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Metabolic and performance effects of Yerba Mate on well-trained cyclists

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Conceptualization: JLA; Analyzed plant material samples: JLA, IA, HW; Conducted clinical trials: JLA; Analyzed data: JLA, IA, HW, CC; Writing, original draft: JLA; Writing, review and editing JLA, IA, HW, CC; Supervision: JLA, CC; Have primary responsibility for the final content: JLA, CC. All authors have read the final version of the manuscript.

Short title: Effects of Yerba Mate on endurance athletes

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ABSTRACT

Introduction. Yerba Mate (YM) is a South-American plant, rich in polyphenols, saponins and xanthines, of growing scientific interest due to its metabolic effects. YM has been shown to increase fat utilization during exercise in untrained humans, but its effects on well-trained individuals during exercise are unknown. Methods. We characterized metabolic and physical performance effects of YM in eleven well-trained male cyclists. In a double-blind crossover design, participants ingested 5 g of YM or placebo (PL; maltodextrin) daily for 5 days, and 1 h prior to experimental trials. Results. Ergometer-based tests included a submaximal step-test (SST) at 30-80% of $\overline{VO}_{2\text{max}}$ (6 x 5 min stages), followed by a cycloergometer-based time-trial test to complete mechanical work (~30min, TT; n=9). Before and during tests, blood and respiratory gas samples were collected. YM increased resting plasma adrenaline concentration (P=0.002), and fat utilization by 23% at 30-50% $\overline{VO}_{2\text{max}}$ vs PL (Effect sizes Glass’ $\Delta$ [ES]+95%CI, 0.8±0.55) correlating strongly with post-SST plasma [glycerol] (r=0.758). Treatment effects on rates of perceived exertion, heart rate and gross efficiency were unclear during SST. Respiratory exchange ratio during TT indicated carbohydrate-dependence and did not differ between treatments (PL, 0.95±0.03[SD]; YM, 0.95±0.02). TT performance showed a small (ES, 0.38±0.33) but significant ($P=0.0278$) improvement with YM (PL, 30.1±1.8[SD]; YM, 29.4±1.4 min; 2.2%±2[95%CI]) with average increase of 7W power-output (ES=0.2±0.19; $P=0.0418$; 2.3%±2[95%CI]) and 2.8% $\overline{VO}_{2}$ ($P=0.019$). Pacing displayed lower power-output after 30% of total TT workload in PL vs YM. Conclusion. YM increased fat utilization during submaximal exercise and improved TT performance, but
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performance-enhancement effect was unrelated to measures of substrate metabolism during maximal exercise.

Key words: *Ilex paraguariensis*, cycling, endurance performance, ergogenic aids, metabolism

**INTRODUCTION.**

*Ilex paraguariensis* is a South American plant of growing scientific interest worldwide, consumed in beverages by millions on a daily basis. Yerba Mate (YM), as named colloquially, has been used since pre-Columbian times ritually and for its alleged medicinal properties by the Guarani ethnic group. YM is rich in phenolics (chlorogenic acid and other caffeoyl derivatives) with high antioxidant potency, saponins and xanthines (caffeine and theobromine) (1). Biomedical research on YM is scarce when compared to coffee and tea (1), but several reports support the role of YM as a modulator of metabolism. For instance, YM has been reported to be anti-obesogenic (2), a regulator of Akt and AMPK signaling pathways in different tissues (3, 4), cardio-protective (4), hypocholesterolemic (5), able to increase thermogenesis (6) and to shift substrate utilization during exercise towards higher fat oxidation (7, 8). Some of these effects position YM as a potential exercise enhancer (9) and ergogenic aid. However, studies to date have only been conducted in rodents and unfit or unhealthy humans. Hence, whether YM can have an effect in an already optimized metabolic machinery of well-trained endurance athletes, is yet unknown.

Specifically, recent reports of YM increasing fat utilization during exercise of different intensities and durations (7, 8), and its potential neurological effects (10, 11),
suggest YM as a suitable supplement to improve endurance performance. Enhancing metabolic flexibility could be an important effect of YM to optimize the use of endogenous fuel: i.e. to increase the use of fat during low-intensity exercise and spare the limited carbohydrate stores for performance-determining high-intensity bouts (12, 13). Additionally, independent of the metabolic effects, a neuro-modulatory effect through synergism between the phenolics and xanthines could drive central nervous system stimulation (10, 11). YM has additionally been reported to have analgesic and anxiolytic effects in rodents (14), which could also be a factor resulting in positive performance outcomes.

Some of the metabolic effects of YM are comparable to those of other well-studied plants also with a high content of phenolics, such as green tea and coffee (15), but the phytochemical profile of YM is unique. Green tea and YM share, among others, compounds such as caffeine, but different from green tea YM contains a large quantity of caffeoyl derivatives (chlorogenic acids) and saponins with different moieties (5). Some of the metabolic effects of phenolics have been attributed to their down-regulation of the catecholamine-O-transferase (COMT), but markers of effects on this enzyme have not been shown in humans (15). Importantly, YM has been reported to lack (-) epigallocatechin-3-gallate (EGCG) and other catechins, allegedly the main compounds for increasing fat oxidation in green tea (15). Therefore, it is possible that the phytochemicals unique to YM, or the effects resulting from their interactions, are the direct key for its metabolic effects.

The aim of the current study was to test the effect of YM ingestion daily for 5 days and 1 hour prior to tests to assess substrate utilization during submaximal exercise and performance during a short (~30 min) simulated cycling time-trial in well-trained cyclists. The dose of YM would be such as to contain a low (non-ergogenic) amount
of caffeine. Our hypotheses were that YM would increase fat utilization during submaximal exercise and improve time-trial performance.
METHODS.

Yerba Mate samples. To determine dosage and due to variability of phytochemical content in plants (16), four lots of YM (leaves mixed with stems) of different origin (Taragüi, Regular Blend, Las Marias, Argentina; La Merced, de Campo, Las Marias, Argentina; Rosamonte, Regular blend, Diez hermanos, Argentina; Guayaqui, Biodynamic, Argentina; Supplementary Tables 1 and 2) were screened for xanthine and total phenol content as well as radical scavenging activity. Based on lower relative caffeine content and higher phenol content and scavenging activity, La Merced, de Campo (lot number L 24515 09:10 A 3370), was selected as treatment and for further analysis of xanthine and phenol content (Table 1). The caloric content of YM doses was estimated to be negligible (<4 kcal).

Subjects. Eleven well-trained competitive male endurance cyclists/triathletes were recruited. The subjects’ age, body mass (BM), maximal oxygen uptake ($\dot{V}O_{2\text{max}}$), peak aerobic power output (PAPO) and regular training load were 30 ± 3 yr, 75 ± 7 kg, 71 ± 6 ml/kg/min, 403 ± 32 W, 11 ± 2 h/week, respectively. Prior to giving their written consent, all subjects were informed of the nature of the study and possible risks involved.

Pretesting: Incremental cycling test. Approximately 2 weeks prior to commencing their first experimental trial, subjects underwent an incremental cycling test to exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) for determination of PAPO and $\dot{V}O_{2\text{max}}$ as previously described (17). During this test, subjects breathed through a two-way, low resistance
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non-rebreathing valve (Hans Rudolph Inc., Kansas City, USA) and mouthpiece attached to a calibrated Oxycon Pro metabolic system (Jaeger, Hochberg, Germany) interfaced to a computer that calculated the instantaneous rates of O\(_2\) consumption (\(\dot{V}O_2\)), CO\(_2\) production (\(\dot{V}CO_2\)), and the respiratory exchange ratio (RER). Before each test, analyzers were calibrated with commercially available gasses of known and certified O\(_2\) and CO\(_2\) content. \(\dot{V}O_{2\text{max}}\) was defined as the highest uptake a subject attained during any 30 s of the test, while PAPO was calculated from the last completed work rate plus the fraction of time spent in the final non-completed work rate (17). All exercise sessions were conducted under standardized laboratory conditions of temperature and humidity, and subjects were fan-cooled (Sealey HVSF30, Suffolk, UK) throughout all tests.

Study overview. An overview of the experimental protocol is shown in Figure 1. The study was evaluated by the Regional Ethics Committee of Norway in accordance to the Norwegian Act on Medical and Health Research, and allowed for implementation. Briefly, each subject completed two experimental trials in a double-blind, placebo controlled, randomized, counterbalanced crossover design. Five days prior to each trial day, subjects ingested per day 5 g of yerba mate (YM) or placebo (PL; maltodextrin). Two days prior to each trial physical activity was controlled, and food for the day prior to each trial was provided. On each trial day, subjects reported to the laboratory fasted at ~7.00 AM and an 18 G catheter (BD, NJ, USA) was inserted into the antecubital vein of one arm to allow for serial blood sampling. Immediately after baseline blood sampling, subjects ingested YM or PL capsules, 1 h before starting a 10 minute warm-up (7 minutes at 70% of \(\dot{V}O_{2\text{max}}\), followed by 3 min ramp-down to 30% of \(\dot{V}O_{2\text{max}}\)) prior to a submaximal step-test (SST). Following SST subjects
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recovered for 5 min and completed a laboratory based cycling time-trial (TT). The
washout period between trials was 1-2 weeks (18, 19).

Submaximal step-test (SST). During this test, participants were instructed to keep
cadence constant at 85 rpm while completing consecutive 5 min long stages of
cycling at power outputs to elicit 30, 40, 50, 60, 70 and 80% of VO_2max. Throughout
SST the respiratory parameters were measured and the last minute of each stage
analyzed. RPE was assessed at the end of each stage.

Time-Trial. Simulated laboratory cycling TTs were undertaken with the ergometer set
in a cadence-dependent power output (linear) mode for participants to take ~30 min to
complete a set amount of mechanical work. A custom-determined alpha value was
assigned to each individual based on the preferred cadence during baseline testing and
the total workload to be completed. Based on previous experiments (20) and extensive
pilot testing, we determined that subjects would average ~80% of PAPO for 30 min
TTs. Accordingly, the mechanical work to be completed for each individual was
determined as:

\[ \text{TT Mechanical Work (joules)} = 0.8 \times \text{PAPO (W)} \times 1800 \text{ s} \]

During TTs, subjects were blinded for time, power output and \( \text{VO}_2/\text{VCO}_2 \) but
provided with cadence and total amount of mechanical work to be completed (as a
percentage of total as well as continuous real-time kJ countdown to zero).

Subjects were instructed to complete TTs as fast as possible, as they would do on a
race. No encouragement was provided during TTs and neutral verbal feedback was
provided at specific time-points. All tests were conducted by the same researcherc (JLA). No music was played during trials and participants had no cues of time elapsed. Participants were tested one at a time on separate days. To assess blinding, immediately after the last trial participants were asked to match first and second experimental trial with ‘YM’, ‘PL’ or ‘I don’t know’.

Respiratory gases were measured 4 times for 3 min at the start of TTs: Start, 1/3 and 2/3 of total workload and during the last ~70 kJ of work. RPE was assessed at the end of each measurement. An overall weighed average for the four TT measuring points was obtained. The weight of each measure was determined based on duration and timing of each sampling point and total duration of the trial, i.e. first and last measures were considered to be only representative of the time during which samples were obtained whereas samples 2 and 3 where considered to be representative of the times where respiratory air was not collected.

**Exercise control.** In the 48 h prior to each trial subjects abstained from any vigorous physical activity, but allowed to exercise at <70% HR_max for a maximum of 2 and 1 h, 2 and 1 days respectively before each trial. The training completed before the first trial was kept on a training log and repeated before the second trial.

**Dietary control.** A custom-made pre-packaged diet containing 45 kcal/kg BM in the form of 8, 0.8 and 1.5 g/kg of high glycaemic index carbohydrates (CHO), fat and protein respectively was provided to each participant for the 24 h prior to each trial. Participants were instructed to ingest a portion of their diet (snack) containing 0.9 g/kg BM CHO prior to sleeping ~9 h before experimental trials to eliminate the chances of hypoglycaemia during experimental trials, which were undertaken in the fasted state. A participant’s checklist of food items was handed back to the researcher
served also as control for dietary compliance. On trial days, *ad libitum* water intake was measured and recorded from the moment of ingestion of the capsules until the end of TT.

**Capsules preparation & delivery.** For each dose dark gelatin capsules (12 units, size 00) were filled with 5 g of YM brought to fine powder in a grinder (MKM6003, Bosch, Germany) or with maltodextrin. All capsules were tasteless and treatments visually undistinguishable from each other (tested in pilot tests prior to study). Participants were indicated to have capsules together with lunch. A reminder was set on participants’ phone with a dedicated app (Medisafe, Medisafe Inc.) and/or on-line calendar which also allowed the main researcher to track compliance with capsule ingestion schedule.

**Blood sampling.** Blood samples were collected in 6 mL EDTA tubes (BD, NJ, USA) upon insertion of catheter (baseline), prior to SST, after SST, every 1/3 of TT and immediately after TT. Following each blood sample and at regular 20-30 min intervals, catheters were flushed with 5 mL saline solution (0.9 % NaCl g/L) to maintain patency in the cannula. Immediately upon collection, samples were spun at 3000 rpm for 10 min at 4 °C. The resultant plasma was aliquoted and stored at -80 °C for later analyses.

**Gastrointestinal distress questionnaire.** A gastrointestinal distress questionnaire as detailed previously (21) was used to assess any potential negative symptoms of the treatment. The questionnaire was completed by participants prior to capsule ingestion, prior to SST and after TTs.
For all calculations the data of last minute of each stage were used. Whole-body rates of CHO and fat oxidation (g/min) were determined for each steady-state gas measurement point from $\dot{V}CO_2$ and $\dot{V}O_2$ values using the non-protein RER calculation (22). Gross efficiency was calculated as reported by Moseley (23). For each SST, a third-degree polynomial line of best fit was incorporated using fat oxidation (g/min) as a function of measured intensity (% of $\dot{V}O_{2\text{max}}$) including origin (0,0) according to previously described methods (24). The turning point (local maximum) of the curve was used to determine Maximal Fat Oxidation (MFAO, g/min) and $\text{FAT}_{\text{max}}$ (the intensity at which MFAO is elicited).

### Analytical techniques

**Plasma.** FFA concentrations were measured using a non-esterified fatty acid assay kit (NEFA-HR (2)), Wako Pure Chemical Industries, Ltd, Osaka, Japan). Glycerol was analyzed using a kit coupling enzyme assay involving glycerol kinase and glycerol phosphate oxidase (MAK117, Sigma-Aldrich, St. Louis, USA). Lactate was analyzed using a YSI 1500 SPORT (Yellow Springs Instruments, Yellow Springs, USA). Glucose was analyzed using a Biosen C-Line (EKF Diagnostics, Magdeburg, Germany). Plasma adrenaline was analyzed using an ELISA kit (EIA-4306, DRG Instruments, Marburg, Germany). Caffeine and paraxanthine were analyzed by validated LC-MS/MS methodology described elsewhere (25). Briefly, the LC-MS/MS system was composed of a Shimadzu (Shimadzu Scientific Instruments, Columbia, USA) LC20AD system and an ABSciex triple quadrupole mass spectrometer.
equipped with a Waters Xbridge C18 column (2.1 x 100 mm, 3.5 µm). MilliQ water (0.1% formic acid) and acetonitrile (0.1% formic acid) were used as mobile phases. Total runtime was 9 minutes. Column oven was operated at 10 °C and 30 °C, respectively. The MS parameters were as follows: CUR 16, CAD 8, IS 5500, temp 575 °C, GS1 60, GS2 50. The mass spectrometer was operated in positive mode with electro spray ionization and multiple reaction monitoring (MRM). Data collection and analysis were handled by Analyst Software 1.5.1 (Applied Biosystems).

Seven concentrations of each analyte were prepared as calibration standards in blank plasma and 4 different concentrations were prepared as quality controls. Limit of quantitation (LOQ) was based on the lowest concentration of the linear calibration curve that gave an acceptable accuracy and precision (+20%). Linear range and LOQ are given in ng/ml; Compound (linear range, LOQ); Caffeine (5-500, 5), Paraxanthine (5-250, 5).

YM extract preparation for phytochemical analysis. Dry YM plant material was grinded to a powder (<1 mm) and extracted on an Accelerated Solvent Extraction system (ASE 350, Dionex, Sunnyvale, CA, USA). Diatomaceous earth (Dionex) was mixed with 3.5 g of plant material and loaded in 100 ml steel cartridges. The cartridges were fitted on to the system and exhaustive extraction was performed with two cycles of 100% methanol followed by two cycles of 50% methanol at 60 °C followed by two cycles of 50% methanol at 100 °C. Preheating time was 5-7 min, static extraction per cycle 5 min and the extraction was carried out under a pressure of 1500 PSI (10 MPa). The extraction was performed three times. The extracts from each cell were combined and diluted to 250 mL with methanol. The diluted extract was used for xanthine determination. For quantification of total phenol content, DPPH
scavenging activity and extraction yield, 150 mL of the diluted extract was taken to dryness in a rotary evaporator.

Quantitative analysis of xanthines in plant extract. One milliliter of 0.525 µg/µl 8-chlorotheophylline 98% (Sigma-Aldrich, MO USA) as internal standard was added to 5 ml of the diluted extract (26), dried on a rotary evaporator and subsequently dissolved in 10 ml of mobile phase before filtering into HPLC vials (PTFT 0.22 µm). Five microliters of the filtrate were analyzed by HPLC (LaChrom Elite, Hitachi, Tokyo, Japan) equipped with a reverse phase C18 column (Atlantis T3, 3 µM, 150 x 4.6 mm, Waters, Ireland), and an L-2455 diode array detector. Elution was performed using isocratic eluent, acetonitrile/0.1% formic acid in distilled water (1:9 v/v) (HPLC grade, Sigma-Aldrich) (26). The flow rate was 1.0 mL/min. The absorbance was recorded at 272 nm and the separation was carried out at 25 °C. The average value of three parallels was used for the amount calculation. Calculation of xanthine amount was based on a linear regression model with internal standard. The calibration curve was obtained using caffeine ReagentPlus® 99% (Sigma-Aldrich) and theobromine ≥ 98.5% (Sigma Life science, MO, USA). Stock solution of caffeine (1.95 µg/µl in methanol) and theobromine (0.53 µg/µl in 50% methanol) were used for a calibration in the of range 0.01-0.12 µg/µl. The calculated amount of caffeine and theobromine were expressed as % (w/w). Theophylline was not detected in the plant material and standard curves therefore not obtained.

Total phenolic content. Dried extract was dissolved in DMSO (5 and 2.5 mg/ml) in triplicates. A linear calibration curve of gallic acid (≥97.5 %, Sigma-Aldrich) was obtained in the range 0.3-2.5 mg/ml. The experiment was performed according to
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Singleton et al. (27). Briefly, 40 µl test solution was mixed with 3160 µl distilled water (MilliQ) and added 200 µl Folin-Ciocalteu reagent (Merck, Darmstadt, Germany). After 5 minutes, 600 µl 20% Na₂CO₃ solution was added and incubated in the dark at room temperature for 2 hours. The absorbance was measured at 765 nm on a Biochrom Libra S32 PC UV/Vis spectrophotometer (Biochrom Ltd., Cambridge, UK).

DPPH radical-scavenging. Reaction with the DPPH radical was carried out as previously described (28). Briefly, the dried extract (0.05 ml, in DMSO) was mixed with a solution of DPPH (Sigma-Aldrich) in methanol (A₅₁₇ = 1.0; 2.95 ml) and the UV absorbance at 517 nm was measured for 5 min. Samples were assayed in triplicate and result given as the effective concentration to give 50% scavenging of the DPPH radical (EC₅₀) ± SD. Quercetin (Sigma-Aldrich) was used as a positive control.

Statistical analysis. Data were analyzed using two-way repeated-measures ANOVA with Student–Newman–Keuls post hoc analysis to correct for the family-wise error during multiple post-hoc tests (Sigmaplot for Windows; Version 13). Grouped data were analyzed using paired t-tests and performance data were analyzed using Glass’ Δ effect-sizes (ES) with an on-line available tool (sportsci.org/resource/stats/xcrossover.xls) following guidelines outlined by Hopkins (29). Linear regressions were calculated using the least-square method. All data are presented as mean ± standard deviation (SD), except for ES -and elsewhere specified-data are reported as ± 95% CI. The level of statistical significance was set at P < 0.05.
RESULTS.

Plant material. Details of plant material are reported in Table 1. Based on a 7.9% water content, the total content per 5 g dose was 52 mg of caffeine (relative to participants’ BM: 0.70 ± 0.06 mg/kg) and 456 mg of total phenolics (relative to participants’ BM 6.1± 0.56 mg/kg). Absence of catechins in the material was confirmed with HPLC-DAD and proton nuclear magnetic resonance analysis.

Participants. Two participants were unable to execute maximal-performance efforts, one on YM and one on PL trial day due to individual incident and to a technical issue, respectively. Because of participants’ reported incapacity for best-performance, large performance variation (in excess of 11%) and being outliers following Chauvenet’s criterion, these were excluded from TT analysis prior to breaking the blinding code and performance data are reported for n = 9. The SST data for these individuals were unaffected and therefore kept for the analysis.

Nutrition. All subjects complied with dietary requirements. Total water ingested on trial days was 948 ± 401 and 894 ± 249 g in PL and YM respectively (P = 0.56).

Performance test. There was a small (ES = 0.38 ± 0.33) but significant (P = 0.028) performance improvement with YM in time to complete TT from 30.1 ± 1.8 to 29.4 ± 1.4 min (delta 0.40 ± 0.45 sec), and a concomitant small but significant 1.7 ± 2.1% increase in average power as percentage of PAPO (ES = 0.36 ± 0.33; P = 0.035) (Figure 2, A) and a 2.3 ± 2.6% increase in absolute power (ES = 0.2 ± 0.19; P = 0.042). Individual performance difference as average percentage of PAPO ranged
from -1.8 to 6.4% (Figure 2, B). Analysis of pacing (Figure 2, C) indicates that there were no differences between treatments in power output until 30% of the total completed workload, from which point the power output in YM was significantly higher ($P < 0.05$) consistently until the end of the TTs. There were no differences in RPE between treatments (Figure 2, D).

Respiratory results.

SST. There was a main effect of treatment ($P = 0.05$) and intensity ($P < 0.001$) on fat oxidation. Fat oxidation was increased in YM compared to placebo at 30, 40 and 50% of $\dot{V}O_{2\text{max}}$ ($P = 0.008$) by 0.10, 0.11 and 0.09 g/min respectively, representing an average 23% higher fat oxidation over this range (Figure 3, A). Fat oxidation tended to be higher in YM ($P = 0.071$) at 60% of $\dot{V}O_{2\text{max}}$. Accordingly, CHO oxidation was higher in PL ($P = 0.01$) over the same range of intensities. MFAO ($0.67 \pm 0.1$ g/min YM, $0.60 \pm 0.16$ g/min PL) and FAT$_{\text{max}}$ ($55 \pm 3\% \dot{V}O_{2\text{max}}$ YM, $55 \pm 3\% \dot{V}O_{2\text{max}}$ PL) were not significantly different, but there was a trend ($P = 0.1$) for higher MFAO in YM. Despite the difference in substrate utilization, there were no differences in efficiency at any workload (Figure 3, B).

TT. Oxygen uptake was higher ($P = 0.018$) in YM ($84.1 \pm 3.4 \% \dot{V}O_{2\text{max}}$) vs PL ($81.8 \pm 4.3 \% \dot{V}O_{2\text{max}}$) (Figure 3, C), but there were no differences between treatments in RER during TTs (PL, $0.95 \pm 0.03$, YM, $0.95 \pm 0.02$) (Figure 3, D), average HR (PL $168 \pm 10$, YM $169 \pm 8$ BPM) or maximal HR (PL $179 \pm 10$, YM $182 \pm 9$ BPM) during TTs.
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SST HR & RPE. There were no differences in heart rate and RPE between treatments during SST (Table 2).

Blinding assessment. Two out of nine participants correctly identified the trial in which they were under the YM treatment. The remaining seven individuals were unable to match the capsules ingested to PL or YM treatment.

Gastrointestinal distress questionnaire. There were no differences between time-points or treatments in any of the items of the gastrointestinal distress questionnaire.

Plasma metabolites. Details of plasma metabolites are outlined in Table 3.

Lactate and Glucose. Lactate showed a trend ($P = 0.07$) for main treatment effects and a clear main effect of time ($P < 0.001$) with a treatment x time interaction ($P = 0.029$). Lactate was significantly higher in YM compared to PL at 2/3 ($P = 0.018$) and 3/3 of TT ($P = 0.001$). Glucose showed a main effect for time ($P < 0.001$) but no main effect for treatment or interactions. Glucose was significantly higher in YM pre-SST compared to PL ($P = 0.029$) and raised above resting values in YM during 2/3 TT ($P = 0.029$) and in both groups at 3/3 TT ($P < 0.001$).

FFA and Glycerol. FFA showed main effects for time ($P = 0.03$) and treatment x time interactions ($P = 0.002$). FFA were lower in YM compared to PL pre-SST ($P = 0.002$) and lower in pre-SST in PL compared to resting values ($P = 0.04$). Glycerol showed main effect of time ($P < 0.001$) and treatment x time interactions ($P = 0.04$). At post-SST ($P = 0.046$) and 1/3 TT ($P = 0.033$) glycerol was higher in YM vs PL. A large
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correlation \((r = 0.59, P = 0.0057); \mu M \text{ FFA ox/kg/min} = 0.012 (\pm 95\% \text{CI, 0.008}) \times [\text{FFA}] + 10.65 (\pm 95\% \text{CI, 4.83})\) was observed between the pre-SST plasma FFA and Fat oxidation during SST and a very large correlation \((r = 0.76, P < 0.001); \mu M \text{ FFA ox/kg/min} = 0.086 (\pm 95\% \text{CI, 0.037}) \times [\text{Glycerol}] + 6.63 (\pm 95\% \text{CI, 4.83})\) between post-SST plasma glycerol and fat oxidation during SST.

Caffeine, Paraxanthine and Adrenaline. Caffeine showed main effects for treatment, time and time x treatment interactions \((P < 0.001)\). Caffeine concentration was higher in YM vs PL at all time-points \((P < 0.001)\). Only in YM there was a marked increase above resting values Pre-SST \((P < 0.001)\) and remained elevated throughout the rest of the trial. Adrenaline concentration was higher in YM vs PL at baseline \((P = 0.049)\), pre-SST \((P = 0.002)\), and at 3/3 TT \((P = 0.002)\). In both treatments adrenaline increased compared to baseline at all time-points after post-SST \((P < 0.001)\).

DISCUSSION.

The main findings of this study were that supplementing well-trained cyclists with 5 g of YM daily for 5 days and 1 h before experimental trials resulted in: 1) a 23% increase in fat oxidation, on average, compared to placebo during cycling at intensities between 30-50% \(\text{VO}_{2\max}\), and 2) a small but significant performance improvement in a ~30 min time-trial compared to placebo. During submaximal exercise we could detect clear metabolic differences between treatments on substrate oxidation, plasma FFA and glycerol, but no differences in gross efficiency. During the performance test there was a clear carbohydrate dependence in both groups with no
differences between treatments, indicating that the performance improvements in YM were due to factors other than a shift in substrate utilization. Additionally, we report for the first time increased plasma adrenaline in humans in response to a supplement low in caffeine and rich in phenolics.

The current study represents the first scientific investigation, to our knowledge, to assess the effects of Yerba Mate on metabolism during submaximal exercise and time-trial performance in well-trained cyclists. Previous studies assessing the metabolic effect of YM during exercise were limited to a single pre-exercise dose of ≤ 2 g in untrained population and included no dietary control, assessment of performance or plasma markers of fat metabolism, focusing mainly on respiratory measures to assess substrate utilization (7, 8). These studies show YM increased fat oxidation by 24% during a step-test and at intensities below 70% $\dot{V}O_{2peak}$ (7) and by ~18% during 30 min continuous exercise at 37% of $\dot{V}O_{2peak}$ (8) compared to placebo treatments. Despite the differences in population and experimental protocols, our findings of 23% increase in fat utilization during submaximal exercise are in line with what has been reported previously. Moreover, it is also possible that effects of YM on increased fat utilization at higher intensities during SST (or indeed at $FAT_{max}$) were not detected because of a small RER over-estimation due to $CO_2$ contribution from body $HCO_3^-$ stores at higher intensities (13, 30). Nonetheless, we provide further insights supporting the use of YM as a new aid to manipulate substrate utilization during submaximal exercise in addition to further novel insights in its potential effect on metabolic regulation.

The metabolic markers assessed in plasma provide new clues on the causes behind changes in substrate utilization. We specifically assessed plasma FFA and glycerol because of their reliability as markers of lipolysis and their relationship to fat
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oxidation (31). We found no effects of 5 days YM supplementation on resting blood FFA or glycerol and plasma FFA remained unchanged 1 h after YM ingestion. Unexpectedly, FFA decreased slightly in PL resulting also in a significant difference from YM at the same time-point. Blood glycerol also showed no differences between groups pre-SST, but instead it was higher in YM compared to placebo post-SST and at 1/3 TT, and proved to have a stronger association with fat oxidation than pre-SST plasma FFA.

Provided that fat metabolism is very sensitive to small increases in blood insulin via a decreasing lipolysis and plasma FFA oxidation (32), it should be considered that the insulin response to 5 g of maltodextrin in PL may have affected substrate utilization and partially explain the drop in plasma FFA pre-SST in PL. However, because this dose represents 1/5th of the minimal dose that has been previously shown to affect substrate utilization (33) and also likely to elicit a minimal insulin response (34), a suppression of fat oxidation seems unlikely. As shown by Achten et al., a dose of 75 g of glucose (13 times the amount we used) seems to be necessary to suppress fat oxidation by ~25% during an incremental test (35). Moreover, fat oxidation does not appear to be affected by changes in FFA in the range observed (31).

Instead, it is possible that the observed increase in lipolysis and fatty acid oxidation is a consequence of a catecholamine-mediated response. Indeed, we observed an increase in baseline and pre-SST adrenaline, which could be mediated by inhibition of the catecholamine-O-methyl-transferase (COMT) enzyme as a consequence of the content of xanthines, chlorogenic acid and other caffeoyl derivatives in mate (11). Such mechanism has been hypothesized in the past, but never observed in humans (15).
Taken together, these results suggest that circulating pre-SST FFA and lipolysis during SST were related to the rates of fat oxidation observed, but do not completely explain our results. Therefore, it is likely that other loci of control and mechanisms such as intermediary metabolism and substrate transport into metabolically active tissues are important to explain our findings. These potential mechanisms should be further evaluated in the future by direct assessment of muscle metabolism in response to YM *in vitro* and *in vivo*. Additionally, future studies assessing the effect of very low doses of CHO prior to exercise on fat oxidation will allow to establish with better precision the magnitude of the effect of YM on fat oxidation reported here and shed further light on the points of metabolic control. In the mean time, our results suggest that YM indeed increases fat oxidation during exercise at submaximal intensities. The enhanced fat oxidation observed during SST, however, does not seem to be a reason behind the performance improvement.

The cycling performance improvement and parameters assessed during TTs provide valuable insights on the physiological response to YM supplementation. Time trials were an average 40 sec faster in YM as a consequence of an average 2.3% increase in absolute power output. The magnitude of improvement observed is in line with a recent meta-analysis showing an average 1.9% increase in performance by short-term polyphenol supplementation (9). Substrate selection was unlikely a factor affecting performance as we show a clear carbohydrate dependence in both groups (Figure 3, D), which is in accordance to the metabolic demands of intense cycling time-trials: mild differences in plasma substrate availability are overridden and normalized by high intensity exercise (20). In relation to physiological and other metabolic variables, a higher power-output was concomitant to higher average \( \dot{V}O_2 \) as well as higher plasma lactate and adrenaline by the end of TTs in YM. Pacing strategy
Effects of Yerba Mate on endurance athletes showed a higher power-output in YM during the last two-thirds of TTs, indicating increased tolerance to fatigue. There were no differences in other physiological parameters such as average or maximum heart-rate, RPE or gross efficiency (measured during the submaximal test). Some of these results, in particular the pacing strategy and increased power output with similar RPE, suggest a performance improvement such as those observed for the effect of caffeine (36), but caffeine on its own is an unlikely candidate for explaining our findings.

The low amounts of naturally occurring caffeine (52 mg, or ~0.7 mg/kg) provided to our participants with YM treatment, were deliberately intended to fall under an ergogenic dose (37). The caffeine content in the plant material was in fact critical to determine treatment dosage so as to provide the highest possible amounts of other phytochemicals together with a non-ergogenic amount of caffeine provided it is widely accepted that 3 mg/kg of caffeine represents an ergogenic dose for endurance sports, an amount that results in plasma caffeine concentration of 15-20 µM. Under this threshold, caffeine is unlikely to have ergogenic effects (37). In the current study we observed a peak plasma caffeine of 2.3 µM, which represents ~1/8 of the alleged minimum plasma concentration representative of an ergogenic dose. Accordingly, it has been shown that doses of caffeine of 1 (38), 1.5 (39) and even 2 (40) mg/kg are not ergogenic in endurance tests lasting ~30 min. Moreover, no difference in the rates of perceived exertion and heart-rate during the submaximal test is another strong indicator of the lack of a caffeine-mediated effect (41). Therefore, our findings match those of previous studies and allow us to conclude that caffeine was not ergogenic, at least not in the way that is commonly observed.

Instead, the ergogenic effects of YM could be explained through a synergism between chlorogenic acids and caffeine in stimulating the central nervous system
(CNS). The effect of phenolic compounds on brain-specific COMT could diminish
the breakdown of dopamine and increase its bioavailability (10). As a consequence of
the increased CNS dopamine levels, doses of caffeine that would normally not be
ergogenic could have a potentiation effect (42) on the -already higher- dopamine
levels, and result ergogenic during exhaustive exercise (10, 42).

Additionally, the ergogenic effects of YM could also be related to its effects on
regulation of blood flow (4). YM has been associated to increased eNOS activation
and consequent endothelial nitric oxide production and vaso-relaxation (4, 43). While
the effect of YM on this pathway has been studied only in rodent cardiac muscle (4),
eNOS is present in human skeletal muscle (43) and it has been proven to be activated
by polyphenols (9). While these ideas remain speculative, they provide suitable
explanations matching our observations to the shown in vivo physiological and
metabolic effects of YM. In particular, the effect of polyphenols on COMT represents
a suitable mechanism linking both the metabolic and performance effects through its
effects on liver and brain COMT isoforms.

In conclusion, YM increased fat oxidation at low exercise intensities (30-50%
$\dot{V}O_{2\text{max}}$) and increased performance in a short (30 min) time-trial. While the
performance improvement in the time-trials were likely due to factors other than
increased fat utilization, enhanced fat oxidation by YM could potentially be of use for
manipulating substrate use during training in conditions of low carbohydrate
availability (44). The performance effect of YM should be addressed in more ‘real
life’ racing conditions including pre- and during-exercise CHO-rich nutrition. In the
meantime, our findings add to a growing body of information regarding the
importance of phytochemicals for performance and we provide valuable new
physiological and metabolic insights to understand the mechanisms behind the response to YM.

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The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by ACSM.
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**FIGURE CAPTIONS**

**Figure 1.** Schematic overview of experimental trials. Participants ingested 5 g of Yerba Mate (YM) or Placebo (PL; maltodextrin) per day for 5 days prior to experimental trials. Exercise was controlled for 48 h prior to trials and diet was provided for 24 h prior to trials. On trial days venous blood samples were obtained at indicated time-points prior to and after ingestion of YM or PL. Submaximal step-test consisted of consecutive 5 min steps at 30, 40, 50, 60, 70 and 80% of \( \dot{V}O_{2\text{max}} \). After 5 min recovery, participants undertook a ~30 min time-trial to complete a pre-set amount of mechanical work. Expired gas samples were collected continuously during the submaximal step-tests and at indicated time-points during time-trials. BM, body mass; CHO, carbohydrates.

**Figure 2.** Performance time-trial test results showing average % PAPO (columns) and individual responses (dots and lines; A), individual differences in performance as delta % PAPO (B), pacing in both groups (C) and rates of perceived exertion at different time-points (D). Data in C and D are Means ± SD. * Significantly different from PL at same point (p<0.05). Data were analyzed using paired t-tests for pairwise comparisons and two-way repeated measures ANOVA with Student–Newman–Keuls post hoc analysis for multiple comparisons. PL, Placebo; PAPO, Peak Aerobic Power Output; RPE, Rate of Perceived Exertion (Borg); YM, Yerba Mate.

**Figure 3.** Respiratory responses during submaximal step test (SST; A and B) and during simulated Time Trials (TTs; C and D). Calculated fat utilization (A) and efficiency (B) during submaximal step-tests at power to elicit 30, 40, 50, 60, 70 and
80% of $\dot{V}O_{2\text{max}}$. TTs oxygen consumption as % of $\dot{V}O_{2\text{max}}$ (A), respiratory exchange ratio (B) and heart rate (C). Data are Means ± SD (n = 11, SST, n = TTs)). *

Significantly different from PL at same point (p<0.05). Data were analyzed using two-way repeated measures ANOVA with Student–Newman–Keuls post hoc analysis.

PL, Placebo; YM, Yerba Mate.
**Supplementary Table 1.** Phytochemical content of 4 brands of Yerba Mate, pre-screened for the study. Total phenols and flavonoids were determined in arbitrary units and are reported as percentage of average values across the 4 brands. Scavenging activity was determined with DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method and is reported as % scavenged. La Merced was selected as plant for the current study due to a higher sum of ratio between total phenols, flavonoids, scavenging activity and caffeine.

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<th>Taragui</th>
<th>La Merced</th>
<th>Rosamonte</th>
<th>Guayaki</th>
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<tbody>
<tr>
<td>Total Phenols (% of average)</td>
<td>87</td>
<td>107</td>
<td>97</td>
<td>110</td>
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<tr>
<td>Flavonoids (% of average)</td>
<td>81</td>
<td>108</td>
<td>103</td>
<td>108</td>
</tr>
<tr>
<td>Scavenging activity (% scavenged)</td>
<td>72</td>
<td>81</td>
<td>72</td>
<td>84</td>
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<tr>
<td>Caffeine (% plant material)</td>
<td>0.93</td>
<td>1.12</td>
<td>1.09</td>
<td>1.14</td>
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**Supplementary Table 2.** Origin details of Yerba Mate brands utilised in this study.

<table>
<thead>
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<th>Brand</th>
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<th>Variety</th>
<th>Country of Origin</th>
<th>Lot number</th>
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<td>Regular Blend</td>
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<tr>
<td>La Merced</td>
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<tr>
<td>Guayaquí</td>
<td>Guayaquí</td>
<td>Biodynamic</td>
<td>Argentina</td>
<td>Not provided</td>
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