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Short-term supplementation with a specific combination of dietary polyphenols increases energy expenditure and alters substrate metabolism in overweight subjects

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Abbrevations

AUC	Area under the curve
CHO	Carbohydrate oxidation
COMT	Catechol-O-Methyl-Transferase
FAT	Fat oxidation
E	Epigallocatechin-gallate
EE	Energy expenditure
N	Nitrogen excretion
PGC-1α	Peroxisome proliferator-activated receptor gamma co-activator 1 alpha
PLA	Placebo
R	Resveratrol
RIA	Radioimmunoassay
RQ	Respiratory quotient
S	Soy isoflavones
SIRT1	Silent mating type information regulation 2 homolog 1
TAG	Triglycerids

Abstract

Background and Objectives: Impaired regulation of lipid oxidation (metabolic inflexibility) is associated with obesity and type 2 diabetes mellitus. Recent evidence has indicated that dietary polyphenols may modulate mitochondrial function, substrate metabolism and energy expenditure in humans. The present study investigated the effects of short-term supplementation of two combinations of polyphenols on energy expenditure (EE) and substrate metabolism in overweight subjects.

Subjects and Methods: 18 healthy overweight volunteers (9 women, 9 men; age 35±2.5 yrs; BMI 28.9±0.4 kg/m^2) participated in a randomized, double-blind cross-over trial. Combinations of Epigallocatechin-gallate (E, 282mg/d) + Resveratrol (R, 200mg/d), and E+R + 80mg/d soy isoflavones (S), or placebo capsules (PLA) were supplemented twice daily for a period of 3 days. On day 3, circulating metabolite concentrations, energy expenditure and substrate oxidation (using indirect calorimetry) were measured during fasting and postprandial conditions for 6 hours (high-fat-mixed meal (2.6MJ, 61.2 E% fat)).

Results: Short-term supplementation of E+R increased resting EE (E+R *vs.* PLA: 5.45±0.24 *vs.* 5.23±0.25 kJ/min, P=0.039), whereas both E+R (699±18 kJ/120min *vs.* 676±20 kJ/120min, P=0.028) and E+R+S (704±18 kJ/120min *vs.* 676±20 kJ/120min, P=0.014) increased 2-4h-postprandial EE compared with PLA. Metabolic flexibility, calculated as the postprandial increase to highest respiratory quotient (RQ) achieved, tended to be improved by E+R compared with PLA and E+R+S only in men (E+R *vs.* PLA: 0.11±0.02 *vs.* 0.06±0.02, P=0.059; E+R+S: 0.03±0.02, P=0.009). E+R+S significantly increased fasting plasma free fatty acid (P=0.064) and glycerol (P=0.021) concentrations compared with PLA.

Conclusions: We demonstrated for the first time that combined E+R supplementation for 3 days significantly increased fasting and postprandial EE, which was accompanied by improved metabolic flexibility in men but not women. Addition of soy isoflavones partially reversed these effects possibly due to their higher lipolytic potential. The present findings may imply that long-term supplementation of these dosages of epigallocatechin-gallate combined with resveratrol may improve metabolic health and body weight regulation.

Introduction

Disturbances in lipid metabolism play a key role in the development of obesity, type 2 diabetes mellitus and cardiovascular disease. A mismatch between energy supply and expenditure as well as intrinsic disturbances in the capacity to adapt fuel oxidation to fuel availability (defined as metabolic inflexibility) in adipose tissue and skeletal muscle are major causes of obesity-related complications (1).

Impairments in the lipid buffering capacity of adipose tissue may lead to lipid accumulation in non-adipose tissues (ectopic fat deposition) in conditions where oxidative capacity is insufficient (2). It is well-established that lipid accumulation in the liver and skeletal muscle is strongly associated with insulin resistance (3). Indeed, decreased fasting lipid oxidation and impaired switching between lipid and carbohydrate fuels in response to insulin, dietary stimuli or exercise has been observed in conditions of insulin resistance (1, 4, 5). Underlying mechanisms for metabolic inflexibility may be a reduced mitochondrial function or capacity (6), although recent studies indicate that glucose disposal rather than mitochondrial dysfunction is a determinant of substrate utilization during insulin stimulation (7).

Lifestyle interventions, aiming at reducing (saturated) fat intake and increasing physical activity have been demonstrated to efficiently counteract disturbances in lipid metabolism, and seem to improve metabolic flexibility (8, 9). However, lifestyle interventions have been shown to be ineffective in about 30% of the subjects, indicating the need for additional preventive strategies.

Reversal of metabolic impairments by means of dietary supplementation may be a good strategy to increase the success of lifestyle interventions. Dietary polyphenols are natural components of fruits and vegetables that have recently been shown to alter substrate and energy metabolism.

Resveratrol (R), an activator of silent mating type information regulation 2 homolog 1 (SIRT1, a member of the NAD+ dependent deacetylases family of sirtuins) and peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC-1α), that is abundantly present in grape skin, has been shown to exert significant effects on whole-body energetics, mitochondrial function and insulin sensitivity in animal models (10, 11). We have recently demonstrated for the first time in humans that 30 days R supplementation (150mg/d) improved metabolic profile, metabolic flexibility and skeletal muscle mitochondrial function, and evoked a reduction in energy expenditure (EE) (12).

In addition to R, it has been shown that the most potent polyphenol from green tea, epigallocatechin-gallate (E), may increase fat oxidation (13-15) and EE (14, 16) in humans, although data are certainly not consistent (13-18). Furthermore, human intervention studies have demonstrated that moderate dosing of green tea extract, containing 900mg polyphenols + 366mg E, may improve glucose tolerance (19) and induce a shift in substrate oxidation towards a more oxidative phenotype in human skeletal muscle (20).

Finally, soy isoflavones, in particular genistein, may beneficially affect lipid and glucose metabolism by reducing lipid accumulation in the liver (21) and adipose tissue (22). Improvements in homeostatic model assessment of insulin resistance (HOMA-IR), fasting glucose and area under the curve (AUC) during an oral glucose tolerance test were attributed to soy isoflavone supplementation in postmenopausal women (23). In line with these findings, insulin-sensitizing effects have been demonstrated in rodents (24). Short-term supplementation studies investigating the effects of soy isoflavones on lipid metabolism are completely lacking.

Altogether, there are strong indications that polyphenols may be attractive candidates

in the prevention of chronic metabolic diseases through modulation of pathways of fatty acid metabolism and mitochondrial function. Importantly, however, rather than increasing the dosages of single supplements, which appeared to deteriorate possible supplementation effects (16, 25), it might be more efficient to combine lower dosages of multiple supplements to achieve metabolic and beneficial health effects. These potential synergies of polyphenols have already been indicated for lipolysis (26) and EE (14, 16).

So far, the additive or possibly synergistic effects of combinations of specific polyphenols on fat oxidation and metabolic profile in humans have not been addressed. The aim of the present study was to examine the effects of short-term supplementation of a combination of specific polyphenols, with partly distinct mechanisms of action, on EE and substrate metabolism in overweight humans.

Subjects and Methods

Subjects

Eighteen healthy, weight-stable overweight (BMI, 25-30 kg/m^2) Caucasian subjects (age, 20-50 yrs) with a normal fasting glucose (<6.1mmol/l) and normal blood pressure (systolic blood pressure, 100-140 mmHg; diastolic blood pressure, 60-90 mmHg) participated in this study. Subjects were assigned to order of treatments according to a computer-generated, randomization plan (block size, n=6). An independent researcher generated the randomization and provided the blinded supplements. Exclusion criteria were (a history of) diabetes, coagulation disorders, pulmonary, cardiovascular, hepatic, renal or gastro-intestinal diseases, liver or thyroid disorders. Furthermore, subjects were excluded when using dietary supplements, amongst others vitamins, electrolytes or antioxidants, or having a high habitual intake of caffeine (>300mg/day), green tea (>1 cup/d) or alcohol (>20g/d). Finally, subjects were excluded from participation if they were on a special diet, donated blood, took antibiotics, followed intense fitness training, were smokers, pregnant or lactating, or were using drugs or medication interfering with the outcomes of the present study, over the 3 months prior to the start of the study. All procedures were according to the Declaration of Helsinki, all subjects gave written informed consent for the study, which was reviewed and approved by the local Medical Ethical Committee of the Maastricht University Medical Center$^+$.

Study design

In this double-blind randomized placebo-controlled cross-over trial, subjects received two combinations of polyphenol supplements and placebo in randomized order: [1] Epigallocatechin-Gallate (E: 282mg/d) + Resveratrol (RSV: 200mg/d); [2] E+R + soy

isoflavones (S: 80mg/d) and [3] placebo (PLA), containing partly hydrolyzed microcrystalline cellulose. Subjects consumed supplements orally for 2 days (twice daily at breakfast and dinner) and during the clinical investigation day (day 3) supplements were taken at arrival and simultaneously with the high-fat mixed meal. There was a wash-out period of at least 7 days between supplementation periods.

Clinical investigation day

Subjects were asked to maintain their habitual eating and physical activity pattern throughout the study. To standardize their eating pattern throughout the supplementation days, subjects were asked to keep a 3-day dietary record during the first supplementation period. Based on these first period records food intake was standardized during the second and third supplementation period. Furthermore, subjects were asked to refrain from drinking alcohol and intensive exercise 48h prior to the study day, and to consume a low-fat, carbohydrate-rich meal at the evening prior to the study day. After an overnight fast of at least 12h, subjects arrived at the laboratory (Maastricht University Medical Center^{+}) by car or bus. After ingestion of half of the daily amount of supplements, a cannula was inserted into an antecubital vein for blood sampling. Blood was sampled under fasting conditions (t=0) and for 6h after the intake of a liquid high-fat mixed meal (consumed within 5min at t=0 min) at t=30, 60, 90, 120, 180, 240, 300 and 360min. The liquid high-fat mixed-meal had an energy content of 2.6MJ (61 E% fat, 33 E% carbohydrate, 6 E% protein). Samples were immediately centrifuged (3000 rpm, 4°C, 10 min) and plasma aliquots were immediately snap-frozen in liquid nitrogen before storage at -80°C until further analysis. Fasting and postprandial energy expenditure and substrate oxidation were

measured by indirect calorimetry, using an open-circuit ventilated hood system (Omnical, Maastricht University, The Netherlands) (27).

Supplements

The test products were commercial available via Pure Encapsulations Inc. (Massachusetts, USA) and were encapsulated for blinding by Wellspring Clinical Services (Placebo Lot 15897, Teavigo Lot 15898, Resveratrol Extra Lot: 15899, Soy Isoflavone 40 Lot: 15900). All capsules were manufactured, tested and checked in accordance to standards of EU GMP requirements.

Teavigo capsules contained 94% E (141mg/capsule), Resveratrol extra (100mg trans-Resveratrol/capsule, combined with 46 mg grape seed polyphenols and 12,5mg red wine polyphenols), and Soy Isoflavone 40 (40mg soy isoflavones/capsule (100mg)). Placebo capsules were filled with microcrystalline cellulose and encapsulated equally as the active supplements. Daily supplemented polyphenol amounts (E, 300mg/d; R, 150mg/d; S, 150mg/d) have been shown to be safe and well-tolerated in humans (12, 28, 29).

Blood analyses

Blood was sampled into pre-chilled EDTA tubes (0.2M EDTA (Sigma, Dorset, UK)). Plasma FFA, triglycerides (TAG) and glucose were measured with enzymatic assays on an automated spectrophotometer (ABX Pentra 400 autoanalyzer, Horiba ABX, Montpellier, France). Plasma free glycerol was measured with an enzymatic assay (Enzytec™ Glycerol, Roche Biopharm, Switzerland) automated on a Cobas Fara spectrophotometric autoanalyzer (Roche Diagnostics, Basel, Switzerland). Circulating plasma insulin, adiponectin and leptin concentrations were determined using

commercially available radioimmunoassay (RIA) kits (Human Insulin specific RIA, Human Adiponectin RIA, Human Leptin RIA, Millipore Corporation, MA, USA). Catecholamin analysis was performed using high performance liquid chromatography with electrochemical detection (RECIPE chemicals & Instruments GmbH, Munich, Germany). Plasma concentrations of inflammatory markers (Interleukin-6 (IL6), Interleukin-8 (IL8) and tumor necrosis factor α (TNFα)) were determined using a multiplex enzyme-linked immuno-sorbent assay (ELISA) (Human ProInflammatory II 4-Plex Ultra-Sensitive Kit, Meso Scale Diagnositics, MD, USA).

Calculations

The equations of Weir (30) and Frayn (31) were used to calculate energy expenditure and the total rate of fat and carbohydrate oxidation, assuming that protein oxidation accounts for 15% of total energy expenditure. Calculations are based on measurements of VO_2 consumption (l/min) and VCO_2 production (l/min), averaged over 20 minutes for each time point.

Energy Expenditure (EE) (kJ/min) = $(3.9*VO_2) + (1.1*VCO_2)$

Carbohydrate oxidation (CHO) (g/min) = $(4.55*VCO_2) – (3.21*VO_2) – (2.87*N)$

Fat oxidation (FAT) (g/min) = $(1.67*VO_2) – (1.67*VCO_2) – (1.92*N)$

N (g/min) = ((0.15* EE)/17)/6.25

Macronutrient oxidation as percentages of energy expenditure:

CHO/EE (%) = (CHO*(17 kJ/g)) / EE

FAT/EE (%) = (FAT* (39 kJ/g)) / EE

Metabolic Flexibility = Postprandial RQ_{Max} - $RQ_{Fasting}$

Statistics

All data are given as means ± standard error of means (SEM). The postprandial response is expressed as AUC and incremental AUC (iAUC), which is calculated by the trapezoid method. iAUC values are used, when differences in resting values are different. AUC and iAUC values are given as total (0-6h), or divided in periods of 2 hours (0-2h, 2-4h, 4-6h). Differences in fasting and postprandial AUC values between treatments were analyzed using analysis of variance (ANOVA), with gender as covariate. Only in case of a trend or a significance of a treatment (treat) effect or treatment-gender (treat*gender) interaction, post-hoc analyses with LSD correction were performed. Trends and significant outcomes of ANOVA and post-hoc tests are summarized in table 2. Statistics was done using SPSS 19.0 for Macintosh. $P<0.05$ was considered statistically significant.

Results

Subjects' characteristics

Eighteen healthy, overweight men and women volunteered to participate in this study. Subjects' characteristics are presented in Table 1. No major differences in macronutrient composition of the diet and energy intake could be identified between subjects (3500kcal/2d (14771kJ/2d), 47.9 E% CHO, 36.5 E% FAT, 14.7 E% PRO).

Energy expenditure and substrate oxidation

Resting EE after an overnight fast was significantly higher during E+R compared to PLA supplementation (5.45±0.24 *vs*. 5.23±0.25 kJ/min, 7843±250 *vs*. 7528±254 kJ/d (1.30±0.04 *vs*. 1.25±0.04 kcal/min, 1873±60 *vs*. 1798±61 kcal/d), P=0.039) (Figure 1A). Postprandial EE remained elevated during E+R as well as E+R+S during the mid-postprandial period compared with PLA ($AUC_{2\text{-}4h}$: PLA, 675±20 kJ/2h (161±5 kcal/2h) *vs.* E+R, 699±18 (167±4 kcal/2h), *P*=0.03; PLA *vs.* E+R+S, 703±18 kJ/2h (168±4 kcal/2h), *P*=0.02) (Figure 1A). No significant effects were observed for respiratory quotient (RQ) (Figure 1B), carbohydrate (Figure 1C) and fat oxidation (Figure 1D) in the fasting and postprandial period. In men, metabolic flexibility (calculated as the difference between fasting and highest postprandial RQ) was increased during E+R treatment as compared to PLA (E+R *vs.* PLA, P=0.059) and as compared to E+R+S supplementation (E+R *vs.* E+R+S, P=0.009) for men (Figure 1E), but not for women (Figure 1F). No differences were observed for the time point, at which the higher RQ was reached (PLA: t=81.7; E+R: t=85.0; E+R+S: t=81.7).

Circulating metabolite concentrations

Fasting and postprandial plasma glucose (Figure 2A) and insulin concentrations (Figure 2B) were not significantly affected by supplementation of combinations of polyphenols. Fasting lactate concentrations were not different between treatments, but overall lactate response was lower during E+R+S compared with PLA ($AUC_{0\text{-}6h}$: 346.0±17.3 *vs.* 374.7±18.8 mmol/l/6h, P=0.024) (Figure 2C). No significant treatment effects were observed for plasma TAG concentrations (Figure 2D), whereas fasting FFA concentrations tended to be increased by E+R+S compared with PLA (508±51 *vs.* 401±28μmol/l, P=0.06). In line, FFA concentrations were higher in the postprandial phase during E+R+S compared with PLA as well as with E+R ($AUC_{0\text{-}360}$: PLA: 146±7, E+R: 148±8, E+R+S: 163±10 mmol/l/6h, P=0.06; E+R+S vs. E+R, P=0.02). E+R had no significant effects on plasma FFA concentrations compared with PLA (Figure 2E). Finally, fasting glycerol concentrations were elevated by E+R+S compared with PLA (E+R+S vs PLA, P=0.02), with no differences in postprandial conditions (Figure 2F).

Circulating adipokine and norepinephrine concentrations

Fasting leptin concentrations were elevated after E+R compared with PLA and E+R+S supplementation in women, but not in men, (women, PLA: 13.6±1.9 (*vs* E+R, p<0.01), E+R: 15.6±1.4, E+R+S: 13.5±1.7 μg/l (*vs* E+R, p<0.01), Figure 3A). There were no significant differences in adiponectin (Figure 3B) and norepinephrine (Figure 3C) concentrations between treatments. Plasma concentrations of IL-6, IL-8 and TNF-α were not changed after polyphenol supplementation compared with PLA (Figures 3D-F).

Discussion

We hypothesized that a combination of polyphenols may act additively or synergistically to enhance EE and fat oxidation. Indeed, the present study demonstrated for the first time that short-term supplementation (3 days) of E+R increased fasting and postprandial EE in humans. This was accompanied by a more pronounced increase in postprandial RQ, metabolic flexibility, in men but not in women. Addition of soy isoflavones partially reversed the beneficial effects of E+R, except for the increase in postprandial EE.

No effects on resting EE have been previously found during human supplementation studies with either E or R alone in the short-term (13, 15, 18) or long-term (12, 25, 32-34). Potent EE-stimulating effects of E in combination with caffeine have been reported during 12h- and 24h-measurements (14, 16), but this effect could not be confirmed in all studies (17). Nevertheless, in the former two studies, E was only effective in combination with caffeine and not specified to either fasting or postprandial conditions (14, 16). Indeed, 3 day supplementation with E alone did not increase resting EE in overweight volunteers (13, 20), indicating that R or a synergistic effect of both components may be responsible for the effect on resting EE, which was found in the present study.

The potential of R to increase EE has been reported in non-human primates after supplementation for 15 weeks (11). Also, studies in obese rodents observed that 6 and 10 weeks R supplementation prevented the development of obesity upon an obesogenic diet (35, 36). However, in the latter studies EE or other measures of energy balance such as food intake and energy excretion were not measured. In contrast, we have recently demonstrated that 30d supplementation with R alone

decreased EE in healthy overweight men, mimicking the effects of caloric restriction (12).

There are several possible mechanisms that may explain the increased EE as a result of short-term E+R supplementation. Rodent studies indicate that E may exert its metabolic effects through an inhibition of the noradrenaline-degrading enzyme Catechol-O-Methyl-Transferase (COMT), leading to an increased norepinephrine concentration (37, 38). In the present study norepinephrine concentrations were not affected by E+R supplementation, indicating that this cannot explain the observed increase in EE. We cannot exclude the possibility that R has counteracted E-induced catecholamine secretion, since R may inhibit catecholamine secretion and signaling in rat cardiomyocytes and adrenal medullary cells (39, 40).

The present results indicate that E+R supplementation increased fasting leptin concentrations. This might have contributed to the increase in EE, since leptin infusion has been shown to stimulate EE in humans (41). In line with our findings, it has been shown that 3-days E supplementation increased leptin expression in adipose tissue in overweight men (20). Since for E supplementation alone no increase in circulating leptin concentrations have been reported in previous short-term studies, it is tempting to postulate that E and R act synergistically to enhance leptin concentration. Interestingly, leptin concentrations remained unchanged after addition of soy isoflavone to E+R supplementation, suggesting that S may impair leptin secretion. Indeed, Szkudelska et al. (42) found that genistein attenuated leptin secretion by rodent adipocytes.

Finally, mitochondrial uncoupling in skeletal muscle, liver and (brown) adipose tissue might contribute to the observed increase in EE following E+R (43). Indeed, studies in rodents reported that E or R alone stimulate expression of uncoupling proteins in

tissues relevant to EE (11, 44). In rodent skeletal muscle, R stimulates PGC-1α, an upstream target of uncoupling genes (11). Thus, it would be highly interesting to examine the effects of combined polyphenol supplementation on mitochondrial function in future studies. In summary, it remains to be determined whether R or the synergistic effect of R+E is responsible for the observed increase in resting EE in humans.

Metabolic flexibility, reflected by the postprandial increase in RQ, was improved by the combination of E+R compared with placebo in men but not women. A recent study performed in our laboratory provided evidence that E supplementation induced a slightly more oxidative phenotype in skeletal muscle of obese men (20), which may improve metabolic flexibility. Additionally, we recently demonstrated that 30 days of R supplementation improved muscle mitochondrial respiration and induced a more oxidative phenotype of skeletal muscle in obese men, which was accompanied by improved metabolic flexibility (12). In line, improved mitochondrial function after R or E treatment was found in rodents (10, 11, 38). Thus, further research has to indicate whether R, E and/or additive or synergistic effects between both components explain the short-term improvement in metabolic flexibility in men.

Interestingly, long-term supplementation of high doses of R (500mg/d, 3-fold higher than in the study of Timmers et al. (12)) had no significant metabolic effects in young mildly insulin resistant obese subjects (25) and that R (75mg/d) has no metabolic effects in non-obese women (34), suggesting that R is mainly beneficial in metabolically compromised states (45) and/or at lower dosages. Therefore, we cannot exclude the possibility that the fact that we only observed an improved metabolic flexibility in men, may be due to the more pronounced insulin resistance in men compared with women at baseline (HOMA, men: 4.21±0.44, women: 2.69±0.35).

Secondly, we cannot exclude that the unaffected metabolic flexibility in women compared with men might be biased by the use of oral contraceptives and/or the phase of the menstrual cycle (46), which were not taken into account in the present study.

Because metabolic flexibility and mitochondrial function are closely associated with insulin sensitivity (1), it is tempting to postulate that prolonged combined E+R supplementation may improve insulin sensitivity. This is strengthened by long-term studies in rodents, demonstrating insulin-sensitizing properties of both E and R (47, 48). Further studies have to address the long-term benefits in both the prevention as well as reversal of obesity-related health complications.

When adding soy isoflavones to the combination of E+R the effects on resting EE and metabolic flexibility was completely reversed. It can be speculated that isoflavones' lipolytic potential, reflected by increased fasting and postprandial FFA concentrations in the present study, may reverse these benefits (49). More specifically, synergistic amplifications of lipolysis after combination of polyphenols (Resveratrol+Genistein) have previously been reported (26). Increased postprandial FFA concentrations may in turn reduce metabolic flexibility in healthy humans (50).

In conclusion, the present study demonstrated for the first time that short-term (3 days) supplementation of low-to-moderate dosages of E+R increased fasting and postprandial EE, which was accompanied by improved metabolic flexibility in men but not women. Soy isoflavones partially reversed the beneficial effects of E+R, except for increased postprandial EE, possibly due to their higher lipolytic potential.

Our data indicate that the impact of polyphenol supplementation is highly dependent on the type or combination of polyphenols and on gender. Furthermore, for resveratrol supplementation it is postulated (51, 52) that supplementation rather prevents and reverses metabolic abnormalities in metabolically high risk subjects than affecting

pathways and phenotypes of originally healthy subjects, which we suggest to account for in future studies.

Independent of gender and metabolic profile appears to be the increased energy expenditure. Assuming the increase achieved in this study by supplementing E+R maintains during long-term supplementation without counter regulatory effects, this may result in a 3.4kg-weight loss over a period of 6 months. According to findings in the US Diabetes Prevention Program, every kilogram of weight loss reduces the risk for developing diabetes by 16% (53). Importantly, long-term follow-up studies should further investigate whether these short-term metabolic effects of combined polyphenol supplementation in fact translate into long-term metabolic benefits. This would yield highly important information to further optimize polyphenol supplementation, thereby improving metabolic health and preventing metabolic disease in humans.

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TABLE 1: Subjects' characteristics.

Subjects	Total (n=18)	Men (n=9)	Women (n=9)
Age (years)	34 ± 2.6	35 ± 2.5	33 ± 2.8
Weight (kg)	86.6 ± 2.9	91.5 ± 3.3	81.6 ± 1.9
Length (m) *	1.73 ± 0.02	1.76 ± 0.02	1.7 ± 0.02
BMI (kg/m^2)	28.9 ± 0.4	29.5 ± 0.4	28.4 ± 0.4
Fat mass (% of total body weight) *	27.3 ± 1.9	21.6 ± 1.1	33 ± 1.5
Fat mass (kg) *	23.25 ± 1.46	19.7 ± 1.2	26.81 ± 1.23
WHR *	0.83 ± 0.03	0.92 ± 0.01	0.76 ± 0.02
SBP (mmHg) *	121 ± 2.2	126 ± 1	116 ± 2.5
DBP (mmHg)	81 ± 1.7	84 ± 1.8	78 ± 1.4
Fglu (mmol/l)	5 ± 0.09	5.07 ± 0.09	4.93 ± 0.09
Fins (mU/l) *	15.38 ± 1.41	18.63 ± 1.32	12.13 ± 1.07
HOMA-IR *	3.45 ± 0.33	4.21 ± 0.31	2.69 ± 0.25

Values are given as means ± SEM (n=18). DBP: diastolic blood pressure; Fglu: fasted plasma glucose; Fins: fasted plasma insulin; HOMA-IR: homeostatic model assessment of insulin resistance; WHR: waist-to-hip ratio; SBP: systolic blood pressure; Statistical significant difference between gender indicated as *, when $P<0.05$;

TABLE 2: ANOVA outcome of selected variables

			ANOVA			Post-hoc			
Variable	Period		p-value treat	p-value treat* sex	group	PLA	E+R	E+R+S	p-value
EE	t=0	kJ/min	0.115	0.587	total	5.23	5.45[1)]	5.27	1) 0.039
	AUC_{0-6h}	kJ/6h	0.122	0.518	total	2046	2072	2094	
	AUC_{0-2h}	kJ/2h	0.512	0.193	total	705	699	712	
	AUC_{2-4h}	kJ/2h	0.01	0.675	total	676	699[1a)]	704[1b)]	1a) 0.028 1b) 0.014
	AUC_{4-6h}	kJ/2h	0.331	0.68	total	664	674	678	
	$iAUC_{0-6h}$	kJ/6h	0.133	0.457	total	164	112	195	
	$iAUC_{0-2h}$	kJ/2h	0.035	0.287	total	78	46[1), 2)]	79	1) 0.037 2) 0.021
	$iAUC_{2-4h}$	kJ/2h	0.226	0.614	total	49	45	71	
	$iAUC_{4-6h}$	kJ/2h	0.226	0.379	total	37	20	45	
ΔRQ			0.308	0.026	total	0.08	0.1	0.07	
					men	0.06	0.11[1), 2)]	0.03	1) 0.059 2) 0.009
Plasma lactate	t=0	mmol/l	0.95	0.414	total	0.9	0.9	0.9	
	AUC_{0-6h}	mmol/l/6h	0.038	0.308	total	382	367	337[1)]	1) 0.024
	AUC_{0-2h}	mmol/l/2h	0.176	0.313	total	169	164	151	
	AUC_{2-4h}	mmol/l/2h	0.165	0.502	total	122	117	106	
	AUC_{4-6h}	mmol/l/2h	0.066	0.163	total	91	86	80	
	$iAUC_{0-6h}$	mmol/l/6h	0.114	0.939	total	58	50	22	
	$iAUC_{0-2h}$	mmol/l/2h	0.159	0.466	total	61	59	46	
	$iAUC_{2-4h}$	mmol/l/2h	0.304	0.836	total	14	11	1	
	$iAUC_{4-6h}$	mmol/l/2h	0.497	0.618	total	-17	-19	-26	
Plasma FFA	t=0	µmol/l	0.042	0.979	total	401	402	508[1), 2)]	1) 0.064 2) 0.061
	AUC_{0-6h}	mmol/l/6h	0.079	0.564	total	146	148	163[1), 2)]	1) 0.088 2) 0.042
	AUC_{0-2h}	mmol/l/2h	0.012	0.35	total	35	37	44[1), 2)]	1) 0.022 2) 0.007
	AUC_{2-4h}	mmol/l/2h	0.401	0.535	total	41	40	45	
	AUC_{4-6h}	mmol/l/2h	0.378	0.905	total	70	71	74	
	$iAUC_{0-6h}$	mmol/l/6h	0.323	0.953	total	0.9	2.2	-20.2	
	$iAUC_{0-2h}$	mmol/l/2h	0.45	0.814	total	-12.8	-11.4	-17	
	$iAUC_{2-4h}$	mmol/l/2h	0.409	0.905	total	-7.8	-8.7	-16.3	
	$iAUC_{4-6h}$	mmol/l/2h	0.257	0.945	total	21.5	22.3	13.2	
Plasma glycerol	t=0	µmol/l	0.058	0.647	total	85.7	93.2	103.3[1)]	1) 0.021
	AUC_{0-6h}	mmol/l/6h	0.709	0.475	total	31.7	32.5	32.8	
	AUC_{0-2h}	mmol/l/2h	0.316	0.145	total	9.2	9.6	9.9	
	AUC_{2-4h}	mmol/l/2h	0.957	0.392	total	10.1	10.1	10.3	
	AUC_{4-6h}	mmol/l/2h	0.815	0.772	total	12.4	12.8	12.6	
	$iAUC_{0-6h}$	mmol/l/6h	0.445	0.6	total	0.045	-1.59	-1.1	
	$iAUC_{0-2h}$	mmol/l/2h	0.103	0.415	total	-1.1	-4.4	-2.5	

	$iAUC_{2-4h}$	mmol/l/2h	0.309	0.851	total	-0.5	-1.1	-2.1	
	$iAUC_{4-6h}$	mmol/l/2h	0.115	0.816	total	2.1	1.6	0.2	
	t=0	mmol/l	0.403	0.107	total	1.4	1.3	1.3	
	AUC_{0-6h}	mmol/l/6h	0.193	0.485	total	714	690	640	
	AUC_{0-2h}	mmol/l/2h	0.343	0.433	total	193	186	173	
	AUC_{2-4h}	mmol/l/2h	0.246	0.84	total	271	261	240	
Plasma TAG	AUC_{4-6h}	mmol/l/2h	0.274	0.347	total	251	242	227	
	$iAUC_{0-6h}$	mmol/l/6h	0.11	0.13	total	206	212	173	
	$iAUC_{0-2h}$	mmol/l/2h	0.236	0.453	total	23	29	18	
	$iAUC_{2-4h}$	mmol/l/2h	0.331	0.269	total	102	99	84	
	$iAUC_{4-6h}$	mmol/l/2h	0.457	0.473	total	82	85	71	
	t=0	ng/l	0.029	0.041	total	13.6	15.6[1), 2)]	13.5	1) 0.043 2) 0.019
Plasma leptin	t=0	ng/l			women	19.1	22.8[1), 2)]	18.7	1) 0.008 2) 0.002
	t=180	ng/l	0.099	0.126	total	14.4	16.3	14.3	
	t=360	ng/l	0.383	0.294	total	14.9	16.2	14.9	

Statistical analysis of selected variables by ANOVA and post-hoc testing. AUC: Area under the curve; iAUC: incremental AUC; t: treatment, t*s: treatment*sex; EE: Energy Expenditure; ΔRQ: Metabolic Flexibility; FFA: free fatty acids; E+R: Epigallocatechin-gallate + Resveratrol; E+R+S: Epigallocatechin-Gallate + Resveratrol + Soy isoflavones; PLA: Placebo; 1): statistically significant different from PLA; 2: statistically significant difference between polyphenol treatments

FIGURE 1. Substrate oxidation and energy expenditure before and after a high-fat mixed meal after 3 day polyphenol supplementation (t=0). Values are given as means ± SEM (n=18). 1A-1D: Dashed line, circles: PLA, Solid line, triangles: E+R, solid line, square: E+R+S; 1E-1F: white: PLA, grey: E+R, black: E+R+S. Statistical significance indicated as *, when post-hoc testing $P<0.05$.

FIGURE 2. Plasma metabolite concentrations after 3 day supplementation before and after a high-fat mixed meal (t=0). Values are given as means ± SEM (n=18). 2.A-2.H: Dashed line, circles: PLA, Solid line, triangles: E+R, solid line, square: E+R+S. Statistical significance indicated as *, when $P<0.05$; trends indicated as #, when $P<0.1$.

FIGURE 3. Plasma adipokine, norepinephrine & cytokine concentrations after 3 day supplementation before and after a high-fat mixed meal (t=0). Values are given as means ± SEM (n=18). 2.A-2.F: Dashed line, circles: PLA, Solid line, triangles: E+R, solid line, square: E+R+S. Statistical significance indicated as *, when $P<0.05$.

Figure 1. Substrate metabolism

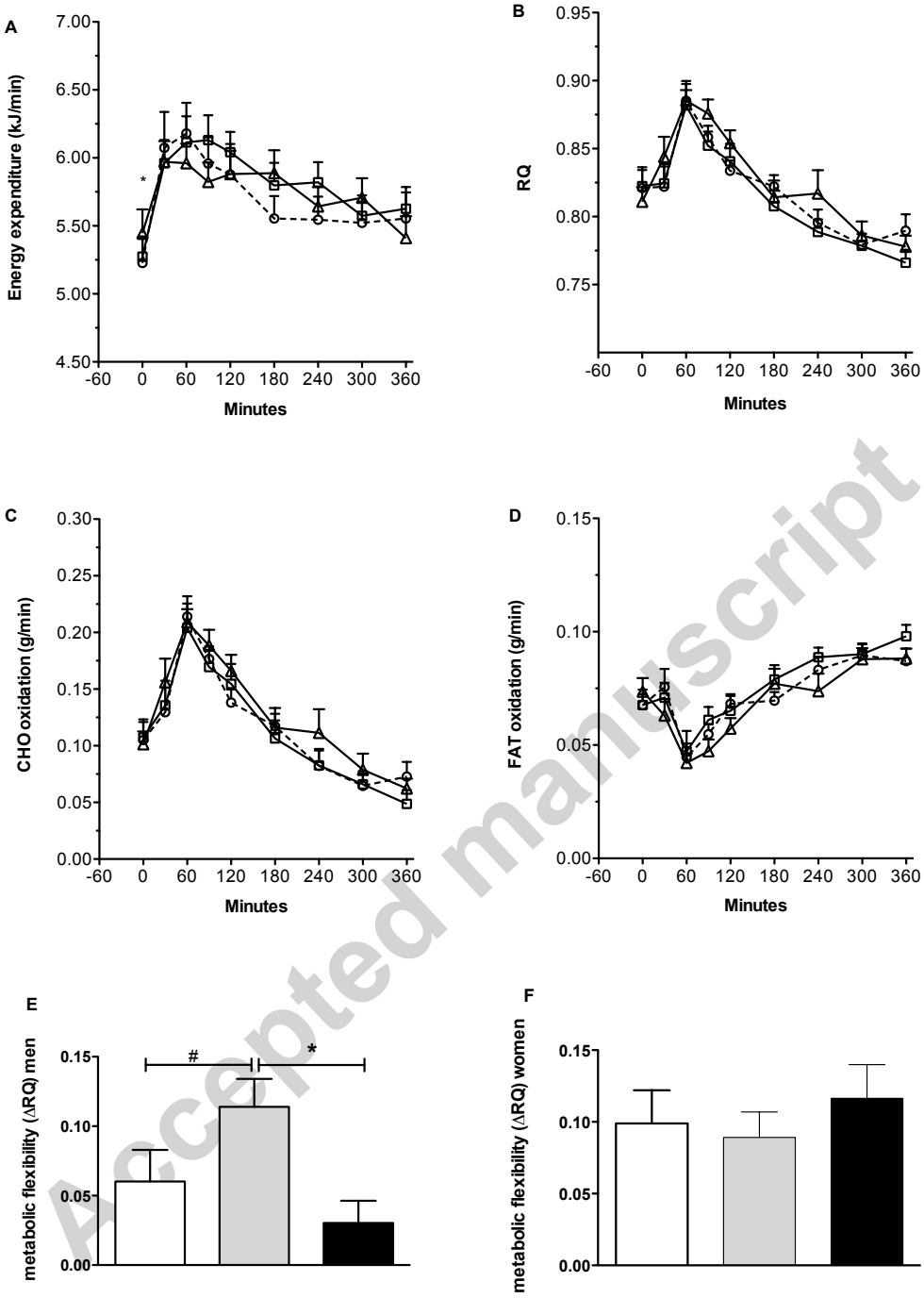

Figure 2. Plasma metabolites

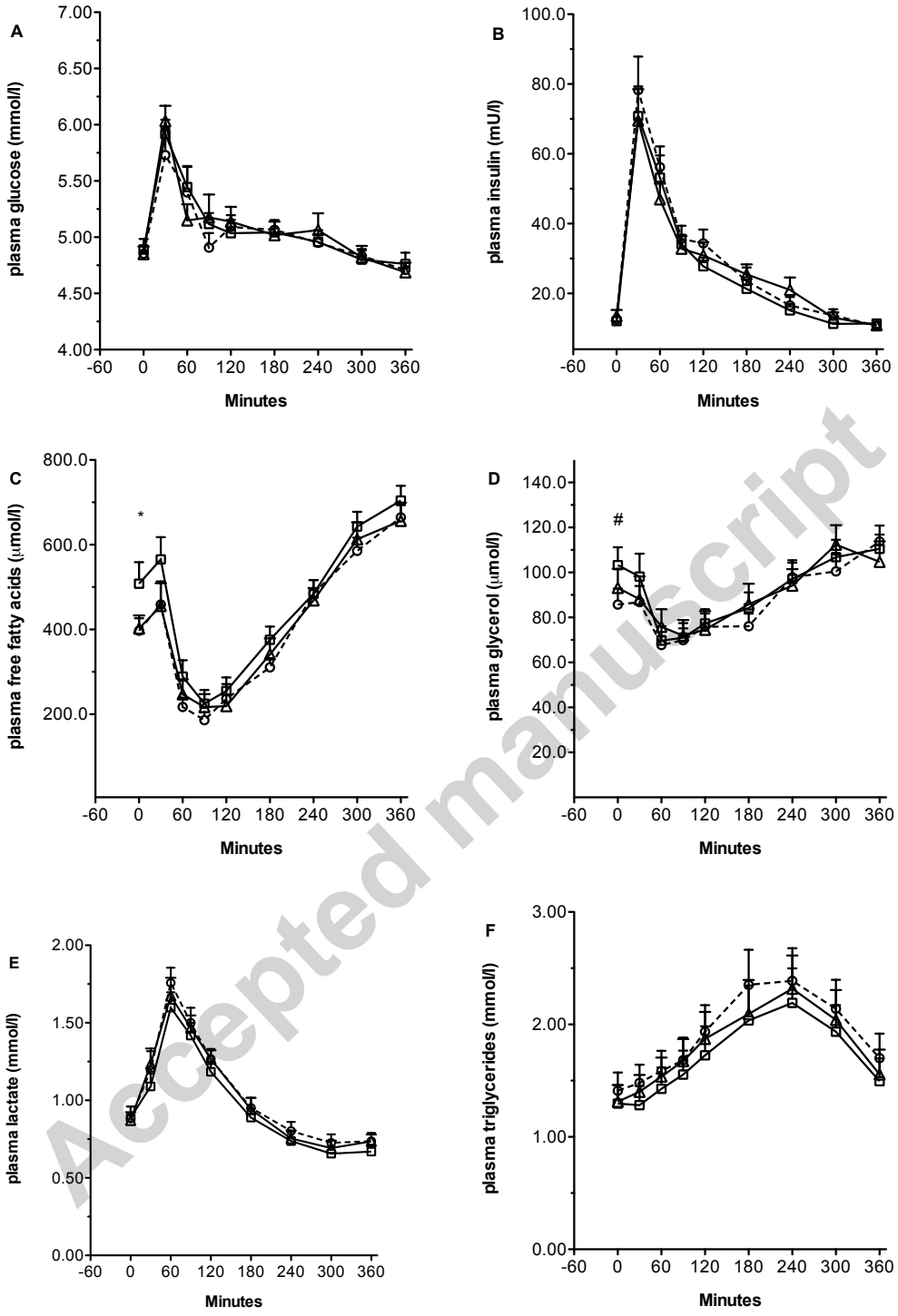

Figure 3. Plasma Norepinephrine and adipokines