Areta, J., Austarheim, I., Wangensteen, H., Capelli, C. (2017). Metabolic and Performance Effects of Yerba Mate on Well-trained Cyclists. *Medicine & Science in Sports & Excercise,* 50, 817-826.

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

J.L. Areta[1], I. Austarheim[2], H. Wangensteen[2], C. Capelli [1]

[1], Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway

[2], Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, Oslo, Norway

Authors contributions to the manuscript

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

Address for correspondence: José L. Areta

Department of Physical Performance

Norwegian School of Sport Sciences

Sognsveien 220, Oslo, Norway (0863)

Phone: +47-94116085

Email: jose.areta@nih.no

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 $\dot{V}O_{2max}$ (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% $\dot{V}O_{2max}$ vs PL (Effect sizes Glass' Δ [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% $\dot{V}O_2$ (*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

50 performance-enhancement effect was unrelated to measures of substrate metabolism
51 during maximal exercise.
52
53 Key words: *Ilex paraguariensis*, cycling, endurance performance, ergogenic aids,
54 metabolism
55
56 **INTRODUCTION.**
57
58 *Ilex paraguariensis* is a South American plant of growing scientific interest
59 worldwide, consumed in beverages by millions on a daily basis. Yerba Mate (YM), as
60 named colloquially, has been used since pre-Columbian times ritually and for its
61 alleged medicinal properties by the Guaraní ethnic group. YM is rich in phenolics
62 (chlorogenic acid and other caffeoyl derivatives) with high antioxidant potency,
63 saponins and xanthines (caffeine and theobromine) (1). Biomedical research on YM is
64 scarce when compared to coffee and tea (1), but several reports support the role of
65 YM as a modulator of metabolism. For instance, YM has been reported to be anti-
66 obesogenic (2), a regulator of Akt and AMPK signaling pathways in different tissues
67 (3, 4), cardio-protective (4), hypocholesterolemic (5), able to increase thermogenesis
68 (6) and to shift substrate utilization during exercise towards higher fat oxidation (7,
69 8). Some of these effects position YM as a potential exercise enhancer (9) and
70 ergogenic aid. However, studies to date have only been conducted in rodents and unfit
71 or unhealthy humans. Hence, whether YM can have an effect in an already optimized
72 metabolic machinery of well-trained endurance athletes, is yet unknown.
73 Specifically, recent reports of YM increasing fat utilization during exercise of
74 different intensities and durations (7, 8), and its potential neurological effects (10, 11),

75 suggest YM as a suitable supplement to improve endurance performance. Enhancing
76 metabolic flexibility could be an important effect of YM to optimize the use of
77 endogenous fuel: i.e. to increase the use of fat during low-intensity exercise and spare
78 the limited carbohydrate stores for performance-determining high-intensity bouts (12,
79 13). Additionally, independent of the metabolic effects, a neuro-modulatory effect
80 through synergism between the phenolics and xanthines could drive central nervous
81 system stimulation (10, 11). YM has additionally been reported to have analgesic and
82 anxiolytic effects in rodents (14), which could also be a factor resulting in positive
83 performance outcomes.

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

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

100 of caffeine. Our hypotheses were that YM would increase fat utilization during
101 submaximal exercise and improve time-trial performance.
102

103 **METHODS.**

104

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

115

116 *Subjects.* Eleven well-trained competitive male endurance cyclists/triathletes were
117 recruited. The subjects' age, body mass (BM), maximal oxygen uptake ($\dot{V}O_{2max}$), peak
118 aerobic power output (PAPO) and regular training load were 30 ± 3 yr, 75 ± 7 kg, 71
119 ± 6 ml/kg/min, 403 ± 32 W, 11 ± 2 h/week, respectively. Prior to giving their written
120 consent, all subjects were informed of the nature of the study and possible risks
121 involved.

122

123 *Pretesting: Incremental cycling test.* Approximately 2 weeks prior to commencing
124 their first experimental trial, subjects underwent an incremental cycling test to
125 exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport,
126 Groningen, The Netherlands) for determination of PAPO and $\dot{V}O_{2max}$ as previously
127 described (17). During this test, subjects breathed through a two-way, low resistance

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. $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$, followed by 3 min ramp-down to 30% of $\dot{V}O_{2max}$) prior to a submaximal step-test (SST). Following SST subjects

153 recovered for 5 min and completed a laboratory based cycling time-trial (TT). The
154 washout period between trials was 1-2 weeks (18, 19).
155
156 *Submaximal step-test (SST).* During this test, participants were instructed to keep
157 cadence constant at 85 rpm while completing consecutive 5 min long stages of
158 cycling at power outputs to elicit 30, 40, 50, 60, 70 and 80% of $\dot{V}O_{2max}$. Throughout
159 SST the respiratory parameters were measured and the last minute of each stage
160 analyzed. RPE was assessed at the end of each stage.
161
162 *Time-Trial.* Simulated laboratory cycling TTs were undertaken with the ergometer set
163 in a cadence-dependent power output (linear) mode for participants to take ~30 min to
164 complete a set amount of mechanical work. A custom-determined alpha value was
165 assigned to each individual based on the preferred cadence during baseline testing and
166 the total workload to be completed. Based on previous experiments (20) and extensive
167 pilot testing, we determined that subjects would average ~80% of PAPO for 30 min
168 TTs. Accordingly, the mechanical work to be completed for each individual was
169 determined as:
170
171 TT Mechanical Work (joules) = 0.8 x PAPO (W) x 1800 s
172
173 During TTs, subjects were blinded for time, power output and $\dot{V}O_2/\dot{V}CO_2$ but
174 provided with cadence and total amount of mechanical work to be completed (as a
175 percentage of total as well as continuous real-time kJ countdown to zero).
176 Subjects were instructed to complete TTs as fast as possible, as they would do on a
177 race. No encouragement was provided during TTs and neutral verbal feedback was

178 provided at specific time-points. All tests were conducted by the same researcher
179 (JLA). No music was played during trials and participants had no cues of time
180 elapsed. Participants were tested one at a time on separate days. To assess blinding,
181 immediately after the last trial participants were asked to match first and second
182 experimental trial with 'YM', 'PL' or 'I don't know'.

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

191

192 *Exercise control.* In the 48 h prior to each trial subjects abstained from any vigorous
193 physical activity, but allowed to exercise at <70% HR_{max} for a maximum of 2 and 1 h,
194 2 and 1 days respectively before each trial. The training completed before the first
195 trial was kept on a training log and repeated before the second trial.

196 *Dietary control.* A custom-made pre-packaged diet containing 45 kcal/kg BM in the
197 form of 8, 0.8 and 1.5 g/kg of high glycaemic index carbohydrates (CHO), fat and
198 protein respectively was provided to each participant for the 24 h prior to each trial.
199 Participants were instructed to ingest a portion of their diet (snack) containing 0.9
200 g/kg BM CHO prior to sleeping ~9 h before experimental trials to eliminate the
201 chances of hypoglycaemia during experimental trials, which were undertaken in the
202 fasted state. A participant's checklist of food items was handed back to the researcher

203 served also as control for dietary compliance. On trial days, *ad libitum* water intake
204 was measured and recorded from the moment of ingestion of the capsules until the
205 end of TT.
206
207 *Capsules preparation & delivery.* For each dose dark gelatin capsules (12 units, size
208 00) were filled with 5 g of YM brought to fine powder in a grinder (MKM6003,
209 Bosch, Germany) or with maltodextrin. All capsules were tasteless and treatments
210 visually undistinguishable from each other (tested in pilot tests prior to study).
211 Participants were indicated to have capsules together with lunch. A reminder was set
212 on participants' phone with a dedicated app (Medisafe, Medisafe Inc.) and/or on-line
213 calendar which also allowed the main researcher to track compliance with capsule
214 ingestion schedule.
215
216 *Blood sampling.* Blood samples were collected in 6 mL EDTA tubes (BD, NJ, USA)
217 upon insertion of catheter (baseline), prior to SST, after SST, every 1/3 of TT and
218 immediately after TT. Following each blood sample and at regular 20-30 min
219 intervals, catheters were flushed with 5 mL saline solution (0.9 % NaCl g/L) to
220 maintain patency in the cannula. Immediately upon collection, samples were spun at
221 3000 rpm for 10 min at 4 °C. The resultant plasma was aliquoted and stored at -80 °C
222 for later analyses.
223
224 *Gastrointestinal distress questionnaire*. A gastrointestinal distress questionnaire as
225 detailed previously (21) was used to assess any potential negative symptoms of the
226 treatment. The questionnaire was completed by participants prior to capsule ingestion,
227 prior to SST and after TTs.

228 *Calculations of substrate utilization, MFAO, FAT_{max} and gross efficiency during SST:*
229 For all calculations the data of last minute of each stage were used. Whole-body rates
230 of CHO and fat oxidation (g/min) were determined for each steady-state gas
231 measurement point from $\dot{V}CO_2$ and $\dot{V}O_2$ values using the non-protein RER calculation
232 (22). Gross efficiency was calculated as reported by Moseley (23). For each SST, a
233 third-degree polynomial line of best fit was incorporated using fat oxidation (g/min)
234 as a function of measured intensity (% of $\dot{V}O_{2max}$) including origin (0,0) according to
235 previously described methods (24). The turning point (local maximum) of the curve
236 was used to determine Maximal Fat Oxidation (MFAO, g/min) and FAT_{max} (the
237 intensity at which MFAO is elicited).
238
239
240 *Analytical techniques*
241
242 *Plasma.* FFA concentrations were measured using a non-esterified fatty acid assay kit
243 (NEFA-HR (2)), Wako Pure Chemical Industries, Ltd, Osaka, Japan). Glycerol was
244 analyzed using a kit coupling enzyme assay involving glycerol kinase and glycerol
245 phosphate oxidase (MAK117, Sigma-Aldrich, St. Louis, USA). Lactate was analyzed
246 using a YSI 1500 SPORT (Yellow Springs Instruments, Yellow Springs, USA).
247 Glucose was analyzed using a Biosen C-Line (EKF Diagnostics, Magdeburg,
248 Germany). Plasma adrenaline was analyzed using an ELISA kit (EIA-4306, DRG
249 Instruments, Marburg, Germany). Caffeine and paraxanthine were analyzed by
250 validated LC-MS/MS methodology described elsewhere (25). Briefly, the LC-MS/MS
251 system was composed of a Shimadzu (Shimadzu Scientific Instruments, Columbia,
252 USA) LC20AD system and an ABSciex triple quadrupole mass spectrometer

253 equipped with a Waters Xbridge C18 column (2.1 x 100 mm, 3.5 µm). MilliQ water
254 (0.1% formic acid) and acetonitrile (0.1% formic acid) were used as mobile phases.
255 Total runtime was 9 minutes. Column oven was operated at 10 ºC and 30 ºC,
256 respectively. The MS parameters were as follows: CUR 16, CAD 8, IS 5500, temp
257 575 ºC, GS1 60, GS2 50. The mass spectrometer was operated in positive mode with
258 electro spray ionization and multiple reaction monitoring (MRM). Data collection and
259 analysis were handled by Analyst Software 1.5.1 (Applied Biosystems).
260 Seven concentrations of each analyte were prepared as calibration standards in blank
261 plasma and 4 different concentrations were prepared as quality controls. Limit of
262 quantitation (LOQ) was based on the lowest concentration of the linear calibration
263 curve that gave an acceptable accuracy and precision (+ 20%). Linear range and LOQ
264 are given in ng/ml; Compound (linear range, LOQ); Caffeine (5-500, 5), Paraxanthine
265 (5-250, 5).
266
267 *YM extract preparation for phytochemical analysis.* Dry YM plant material was
268 grinded to a powder (< 1 mm) and extracted on an Accelerated Solvent Extraction
269 system (ASE 350, Dionex, Sunnyvale, CA, USA). Diatomaceous earth (Dionex) was
270 mixed with 3.5 g of plant material and loaded in 100 ml steel cartridges. The
271 cartridges were fitted on to the system and exhaustive extraction was performed with
272 two cycles of 100% methanol followed by two cycles of 50% methanol at 60 °C
273 followed by two cycles of 50% methanol at 100 °C. Preheating time was 5-7 min,
274 static extraction per cycle 5 min and the extraction was carried out under a pressure of
275 1500 PSI (10 MPa). The extraction was performed three times. The extracts from
276 each cell were combined and diluted to 250 mL with methanol. The diluted extract
277 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

303 Singleton et al. (27). Briefly, 40 µl test solution was mixed with 3160 µl distilled
304 water (MilliQ) and added 200 µl Folin-Ciocalteu reagent (Merck, Darmstadt,
305 Germany). After 5 minutes, 600 µl 20% Na_2CO_3 solution was added and incubated in
306 the dark at room temperature for 2 hours. The absorbance was measured at 765 nm on
307 a Biochrom Libra S32 PC UV/Vis spectrophotometer (Biochrom Ltd., Cambridge,
308 UK).
309 *DPPH radical-scavenging.* Reaction with the DPPH radical was carried out as
310 previously described (28). Briefly, the dried extract (0.05 ml, in DMSO) was mixed
311 with a solution of DPPH (Sigma-Aldrich) in methanol (A_{517} = 1.0; 2.95 ml) and the
312 UV absorbance at 517 nm was measured for 5 min. Samples were assayed in triplicate
313 and result given as the effective concentration to give 50% scavenging of the DPPH
314 radical (EC_{50}) ± SD. Quercetin (Sigma-Aldrich) was used as a positive control.
315
316 *Statistical analysis*. Data were analyzed using two-way repeated-measures ANOVA
317 with Student–Newman–Keuls post hoc analysis to correct for the family-wise error
318 during multiple post-hoc tests (Sigmaplot for Windows; Version 13). Grouped data
319 were analyzed using paired t-tests and performance data were analyzed using Glass' Δ
320 effect-sizes (ES) with an on-line available tool
321 (sportsci.org/resource/stats/xcrossover.xls) following guidelines outlined by Hopkins
322 (29). Linear regressions were calculated using the least-square method. All data are
323 presented as mean ± standard deviation (SD), except for ES -and elsewhere specified-
324 data are reported as ± 95% CI. The level of statistical significance was set at $P < 0.05$.
325

326 **RESULTS.**

327

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

333

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

341

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

344

345 *Performance test.* There was a small (ES = 0.38 ± 0.33) but significant ($P = 0.028$)
346 performance improvement with YM in time to complete TT from 30.1 ± 1.8 to 29.4 ±
347 1.4 min (delta 0.40 ± 0.45 sec), and a concomitant small but significant 1.7 ± 2.1%
348 increase in average power as percentage of PAPO (ES = 0.36 ± 0.33; $P = 0.035$)
349 (**Figure 2, A**) and a 2.3 ± 2.6% increase in absolute power (ES = 0.2 ± 0.19; $P =$
350 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 $\dot{V}O_{2max}$ ($P = 0.008$) 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 $\dot{V}O_{2max}$. Accordingly, CHO oxidation was higher in PL ($P = 0.01$) over the same range of intensities. MFAO (0.67 ± 0.1 g/min YM, 0.60 ± 0.16 g/min PL) and FAT_{max} (55 ± 3% $\dot{V}O_{2max}$ YM, 55 ± 3% $\dot{V}O_{2max}$ 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 ± 3.4 % $\dot{V}O_{2max}$) vs PL (81.8 ± 4.3 % $\dot{V}O_{2max}$) (**Figure 3, C**), but there were no differences between treatments in RER during TTs (PL, 0.95±0.03, YM, 0.95±0.02) (**Figure 3, D**), average HR (PL 168 ± 10, YM 169 ± 8 BPM) or maximal HR (PL 179 ± 10, YM 182 ± 9 BPM) during TTs.

375 *SST HR & RPE.* There were no differences in heart rate and RPE between treatments
376 during SST (**Table 2**).
377
378 *Blinding assessment.* Two out of nine participants correctly identified the trial in
379 which they were under the YM treatment. The remaining seven individuals were
380 unable to match the capsules ingested to PL or YM treatment.
381
382 *Gastrointestinal distress questionnaire.* There were no differences between time-
383 points or treatments in any of the items of the gastrointestinal distress questionnaire.
384
385 *Plasma metabolites.* Details of plasma metabolites are outlined in **Table 3**.
386
387 *Lactate and Glucose.* Lactate showed a trend ($P = 0.07$) for main treatment effects
388 and a clear main effect of time ($P < 0.001$) with a treatment x time interaction ($P =$
389 0.029). Lactate was significantly higher in YM compared to PL at 2/3 ($P = 0.018$) and
390 3/3 of TT ($P = 0.001$). Glucose showed a main effect for time ($P < 0.001$) but no main
391 effect for treatment or interactions. Glucose was significantly higher in YM pre-SST
392 compared to PL ($P = 0.029$) and raised above resting values in YM during 2/3 TT (P
393 $= 0.029$) and in both groups at 3/3 TT ($P < 0.001$).
394
395 *FFA and Glycerol.* FFA showed main effects for time ($P = 0.03$) and treatment x time
396 interactions ($P = 0.002$). FFA were lower in YM compared to PL pre-SST ($P = 0.002$)
397 and lower in pre-SST in PL compared to resting values ($P = 0.04$). Glycerol showed
398 main effect of time ($P < 0.001$) and treatment x time interactions ($P = 0.04$). At post-
399 SST ($P = 0.046$) and 1/3 TT ($P = 0.033$) glycerol was higher in YM vs PL. A large

correlation (r = 0.59, P = 0.0057; µM FFA ox/kg/min = 0.012 (±95%CI, 0.008) x [FFA] + 10.65 (±95%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; µM FFA ox/kg/min = 0.086 (±95%CI, 0.037) x [Glycerol] + 6.63 (±95%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% $\dot{V}O_{2max}$, 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

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

499 showed a higher power-output in YM during the last two-thirds of TTs, indicating
500 increased tolerance to fatigue. There were no differences in other physiological
501 parameters such as average or maximum heart-rate, RPE or gross efficiency
502 (measured during the submaximal test). Some of these results, in particular the pacing
503 strategy and increased power output with similar RPE, suggest a performance
504 improvement such as those observed for the effect of caffeine (36), but caffeine on its
505 own is an unlikely candidate for explaining our findings.

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

522 Instead, the ergogenic effects of YM could be explained through a synergism
523 between chlorogenic acids and caffeine in stimulating the central nervous system

524 (CNS). The effect of phenolic compounds on brain-specific COMT could diminish
525 the breakdown of dopamine and increase its bioavailability (10). As a consequence of
526 the increased CNS dopamine levels, doses of caffeine that would normally not be
527 ergogenic could have a potentiation effect (42) on the -already higher- dopamine
528 levels, and result ergogenic during exhaustive exercise (10, 42).

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

539 In conclusion, YM increased fat oxidation at low exercise intensities (30-50%
540 $\dot{V}O_{2max}$) and increased performance in a short (30 min) time-trial. While the
541 performance improvement in the time-trials were likely due to factors other than
542 increased fat utilization, enhanced fat oxidation by YM could potentially be of use for
543 manipulating substrate use during training in conditions of low carbohydrate
544 availability (44). The performance effect of YM should be addressed in more 'real
545 life' racing conditions including pre- and during-exercise CHO-rich nutrition. In the
546 meantime, our findings add to a growing body of information regarding the
547 importance of phytochemicals for performance and we provide valuable new

548 physiological and metabolic insights to understand the mechanisms behind the
549 response to YM.
550
551 **Acknowledgments.**
552 We want to thank Prof. Jostein Hallén for assistance during the preparation of the
553 study, Øyvind Skattebo for language assistance in ethics application process and Bent
554 Håvard Hellum from PROMEC (Norwegian University of Science and Technology)
555 for plasma caffeine and paraxanthine analyses.
556 The current study was internally funded by the department of physical performance of
557 the Norwegian School of Sport Sciences. All authors report no conflicts of interest.
558 The results of the study are presented clearly, honestly, and without fabrication,
559 falsification, or inappropriate data manipulation. The results of the present study do
560 not constitute endorsement by ACSM.
561

562

563 **References:**

564

565 1. Bracesco N, Sanchez AG, Contreras V, Menini T, Gugliucci A. Recent
566 advances on *Ilex paraguariensis* research: minireview. *J. Ethnopharmacol.*
567 2011;136(3):378-84.
568 2. Gambero A, Ribeiro ML. The positive effects of yerba mate (*Ilex*
569 *paraguariensis*) in obesity. *Nutrients*. 2015;7(2):730-50.
570 3. Arcari DP, Santos JC, Gambero A, Ferraz LF, Ribeiro ML. Modulatory
571 effects of yerba mate (*Ilex paraguariensis*) on the PI3K-AKT signaling
572 pathway. *Mol. Nutr. Food Res.* 2013;57(10):1882-5.
573 4. Gonzalez Arbelaez LF, Fantinelli JC, Ciocci Pardo A et al. Effect of an *Ilex*
574 *paraguariensis* (yerba mate) extract on infarct size in isolated rat hearts: the
575 mechanisms involved. *Food Funct.* 2015;7(2):816-24.
576 5. Heck CI, de Mejia EG. Yerba Mate Tea (*Ilex paraguariensis*): a
577 comprehensive review on chemistry, health implications, and technological
578 considerations. *J. Food Sci.* 2007;72(9):R138-51.
579 6. Martinet A, Hostettmann K, Schutz Y. Thermogenic effects of commercially
580 available plant preparations aimed at treating human obesity. *Phytomedicine*.
581 1999;6(4):231-8.
582 7. Alkhatib A. Yerba Mate (*Illex Paraguariensis*) ingestion augments fat
583 oxidation and energy expenditure during exercise at various submaximal
584 intensities. *Nutr. Metab. (Lond.)*. 2014;11:42.
585 8. Alkhatib A, Atcheson R. Effects of Yerba Maté ingestion on fat utilisation
586 during prolonged moderate intensity exercise. In. *The Nutrition Society*
587 *Summer Meeting* 2015, p E297.

588 9. Somerville V, Bringans C, Braakhuis A. Polyphenols and Performance: A
589 Systematic Review and Meta-Analysis. *Sports Med.* 2017:1-11.
590 10. Roelands B, Meeusen R. Alterations in Central Fatigue by Pharmacological
591 Manipulations of Neurotransmitters in Normal and High Ambient
592 Temperature. *Sports Med.* 2010;40(3):229-46.
593 11. Jatana N, Apoorva N, Malik S, Sharma A, Latha N. Inhibitors of catechol-O-
594 methyltransferase in the treatment of neurological disorders. *Cent. Nerv. Syst.*
595 *Agents Med. Chem.* 2013;13(3):166-94.
596 12. Burke LM. Re-Examining High-Fat Diets for Sports Performance: Did We
597 Call the 'Nail in the Coffin' Too Soon? *Sports Med.* 2015;45 Suppl 1:33-49.
598 13. Rowlands DS, Hopkins WG. Effects of high-fat and high-carbohydrate diets
599 on metabolism and performance in cycling. *Metabolism*. 2002;51(6):678-90.
600 14. Santos EC, Bicca MA, Blum-Silva CH et al. Anxiolytic-like, stimulant and
601 neuroprotective effects of *Ilex paraguariensis* extracts in mice. *Neuroscience*.
602 2015;292:13-21.
603 15. Hodgson AB, Randell RK, Jeukendrup AE. The effect of green tea extract on
604 fat oxidation at rest and during exercise: evidence of efficacy and proposed
605 mechanisms. *Adv. Nutr.* 2013;4(2):129-40.
606 16. Marx F, Janssens M, Urfer P, Scherer R. Caffeine and theobromine
607 composition of mate (*Ilex paraguariensis*) leaves in five plantations of
608 Misiones, Argentina. *Plant Foods Hum. Nutr.* 2003;58(3):1-8.
609 17. Hawley JA, Noakes TD. Peak power output predicts maximal oxygen uptake
610 and performance time in trained cyclists. *Eur. J. Appl. Physiol.* 1992;65(1):79-
611 83.

612 18. Harwood M, Danielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams
613 GM, Lines TC. A critical review of the data related to the safety of quercetin
614 and lack of evidence of in vivo toxicity, including lack of
615 genotoxic/carcinogenic properties. *Food Chem. Toxicol.* 2007;45(11):2179-
616 205.
617 19. Davis JM, Carlstedt CJ, Chen S, Carmichael MD, Murphy EA. The dietary
618 flavonoid quercetin increases VO(2max) and endurance capacity. *Int. J. Sport*
619 *Nutr. Exerc. Metab.* 2010;20(1):56-62.
620 20. Torrens SM, Areta JL, Parr EV, Hawley JA. Carbohydrate dependence during
621 prolonged cycling time-trials. *Eur. J. Appl. Physiol.* 2016;(electronic
622 publication ahead of print).
623 21. Pfeiffer B, Cotterill A, Grathwohl D, Stellingwerff T, Jeukendrup AE. The
624 effect of carbohydrate gels on gastrointestinal tolerance during a 16-km run.
625 *Int. J. Sport Nutr.* 2009;19(5):485.
626 22. Peronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update.
627 *Can. J. Sport Sci.* 1991;16(1):23-9.
628 23. Moseley L, Jeukendrup AE. The reliability of cycling efficiency. *Med. Sci.*
629 *Sports Exerc.* 2001;33(4):621-7.
630 24. Stisen AB, Stougaard O, Langfort J, Helge JW, Sahlin K, Madsen K. Maximal
631 fat oxidation rates in endurance trained and untrained women. *Eur. J. Appl.*
632 *Physiol.* 2006;98(5):497-506.
633 25. Thu OK, Spigset O, Nilsen OG, Hellum B. Effect of commercial Rhodiola
634 rosea on CYP enzyme activity in humans. *Eur. J. Appl. Physiol.*
635 2016;72(3):295-300.

636 26. Hackett J, Telepchak MJ, Coyer MJ. Analysis of total caffeine and other
637 xanthines in specialty coffees using mixed mode solid-phase extraction and
638 liquid chromatography-diode-array detection after microwave digestion. *J*
639 *Anal Toxicol*. 2008;32(8):695-701.
640 27. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with
641 Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of*
642 *Enology and Viticulture*. 1965;16(3):144-58.
643 28. Wangensteen H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts
644 from coriander. *Food Chemistry*. 2004;88(2):293-7.
645 29. Hopkins WG. A Spreadsheet for Analysis of Straightforward Controlled
646 Trials. *Sportscience*. 2003;7.
647 30. Rowlands DS. Model for the behaviour of compartmental CO2 stores during
648 incremental exercise. *Eur. J. Appl. Physiol.* 2005;93(5):555-68.
649 31. Montain SJ, Hopper M, Coggan AR, Coyle EF. Exercise metabolism at
650 different time intervals after a meal. *J. Appl. Physiol.* 1991;70(2):882-8.
651 32. Bonadonna RC, Groop LC, Zych K, Shank M, DeFronzo RA. Dose-dependent
652 effect of insulin on plasma free fatty acid turnover and oxidation in humans.
653 *Am. J. Physiol. Endocrinol. Metab.* 1990;259(5):E736-E50.
654 33. Jentjens R, Cale C, Gutch C, Jeukendrup A. Effects of pre-exercise ingestion
655 of differing amounts of carbohydrate on subsequent metabolism and cycling
656 performance. *Eur. J. Appl. Physiol.* 2003;88(4-5):444-52.
657 34. Martin-Du Pan R, Mauron C, Glaeser B, Wurtman R. Effect of various oral
658 glucose doses on plasma neutral amino acid levels. *Metabolism*.
659 1982;31(9):937-43.

660 35. Achten J, Jeukendrup AE. The effect of pre-exercise carbohydrate feedings on
661 the intensity that elicits maximal fat oxidation. *J. Sports Sci.*
662 2003;21(12):1017-25.
663 36. Stadheim HK, Kvamme B, Olsen R, Drevon CA, Ivy JL, Jensen J. Caffeine
664 increases performance in cross-country double-poling time trial exercise. *Med.*
665 *Sci. Sports Exerc.* 2013;45(11):2175-83.
666 37. Spriet LL. Exercise and sport performance with low doses of caffeine. *Sports*
667 *Med.* 2014;44(2):175-84.
668 38. Jenkins NT, Trilk JL, Singhal A, O'Connor PJ, Cureton KJ. Ergogenic effects
669 of low doses of caffeine on cycling performance. *Int. J. Sport Nutr. Exerc.*
670 *Metab.* 2008;18(3):328.
671 39. Desbrow B, Barrett CM, Minahan CL, Grant GD, Leveritt MD. Caffeine,
672 cycling performance, and exogenous CHO oxidation: a dose-response study.
673 *Med. Sci. Sports Exerc.* 2009;41(9):1744-51.
674 40. Ryan EJ, Kim C-H, Muller MD et al. Low-dose caffeine administered in
675 chewing gum does not enhance cycling to exhaustion. *J. Strength Cond. Res.*
676 2012;26(3):844-50.
677 41. Doherty M, Smith P. Effects of caffeine ingestion on rating of perceived
678 exertion during and after exercise: a meta‐analysis. *Scand. J. Med. Sci.*
679 *Sports*. 2005;15(2):69-78.
680 42. Solinas M, Ferré S, You Z-B, Karcz-Kubicha M, Popoli P, Goldberg SR.
681 Caffeine Induces Dopamine and Glutamate Release in the Shell of the Nucleus
682 Accumbens. *J. Neurosci.* 2002;22(15):6321-4.

683 43. Frandsen U, Lopez-Figueroa M, Hellsten Y. Localization of nitric oxide
684 synthase in human skeletal muscle. *Biochem. Biophys. Res. Commun.*
685 1996;227(1):88-93.
686 44. Lane SC, Areta JL, Bird SR et al. Caffeine ingestion and cycling power output
687 in a low or normal muscle glycogen state. *Med. Sci. Sports Exerc.*
688 2013;45(8):1577-84.
689
690

691 **FIGURE CAPTIONS**

692

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

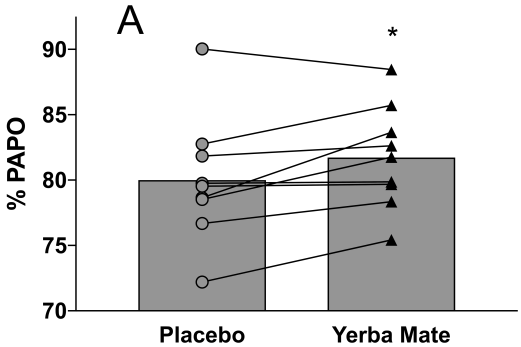
703

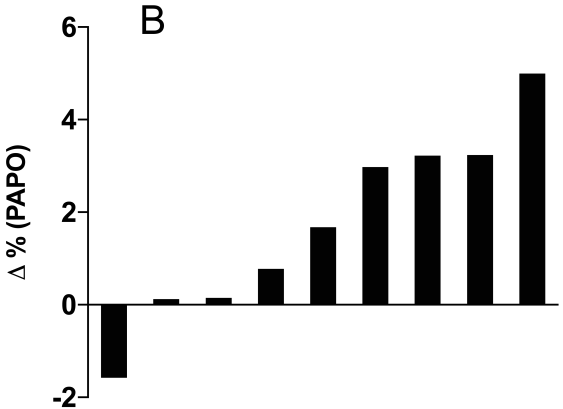
704 **Figure 2.** Performance time-trial test results showing average % PAPO (columns) and
705 individual responses (dots and lines; A), individual differences in performance as
706 delta % PAPO (B), pacing in both groups (C) and rates of perceived exertion at
707 different time-points (D). Data in C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

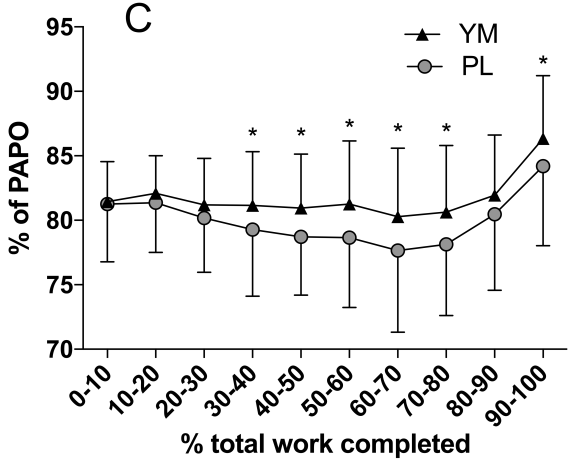
712

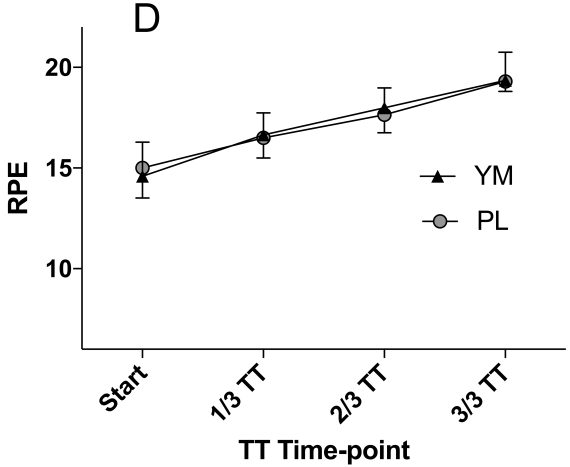
713 **Figure 3.** Respiratory responses during submaximal step test (SST; A and B) and
714 during simulated Time Trials (TTs; C and D). Calculated fat utilization (A) and
715 efficiency (B) during submaximal step-tests at power to elicit 30, 40, 50, 60, 70 and

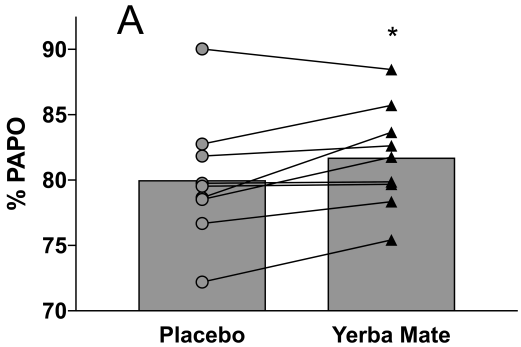
716 80% of $\dot{V}O_{2max}$. TTs oxygen consumption as % of $\dot{V}O_{2max}$ (A), respiratory exchange
717 ratio (B) and heart rate (C). Data are Means ± SD (n = 11, SST, n = TTs)). *
718 Significantly different from PL at same point ($p<0.05$). Data were analyzed using
719 two-way repeated measures ANOVA with Student–Newman–Keuls post hoc analysis.
720 PL, Placebo; YM, Yerba Mate.

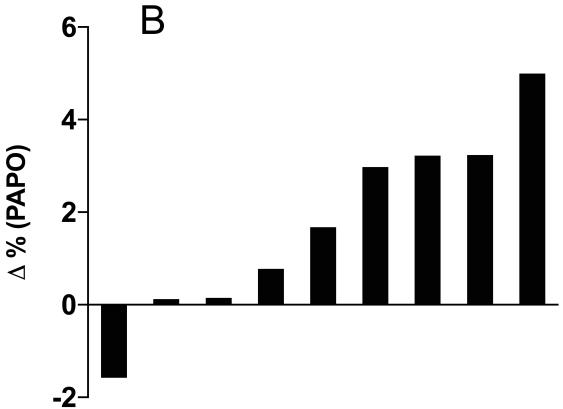

A
% PAPO
90
85
80
75
70
*
Placebo
Yerba Mate

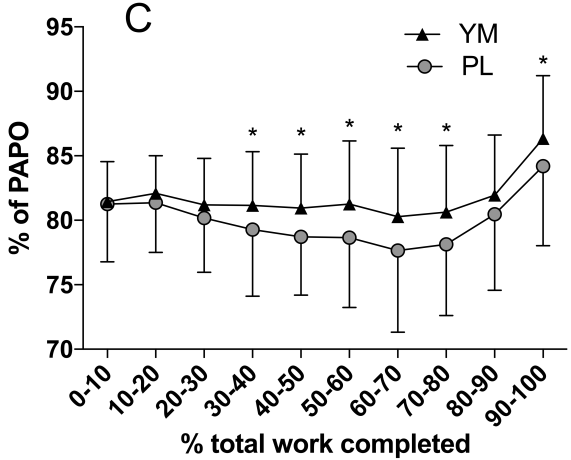

B
Δ % (PAPO)
6
4
2
0
-2

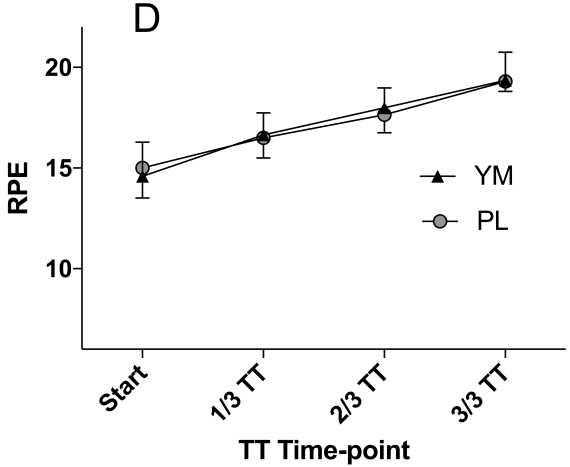

C
YM
PL
% of PAPO
95
90
85
80
75
70
*
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
% total work completed

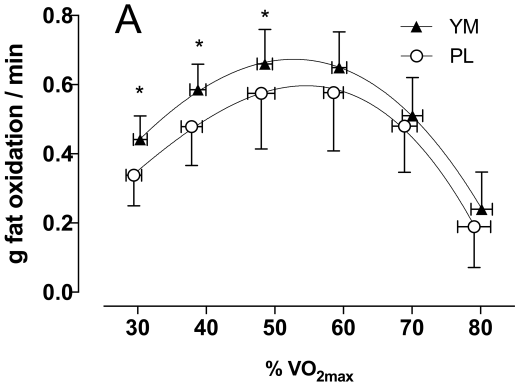

D
RPE
20
15
10
YM
PL
Start
1/3 TT
2/3 TT
3/3 TT
TT Time-point

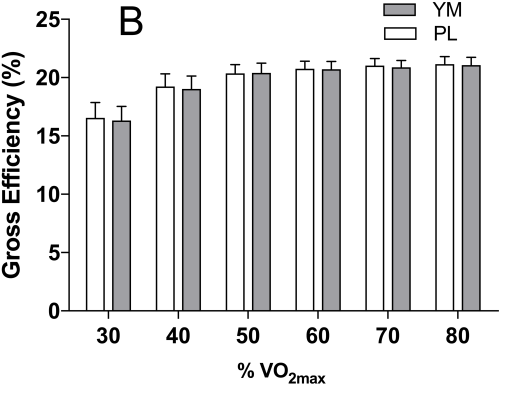

A
% PAPO
90
85
80
75
70
*
Placebo
Yerba Mate

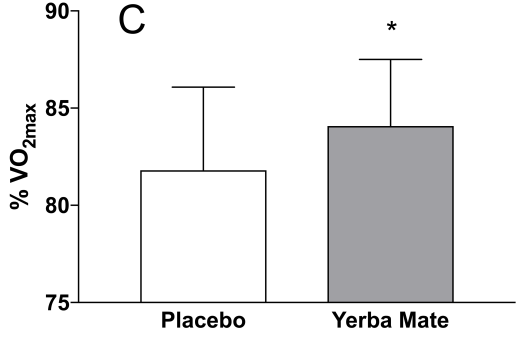

B
Δ % (PAPO)
6
4
2
0
-2

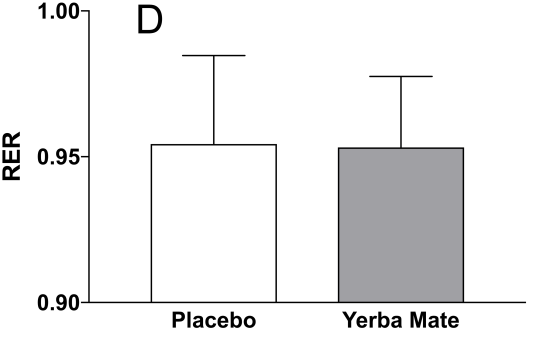

C
YM
PL
% of PAPO
95
90
85
80
75
70
*
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
% total work completed


D
RPE
20
15
10
YM
PL
Start
1/3 TT
2/3 TT
3/3 TT
TT Time-point


A
YM
PL
g fat oxidation / min
0.0
0.2
0.4
0.6
0.8
30
40
50
60
70
80
% VO2max
*
*
*


B
YM
PL
Gross Efficiency (%)
0
5
10
15
20
25
30
40
50
60
70
80
% VO2max


C
% VO2max
75
80
85
90
Placebo
Yerba Mate
*


D
RER
0.90
0.95
1.00
Placebo
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.

	Taragui	La Merced	Rosamonte	Guayaki
Total Phenols (% of average)	87	107	97	110
Flavonoids (% of average)	81	108	103	108
Scavenging activity (% scavenged)	72	81	72	84
Caffeine (% plant material)	0.93	1.12	1.09	1.14

Supplementary Table 2. Origin details of Yerba Mate brands utilised in this study.

Brand	**Producer (Farm)**	**Variety**	**Country of Origin**	**Lot number**
Taragui	Las Marias	Regular Blend	Argentina	L 21015 53234 12:04
La Merced	Las Marias	de campo	Argentina	L 24515 09:10 A 3370
Rosamonte	Diez Hermanos	Regular Blend	Argentina	9215 RP 18:20
Guayaquí	Guayaquí	Biodynamic	Argentina	Not provided