose, lactate, pyruvate, and glycerol concentrations were measured with the ISCUS^{flex} microdialysis analyzer (M Dialysis AB). In situ dialysate recovery for these metabolites was about 30% in adipose tissue and 50% in skeletal muscle, respectively, as assessed by near-equilibrium dialysis (24).

Sample size calculation and data analysis

Sample size calculation was based on the finding that EGCG increased postprandial FAOx in overweight/obese men by 33%

compared with placebo (25). We assumed that this would also be true for patients with MS and estimated an increase of 40% because of the longer treatment period (3 d compared with 12 wk). In obese men, mean \pm SD fat oxidation on placebo was 3.42 \pm 2.09 g/h. For patients with MS, we estimated an effect size of 1.37 g/h (α = 0.05, β = 0.20). From this, a total sample size of 21 was calculated when using nonparametric Wilcoxon's signed rank test for matched pairs.

Statistical analyses and graphics were performed with GraphPad Prism version 5.01 (GraphPad Software) and R version 3.1.1 (packages "lme4" and "nlme"; Revolution Analytics). Data in figures are shown as means \pm SEMs. Group differences (EGCG and placebo) were compared by nonparametric Wilcoxon's signed rank test for matched pairs. To test the effect of variables on various continuous outcome measures, we conducted multilevel modeling by using linear mixed models to account for dependent variables attributed to time-related measurements. Random intercept and random intercept-slope models were tested, with the best-fit model selected. If there were no significant interactions between factors, analysis was performed within related subgroups. Association between variables was assessed by using nonparametric Spearman's rank correlation coefficients (r_s). Percent variability of one variable accounted for by the other one was determined by squaring r_s and multiplying by 100. P < 0.05 indicated statistical significance.

RESULTS

Demographic and anthropometric characteristics of the 18 patients with MS (all Caucasians) who completed the study are summarized in **Table 1**. In men, BMI was significantly higher after EGCG (24.7) than after placebo (24.0) (P = 0.04).

Protocol I

Systemic metabolism at rest

Postprandial FAOx was 40% higher in men and 21% lower in women who received EGCG compared with placebo (**Table 2**). Because we did not find an overall treatment effect of EGCG, we tested for treatment and sex interaction within the mixed model. P values for absolute and relative lipid oxidation were 0.005 and 0.025, respectively, indicating a significantly different response to treatment in men and women. Therefore, all data will be presented and discussed in a sex-specific manner and considered exploratory rather than confirmatory.

Fasting EE did not differ between placebo and EGCG in both men and women. In men, EE increased less strongly within the first 30 min after the meal and was lower with EGCG than with placebo (P = 0.02, **Figure 2A**) until the end of testing. In women, EE increased similarly in both treatments within the first 30 min and was higher with EGCG than with placebo (P = 0.03, **Figure 2A**) until the end of testing. Fasting RER did not differ between placebo and EGCG in both men and women. However, postprandial changes of RER in response to EGCG were significantly different in men and women (P = 0.01 for treatment and sex interaction). In men taking placebo, RER increased steadily within 60 min after the meal, remained elevated during the next 60 min, and decreased finally until the end of testing, whereas with EGCG, RER remained on the fasting

TABLE 1
Characteristics of patients with MS at baseline and after a 12-wk intake of placebo and EGCG¹

Characteristic	Baseline		Placebo		EGCG	
	Men (n = 8)	Women (n = 10)	Men (n = 8)	Women (n = 10)	Men (n = 8)	Women (n = 10)
Age, y	40 ± 8 ²	45 ± 7	41 ± 8	45 ± 7	41 ± 8	45 ± 7
BMI, kg/m ²	24.2 ± 3.0	25.9 ± 2.8	24.0 ± 3.0	25.8 ± 3.2	24.7 ± 3.1*	25.9 ± 3.0
Body fat, %	ND	ND	22.3 ± 6.7	39.7 ± 7.4	23.3 ± 7.6	39.0 ± 6.3
Disease duration, mo	89 (7–192) ³	60 (25–208)	92 (10–199)	61 (28–216)	96 (13–195)	68 (31–211)
EDSS	2.0 (0.0–3.5)	3.0 (2.0–3.5)	2.0 (0.0–3.5)	2.8 (2.0–3.5)	2.0 (0.0–3.5)	3.3 (2.0–4.0)

¹*P* = 0.04 compared with placebo (Wilcoxon's signed rank test for matched pairs). EDSS, expanded disability status scale; EGCG, epigallocatechin-3-gallate; MS, multiple sclerosis; ND, not determined.

²Mean ± SD (all such values).

³Median; range in parentheses (all such values).

level over the complete time of postprandial testing (*P* < 0.001 compared with placebo; Figure 2B). In women, RER increased in almost the same way with both treatments during the early postprandial phase (first 2 h) but was significantly higher after 120 min with EGCG than with placebo. Then, in the late postprandial phase (second 2 h), RER decreased, but the values remained significantly higher at almost all time points with EGCG than with placebo until the end of testing (*P* = 0.03; Figure 2B).

Fasting values and postprandial profiles of plasma glucose, insulin, and free fatty acids were within the normal range and almost identical for EGCG compared with placebo in both men and women (Supplemental Figure 1A–C). For placebo, mean ± SD fasting triglyceride concentrations were 97 ± 46 mg/dL and 95 ± 28 mg/dL in men and women, respectively (Supplemental Figure 1D). After the meal, triglyceride concentrations increased up to 131 ± 68 mg/dL and 101 ± 21 mg/dL in men and women, respectively. For EGCG, fasting and postprandial triglyceride concentrations were almost identical in women (Supplemental Figure 1D). In men, however, triglyceride concentrations were significantly lower for EGCG than for placebo (*P* = 0.005). Fasting norepinephrine and leptin concentrations were not significantly different for EGCG compared with placebo in both men and women (Supplemental Figure 2A,B). Correlation analysis of all patients revealed that ~80% of leptin variability was associated with body fat variability for placebo and EGCG (Supplemental Figure 2C). However, for EGCG, leptin was rather lower in some men and rather higher in some women (both nonsignificant; Supplemental Figure 2B). Ninety min after intake of 300 mg EGCG in the morning, mean ± SD plasma EGCG concentrations were 0.19 ± 0.13 μmol/L and 0.19 ± 0.21 μmol/L in men and women, respectively (data not shown).

Local tissue metabolism at rest

In adipose tissue, baseline and postprandial ethanol ratios were higher in men and lower in women receiving EGCG compared with placebo (*P* < 0.001 and *P* = 0.003; Figure 3A), indicating lower and higher tissue perfusion in men and women, respectively. After the meal, ethanol ratios slightly decreased with both treatments in men and women, indicating an increased postprandial tissue perfusion. According to the differences in tissue perfusion, fasting and postprandial dialysate glucose concentrations were lower in men and higher in women receiving EGCG (men, *P* = 0.002 and women, *P* < 0.001 for EGCG compared with placebo; Figure 3B), indicating a lower and higher glucose supply, respectively. After the meal, time courses of dialysate glucose were almost identical and regular for both treatments in men and women, with an increase during the first 60 min after the meal followed by a constant decrease during the next 3 h. However, glucose values were always significantly lower and higher with EGCG than with placebo in men and women, respectively. Interestingly, fasting dialysate lactate concentrations were markedly lower with EGCG (~0.5 mmol/L) than with placebo (~1 mmol/L) in men but not in women (Figure 3C). Considering the higher tissue perfusion and therefore also higher product (lactate) removal for placebo compared with EGCG in men, actual lactate production should be higher than indicated by the dialysate concentrations. After the test meal, lactate did not further increase with placebo but increased regularly with EGCG in men. In women, fasting dialysate lactate concentrations were comparable with placebo and EGCG and increased similarly during the first 60 min after the test meal (Figure 3C). Then, lactate concentrations remained at the higher level with placebo but decreased slowly with EGCG. Fasting dialysate glycerol concentrations were slightly higher in women than in men but did not differ significantly between

TABLE 2
Primary and secondary outcome measure¹

Characteristic	All		Men		Women	
	Placebo	EGCG	Placebo	EGCG	Placebo	EGCG
Postprandial FAOx, g/4 h	8.3 ± 4.3	8.6 ± 5.0	9.2 ± 4.9	12.9 ± 5.7	7.8 ± 4.2	6.2 ± 3.6
Working efficiency, %	20 ± 3	25 ± 6**	21 ± 3	27 ± 6*	20 ± 3	25 ± 7 [§]

¹Values are means ± SDs. ***P* = 0.004, **P* = 0.02, [§]*P* = 0.08 all compared with placebo (Wilcoxon's signed-rank test for matched pairs). EGCG, epigallocatechin-3-gallate; FAOx, fat oxidation.

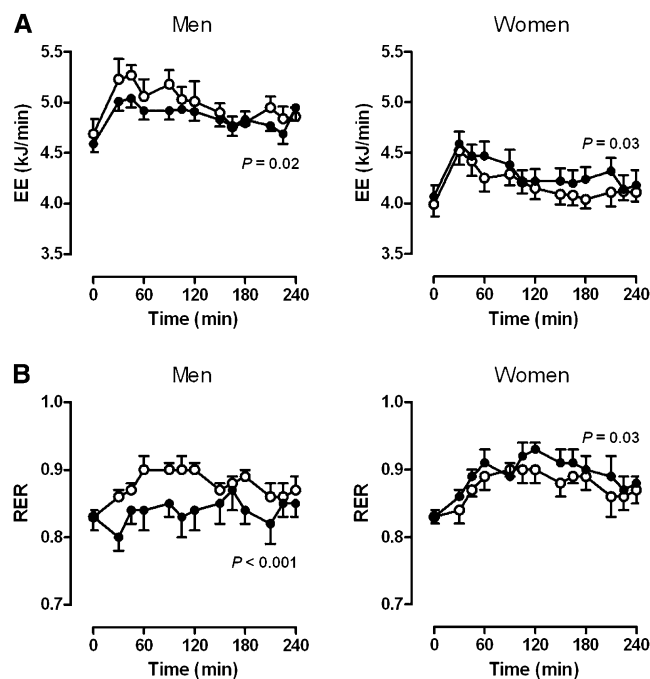


FIGURE 2 Systemic energy metabolism at rest (canopy device). Mean (\pm SEM) EE (A) and RER (B) before and after a test meal in men ($n = 5$) and women ($n = 9$) with multiple sclerosis after a 12-wk intake of placebo (open circles) and epigallocatechin-3-gallate (closed circles). P -treatment by mixed model. EE, energy expenditure; RER, respiratory exchange ratio.

treatments (Figure 3D). After the test meal, glycerol concentrations decreased similarly in men and women during the first hour, remained at a lower level in men, but increased slowly in women during the next 3 h without significant differences between treatments.

In skeletal muscle, baseline and postprandial ethanol ratios were also higher in men and lower in women receiving EGCG ($P = 0.003$ and $P = 0.006$ compared with placebo; **Figure 4A**), indicating lower and higher tissue perfusion in men and women, respectively ($P = 0.04$ for treatment and sex interaction). After the meal, ethanol ratios slightly decreased for both treatments in men and women, indicating an increased postprandial tissue perfusion. Fasting marker metabolite concentrations did not differ significantly for EGCG compared with placebo in both men and women. After the meal, dialysate glucose, lactate, and pyruvate concentrations increased and glycerol concentrations decreased regularly without greater differences between treatments in both men and women (Figure 4B–E).

Protocol II

Resting EE did not differ significantly between treatments in both men and women (**Figure 5A**). After starting exercise, EE increased rapidly within the first 10 min (placebo = EGCG) and was less accelerated during the following 20 min. However, total increase in EE was lower in men ($P = 0.01$) and women (nonsignificant) receiving EGCG compared with placebo. During the recovery period, EE decreased regularly and reached almost baseline levels with both treatments (men = women, Figure 5A).

Resting RER was lower in men but higher in women receiving EGCG compared with placebo, indicating a lower CHOx and therefore a higher FAOx in men and vice versa in women (Figure 5B). With placebo, RER increased strongly within the first 10 min of exercise, followed by an immediate and nearly linear decrease (men > women) down to almost baseline values until the end of exercise and even below resting values during the recovery period. With EGCG, RER increased also within the first 10 min of exercise but remained at the peak level during the next 30 min in

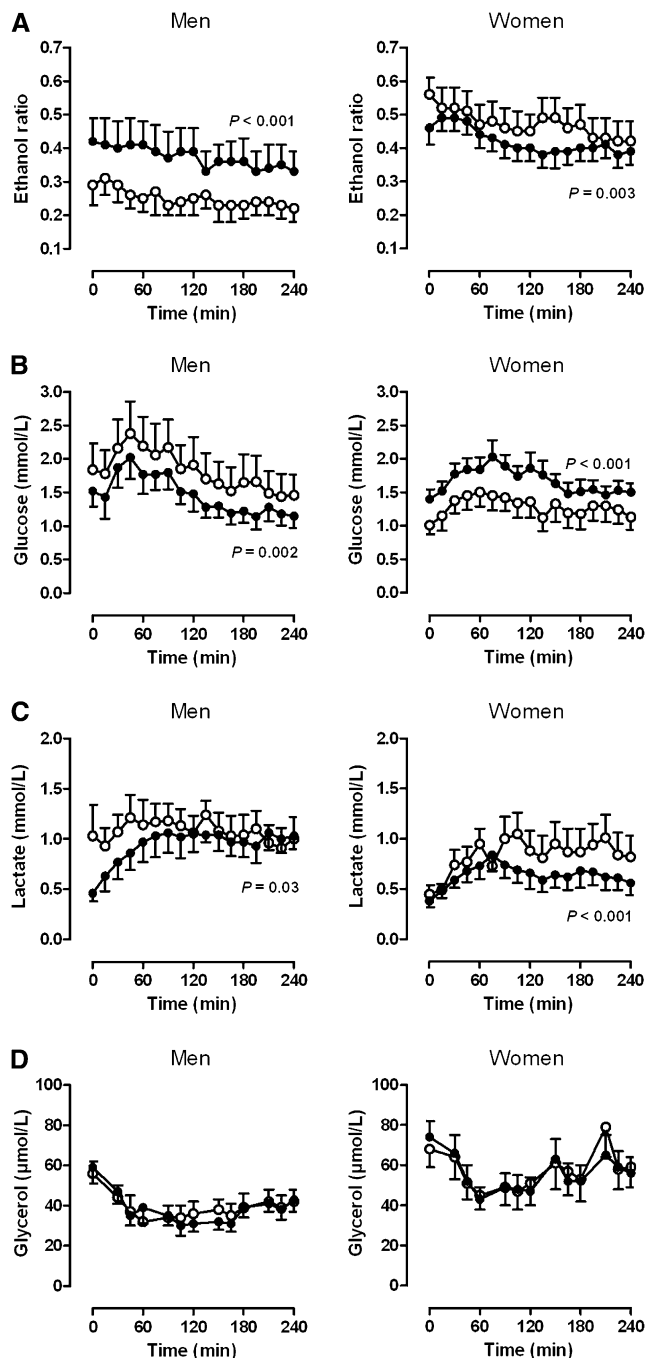


FIGURE 3 Adipose tissue microdialysis. Mean (\pm SEM) ethanol ratio (A) and dialysate concentrations of glucose (B), lactate (C), and glycerol (D) in adipose tissue before and after a test meal in men ($n = 6$) and women ($n = 10$) with multiple sclerosis after a 12-wk intake of placebo (open circles) and epigallocatechin-3-gallate (closed circles). P -treatment by mixed model.

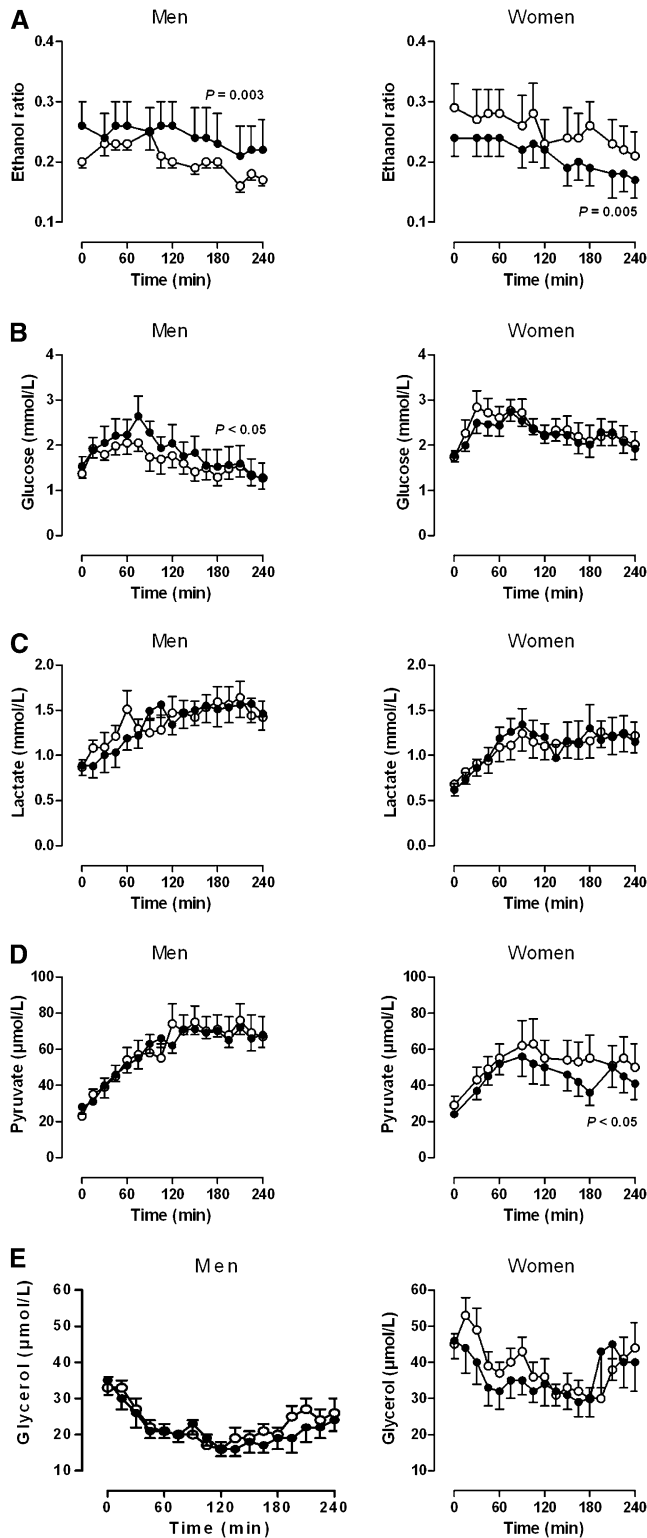


FIGURE 4 Skeletal muscle microdialysis. Mean (\pm SEM) ethanol ratio (A) and dialysate concentrations of glucose (B), lactate (C), pyruvate (D), and glycerol (E) in skeletal muscle before and after a test meal in men ($n = 7$) and women ($n = 10$) with multiple sclerosis after a 12-wk intake of placebo (open circles) and epigallocatechin-3-gallate (closed circles). P -treatment by mixed model.

men, whereas it started to decrease in women as with placebo. During recovery, RER reached resting values in both men and women (Figure 5B).

Efficiency of muscle work (work load/EE during exercise) increased from 21% with placebo to 27% with EGCG in men and from 20% to 25% in women (Table 2).

DISCUSSION

We tested the hypothesis that EGCG improves systemic and tissue energy metabolism and finally metabolic capacity at rest and during exercise in patients with MS.

At rest, postprandial EE and CHOx were lower in men but higher in women receiving EGCG compared with placebo after a test meal. Adipose tissue and skeletal muscle perfusion were lower in men but higher in women receiving EGCG compared with placebo. Also, fasting adipose tissue lactate concentrations were about 2-fold higher in men receiving placebo, indicating an increased lipolytic activity, but normalized with EGCG. During exercise, energy metabolism became more efficient with EGCG than with placebo (men > women).

We did not observe clinically relevant changes in body weight and body composition. Some studies showed favorable effects of EGCG on body weight in rodent obesity models due to inhibition of adipocyte differentiation and lipogenic enzyme expression (26) and reduced diet digestibility (27). However, this effect might be smaller or even absent in normal-weight persons.

Systemic responses of glucose, insulin, and free fatty acids to the test meal did not indicate any striking abnormalities with placebo or significant changes with EGCG. However, fasting and postprandial triglyceride concentrations were also significantly higher in men (not women) taking placebo but normalized with EGCG. Such an effect of tea catechins on postprandial triglyceride

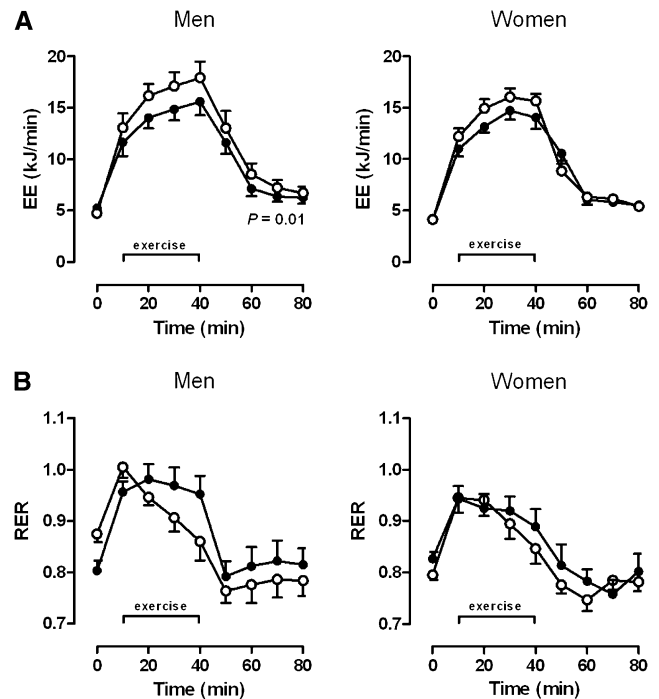


FIGURE 5 Systemic energy metabolism during exercise (metabolic chamber). Mean (\pm SEM) EE (A) and RER (B) at rest and during bicycle exercise after a test meal in men ($n = 8$) and women ($n = 10$) with multiple sclerosis after a 12-wk intake of placebo (open circles) and epigallocatechin-3-gallate (closed circles). P -treatment by mixed model. EE, energy expenditure; RER, respiratory exchange ratio.

concentrations has also been reported in a randomized controlled trial on 9 men with mild hypertriglyceridemia (28). This beneficial lipid-lowering effect (at least in men) is noteworthy with respect to the increased risk for cardiovascular diseases in patients with MS compared with the general population (29).

EGCG is known to inhibit catechol-*O*-methyltransferase enzyme activity in vitro (30), but supplementation of a single high dose of EGCG (750 mg) did not impair catechol-*O*-methyltransferase activity in healthy volunteers, at least in red blood cells (31). Because of this, we and others (14) did not find any effect of EGCG on plasma norepinephrine and leptin concentrations.

Green tea, GTEs high in EGCG, and oolong tea increased EE in healthy men and women (32–35) and FAOx in men (34, 35) but not in women (33). In all of these studies, tea preparations or GTEs contained caffeine, which is also known to increase EE and FAOx. Therefore, the effect of EGCG alone remained unclear. We found that 150 mg EGCG twice daily increased FAOx in overweight men, specifically during the first 2 h after a fat-enriched test meal (25, 36). In the present study on patients with MS, 300 mg EGCG twice daily increased FAOx in men and CHOx in women over 4 h after a similar test meal. Interestingly, this different metabolic response was also found after drinking 500 mL water (37). The increase of EE after a meal (i.e., diet-induced thermogenesis) depends on the nutrients tested for and is much lower for lipids than for carbohydrates (38). Because of the higher postprandial FAOx in men (and lower in women), postprandial EE was therefore lower in men (and higher in women) receiving EGCG compared with placebo. Possibly, EGCG targets the autonomic system in men but the endocrine system in women (37, 39).

Adipose tissue and muscle perfusion were lower in men but higher in women receiving EGCG compared with placebo. However, lower skeletal muscle perfusion in men taking EGCG did not limit tissue metabolism, as indicated by the unchanged pattern of dialysate marker metabolites for glucose and lipid metabolism. EGCG inhibits angiogenesis via vascular endothelial growth factor in several cancer cell models (e.g., gastric cancer) (16) and human umbilical vein endothelial cells (40). Whether this effect is also clinically relevant is unclear.

Recently, we reported an increased fasting lipolytic state of adipose tissue in patients with MS compared with healthy controls, which we attributed, in part, to long-time daily injections of glatiramer acetate into adipose tissue (12). In our present study, we could confirm these findings because patients were almost the same as in the recent study. However, because of the higher tissue perfusion and therefore higher product (glycerol) removal with placebo, lipolytic activity was possibly higher in women than in men. Because of the lower and higher tissue perfusion with EGCG in men and women, respectively, we would expect higher dialysate glycerol values in men and lower ones in women. But, fasting and postprandial glycerol values were almost identical with EGCG compared with placebo in both men and women. Therefore, adipose tissue lipolytic activity decreased in men and increased in women receiving EGCG compared with placebo. Considering the same for lactate, fasting and postprandial dialysate lactate should be higher in men and lower in women receiving EGCG compared with placebo. However, the lower fasting and early postprandial values in men and rather unchanged ones in women argue for a decreased and rather unchanged

glycolytic activity with EGCG compared with placebo in men and women, respectively. The chronic inflammatory process in MS might affect adipose tissue metabolism by interfering with local insulin responsiveness (12, 41). At least in men, EGCG possibly improves insulin sensitivity because of the reduced lipolytic activity and lactate production.

We also studied the metabolic response to EGCG during moderate exercise. In mice, a GTE improved endurance capacity and increased muscle fat oxidation (42). However, in moderately trained men, 1 and 7 d of GTE ingestion did not increase fat oxidation rates during 60 min of moderate-intensity exercise (43). In our present study, MS patients with showed almost the same deviations with placebo during moderate-intensity exercise as reported in our recent study (12), despite different nutritional interventions (test meal compared with oral glucose load). Whereas patients used almost exclusively carbohydrates in the early but fat in the late phase of exercise, healthy controls used constantly more carbohydrates than fat. Interestingly, EGCG shifted the substrate utilization from fat to carbohydrate oxidation in men but not in women. A more efficient postprandial glucose oxidation during moderate-intensity exercise indicates an improved whole-body glucose homeostasis and insulin sensitivity. Interestingly, patients (men > women) needed less energy with EGCG compared with placebo to handle the moderate exercise workload, indicating a higher efficiency of muscle work (44). This might help patients with MS to manage physical demands of everyday life.

Our study has clear strengths but also limitations. The strength of this study is the group homogeneity of patients with MS regarding the ratio of men to women, age, body composition, and medication. Although small, sample size was obviously sufficient to detect significant differences in metabolic responses to EGCG in men and women in this crossover study. Having found a differential treatment effect on the primary outcome measure in men and women, we performed an exploratory subgroup analysis in which no α adjustment was made. This increases the probability of false significant findings due to multiple testing. Thus, results should be interpreted with caution. Furthermore, we did not assess metabolic profiles before starting supplementations. Therefore, carryover effects from the EGCG to the placebo phase cannot be ruled out completely. However, most of the proposed mechanisms of EGCG on metabolism (changes of transcription factor and enzyme activities) do not indicate prolonged treatment effects after EGCG withdrawal (36). This would be true for a decrease in adipose tissue mass, which was, however, not present in our study, as indicated by the body composition data. Also, we did not standardize women for menstrual cycle phase. However, cycle length shows a great variability. Even if we had started drug treatment always in the follicular phase, it would have been unclear if we would have also met in the same phase after 12 wk on EGCG. Therefore, the potential influence of sex hormones on our results remains unknown. Nevertheless, there are few reports about the effect of the menstrual cycle on energy expenditure, indicating no or rather small effects, with a great variability depending on age and cycle length and also on the activity state (i.e., resting or exercising) (45–50).

In conclusion, 3-mo EGCG treatment affects energy metabolism in patients with MS in a sex-specific manner. After an overnight fast and a test meal, postprandial FAOx is higher at rest but lower during exercise with EGCG compared with placebo in

men and vice versa in women. This might be caused by different effects of EGCG on autonomic and endocrine control at tissue levels.

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