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The effect of astaxanthin supplementation on performance and fat oxidation during a 40 km cycling time trial
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1   **The effect of astaxanthin supplementation on performance and fat oxidation during a 40 km**

2   **cycling time trial**

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20 **Abstract**

21 **Objectives**

22 This study aimed to investigate whether supplementation with 12 mg·day^{-1} astaxanthin for 7 days can

23 improve exercise performance and metabolism during a 40 km cycling time trial.

24 **Design**

25 A randomised, double-blind, crossover design was employed.

26 **Methods**

27 Twelve recreationally trained male cyclists (VO$_{2peak}$: 56.5 ± 5.5 mL·kg^{-1}·min^{-1}, W$_{max}$: 346.8 ± 38.4 W)

28 were recruited. Prior to each experimental trial, participants were supplemented with either 12 mg·day^{-1}

29 astaxanthin or an appearance-matched placebo for 7 days (separated by 14 days of washout). On day 7

30 of supplementation, participants completed a 40 km cycling time trial on a cycle ergometer, with indices

31 of exercise metabolism measured throughout.

32 **Results**

33 Time to complete the 40 km cycling time trial was improved by 1.2 ± 1.7% following astaxanthin

34 supplementation, from 70.76 ± 3.93 min in the placebo condition to 69.90 ± 3.78 min in the astaxanthin

35 condition (mean improvement = 51 ± 71 s, p = 0.029, g = 0.21). Whole-body fat oxidation rates were

36 also greater (+0.09 ± 0.13 g·min^{-1}, p = 0.044, g = 0.52), and the respiratory exchange ratio lower (-0.03

37 ± 0.04, p = 0.024, g = 0.60) between 39-40 km in the astaxanthin condition.

38 **Conclusion**

39 Supplementation with 12 mg·day^{-1} astaxanthin for 7 days provided an ergogenic benefit to 40 km

40 cycling time trial performance in recreationally trained male cyclists and enhanced whole-body fat

41 oxidation rates in the final stages of this endurance-type performance event.

42 **Key words**

43 Antioxidants; Dietary Supplements; Substrate Utilisation; Sports Performance; Sports Nutrition

Introduction

Dietary supplementation strategies that can modify substrate utilisation patterns during exercise have received widespread attention in the literature [1-3]. One such supplement is astaxanthin, a liposoluble carotenoid usually supplemented through the intake of *Haematococcus pluvialis*-derived antioxidant products. Based upon research on mice, improvements in endurance performance are reported following 3-5 weeks of astaxanthin intake [4-6]. This is attributed to the potential for astaxanthin to protect and upregulate key metabolic enzymes, such as carnitine palmitoyltransferase 1 (CPT1) and 5'adenosine monophosphate-activated protein kinase (AMPK), that are implicated in the oxidation of fatty acids as a viable energy source [5,6].

A similar ergogenic benefit was reported in trained cyclists, with 4 weeks of 4 mg·day^{-1} astaxanthin improving 20 km cycling time trial (TT) performance when compared to a placebo (mean improvement (MI) = astaxanthin: 121 s (5.1%) vs. placebo: 18 s (0.8%)) [7]. Conversely, in a 1.0 h cycling TT an ergogenic benefit was not reported following a 4 week supplementation with either 20 mg·day^{-1} astaxanthin (MI = 74 s (2.1%)) or a placebo (MI = 52 s (1.4%)) in trained cyclists or triathletes [8]. Interestingly, astaxanthin did not influence measures of substrate utilisation obtained in either study [7,8]. The absence of a metabolic effect may be explained by the use of a parallel group design in both studies [7,8], with substrate utilisation rates known to vary considerably between individuals of a similar fitness demographic, even at the same absolute and relative exercise intensities [9,10].

A 3-5 week supplementation strategy is seemingly advocated in mice-models when seeking to elicit the ergogenic potential of astaxanthin [4-6]. In research on humans, one key methodological consistency to that of animal studies is the 3-5 week supplementation strategy implemented [7,8]. Plasma astaxanthin concentrations are, however, reported to peak within the first week of intake, even when consumption is chronic. Rüfer et al. [11], for example, quantified the uptake of ~ 1.25 mg·day^{-1} astaxanthin in the plasma of 28 healthy males over a 4 week period and reported a peak in concentration following 6 days of intake [11]. This finding enables shorter supplementation periods to be advocated, which in turn may allow the use of a randomised crossover design.

70   As such, the current study implements a 7 day supplementation period to ensure that participants could

71   act as their own control, mitigating the potential impact inter-individual differences could have upon

72   the outcome variable [12,13]. A 40 km cycling TT was used as a reliable measure of endurance performance

73   obtained during a distance that is common in competitive cycling events [14–16]. Therefore, the aim of the

74   current study was to investigate whether supplementation with 12 mg·day^{-1} astaxanthin for 7 days can

75   improve exercise performance and metabolism during a 40 km cycling TT using a randomised crossover

76   design. It was hypothesised that astaxanthin supplementation would improve cycling TT performance,

77   an ergogenic effect underpinned by the ability of astaxanthin to enhance fat oxidation during exercise.

78   **Methods**

79   Twelve recreationally trained male cyclists (age: 27.5 ± 5.7 years, height: 1.78 ± 0.07 m, body mass:

80   78.3 ± 7.6 kg, body fat: 13.7 ± 2.6%, VO_{2peak}: 56.5 ± 5.5 mL·kg^{-1}·min^{-1}, W_{max}: 346.8 ± 38.4 W)

81   volunteered to participate in the study, with prior ethical approval attained from the institutional ethics

82   committee (SPA-REC-2017-323). The term "recreationally trained cyclist" was deemed most

83   appropriate for the sample recruited, as although performance criteria for a "trained cyclist" was met

84   (VO_{2peak}: 55-64.9 mL·kg^{-1}·min^{-1}; W_{max}: 320-379 W), training load criteria was not (distance covered:

85   60-290 km·week^{-1}; cycling frequency ≥ 3 times·week^{-1}) [17].

86   Supplementation with additional antioxidants/vitamins was not permitted alongside those provided in

87   the current study, with a list of astaxanthin-rich foods to avoid also provided to limit the additional

88   dietary intake of astaxanthin. Participants refrained from strenuous exercise and the consumption of

89   alcohol and caffeine in the 24 h preceding each visit [18,19]. Habitual dietary intake was maintained;

90   however, participants entered the laboratory in a 4 h postprandial state, except for the ingestion of water

91   to ensure euhydration. Compliance with the above procedures was checked via 24 h dietary recall, with

92   dietary intake replicated prior to each trial. All participants visited the laboratory (temperature: 18.0 ±

93   1.2 °C; pressure: 754.4 ± 8.8 mmHg, humidity 44.7 ± 3.5%) on four occasions (two preliminary trials

94   and two experimental trials) at a similar time of day (± 1.0 h). A randomised, double-blind, crossover

95   design was employed.

During the first preliminary visit participants completed a graded exercise test to volitional exhaustion using an electromagnetically-braked cycle ergometer (Lode Excalibur Sport, The Netherlands). The test commenced at 75.0 W, increasing by 30.0 W every 1 min until volitional exhaustion. Breath-by-breath expired air was collected for VO_{2peak} determination and was defined as the highest 30 s average of VO_2 recorded during the test. A full familiarisation with the 40 km TT was then undertaken during a second preliminary visit to ensure participants were accustomed to procedures employed during each experimental trial.

Prior to each experimental trial, participants supplemented with one of two randomly assigned supplements for 7 days, with supplementation separated by a 14-day washout period. Estimations were made based upon calculations that > 99.9% of a treatment is eliminated after a time period equivalent to 10 half-lives [20]. Using a half-life of 15.9 ± 5.3 h [21], it was estimated that > 99.9% of total astaxanthin consumed would be eliminated following ~ 7 days of washout. As this was an estimation, a more conservative 14-day washout period was decided upon in the current study. Supplementation consisted of either 12 mg·day^{-1} astaxanthin (AstaReal®, Sweden) or an appearance-matched placebo with no viable constituents (AstaReal®, Sweden). Participants ingested two capsules daily (one morning and one evening), with compliance ensured via daily text message reminders and a pill count post-ingestion. To ensure the study remained double-blind, each supplement was assigned a randomised alphanumerical code until after data analysis was complete.

Each experimental trial required participants to undertake a 5 min warm-up before completing a 40 km TT on a Velotron Racermate™ cycle ergometer (Velotron, USA). Preferred frame geometry was selected and replicated between trials. Information regarding cadence, gear and distance covered was received, with no other information or external encouragement provided. Participants were permitted to drink water *ad libitum* during the first experimental trial, with the volume of water consumed recorded and kept constant during the second experimental trial. Time to complete and mean power were recorded for both the total distance and for each 10 km quartile during the TT. Heart rate (HR), ratings of fatigue (ROF) [22] and ratings of perceived exertion (RPE) [23] for the whole-body (RPE_O) and the lower limbs (RPE_L) were measured every 10 km. A finger prick capillary blood sample was taken

123   at rest and every 10 km during the TT to determine blood lactate (Lactate Pro 2, Japan), glucose

124   (Hemocue, Sweden) and triglycerides (Reflotron, USA). Breath-by-breath expired air was obtained

125   during the 10th, 20th, 30th and 40th km of the TT. Respiratory gas data were then used to calculate whole-

126   body fat and carbohydrate oxidation rates (FATox and CHox, respectively) using the method of

127   Jeukendrup and Wallis [24].

128   As assumptions of normality and homogeneity were met, a paired *t*-test was used to compare differences

129   in performance time and mean power between conditions, and to determine whether a trial order effect

130   was present. A two-way [condition x time] analysis of variance (ANOVA) was used to determine

131   differences in performance, respiratory and perceptual variables, blood metabolites and HR. *Post-hoc*

132   analysis was performed with a Bonferroni adjustment. Effect sizes were calculated using Hedge's *g* and

133   were interpreted as trivial (< 0.20), small (0.20-0.49), moderate (0.50-0.79) or large (≥ 0.80) [25].

134   Confidence intervals (CI) ($\pm 95.0\%$) were also calculated and are reported where necessary. Descriptive

135   data are displayed as mean $\pm$ standard deviation (SD). Statistical analysis was conducted using a

136   statistical software package (SPSS, Version 25, USA), with significance accepted at $p < 0.05$.

137   **Results**

138   Time to complete the 40 km TT (Figure 1a) was improved from 70.76 ± 3.93 min in the placebo

139   condition to 69.90 ± 3.78 min in the astaxanthin condition, which equates to a $1.2 \pm 1.7\%$ improvement

140   (MI = 51 ± 71 s, 95.0% CI = 6-96 s, $p = 0.029$, $g = 0.21$). Mean power (Figure 1c) was also improved

141   from 213.8 ± 29.0 W in the placebo condition to 219.9 ± 28.7 W in the astaxanthin condition, which

142   equates to a $2.8 \pm 4.1\%$ improvement (MI = 6.1 ± 9.5 W, 95.0% CI = 0.1-12.1 W, $p = 0.047$, $g = 0.20$).

143   No trial order was present for performance time ($p = 0.993$, $g = 0.04$) or mean power ($p = 0.996$, $g = $

144   0.02). There was also no [condition x time] interaction observed across each 10 km quartile for either

145   performance time ($p = 0.158$; Figure 1b) or mean power ($p = 0.242$; Figure 1d), suggesting that the

146   general pacing profile of the 40 km TT was similar across conditions.

147   A [condition x time] interaction was observed for FATox ($p = 0.037$), whereby FATox was greater

148   between 39-40 km following astaxanthin supplementation (Figure 2c), increasing from 0.13 ± 0.04

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149    $g \cdot min^{-1}$ in the placebo condition to 0.22 ± 0.05 $g \cdot min^{-1}$ in the astaxanthin condition (+0.09 ± 0.13

150    $g \cdot min^{-1}$, 95.0% CI = 0.00-0.17 $g \cdot min^{-1}$, p = 0.044, g = 0.52). A similar [condition x time] interaction

151   was also observed for the respiratory exchange ratio (RER) (p = 0.007), whereby RER was lower

152   between 39-40 km following astaxanthin supplementation (Figure 2a), decreasing from 0.99 ± 0.02 in

153   the placebo condition to 0.96 ± 0.01 in the astaxanthin condition (-0.03 ± 0.04, 95.0% CI = -0.01 to -

154   0.06, p = 0.024, g = 0.60). For CHox a [condition x time] interaction was present (p = 0.037), with

155   CHox greater at 39-40 km in both conditions (p < 0.045). There were, however, no differences reported

156   between conditions for CHox at any time point during the TT (p ≥ 0.118; Figure 2e).

157   Lactate (Figure 2b) was increased above baseline throughout the TT (p ≤ 0.001) and was greater at 40

158   km compared to 30 km (p = 0.002). Glucose (Figure 2f) was lower throughout the TT when compared

159   to baseline (p ≤ 0.003), and triglycerides (Figure 2d) were increased above baseline at 30 km (p = 0.027)

160   and 40 km (p = 0.002), as well as being greater at 40 km than at any other time point (p ≤ 0.003). There

161   were no differences between conditions for each of these blood metabolites (p ≥ 0.346).

162   Ratings of fatigue (p < 0.001), RPE_O (p < 0.001) and RPE_L (p < 0.001) all increased progressively over

163   time with no effect of condition (p ≥ 0.131). A main effect of time was also present for HR (p < 0.001)

164   and VO_2 (p < 0.001) in both conditions (p ≥ 0.338), with HR greater at 40 km than at each previous time

165   point (p ≤ 0.001) and VO_2 greater at 30 km than at 20 km (p = 0.029) and at 40 km when compared to

166   each previous time point (p ≤ 0.002) (Table 1).

167   **Discussion**

168   The current investigation is the first to demonstrate an increase in whole-body fat oxidation (FATox)

169   and a corresponding reduction in RER during endurance exercise in humans supplementing with

170   astaxanthin. This study also reports a small, yet significant, ergogenic benefit from 12 $mg \cdot day^{-1}$

171   astaxanthin supplementation for 7 days in recreationally trained male cyclists completing a 40 km

172   cycling TT. This equates to a mean 51 s (1.2%) time improvement when compared to the placebo.

173   The performance findings of this study are, therefore, consistent with those reported by Earnest et al. [7],

174   as 4 weeks of 4 $mg \cdot day^{-1}$ astaxanthin improved 20 km cycling TT performance in trained male cyclists

175   [7]. Furthermore, the 121 s time improvement (5.1%) reported in the astaxanthin group was greater than

176   the corresponding 18 s improvement (0.8%) reported in the placebo, suggesting a treatment effect was

177   present [7]. In contrast, an ergogenic benefit was not reported during a 1.0 h cycling TT in trained male

178   cyclists or triathletes following 4 weeks of supplementation with either 20 mg·day^{-1} astaxanthin (MI =

179   74 s (2.1%)) or a placebo (MI = 52 s (1.4%)) [8]. Although there is no clear explanation for the disparity

180   between the two studies [7,8], neither Earnest et al. [7] nor Res et al. [8] reported differences in substrate

181   utilisation during exercise. Four weeks of 4 mg·day^{-1} astaxanthin supplementation, for example, did not

182   influence measures of RER, CHox or FATox obtained during a 2 h submaximal cycle at 5.0% below

183   the lactate threshold [7]. Likewise, 20 mg·day^{-1} astaxanthin for 4 weeks did not influence measures of

184   RER, CHox or FATox obtained during the completion of a 1.0 h steady-state cycle at 50.0% W_{max} [8]. As

185   such, the increase in FATox and the decrease in RER reported in the latter stages of exercise in the

186   current study are in contrast with previous research [7,8].

187   The shorter 7-day supplementation strategy implemented in the current study, which enabled the use of

188   a randomised crossover design, may provide a methodological insight as to why a metabolic effect of

189   astaxanthin has been observed. In previous research the application of a prolonged supplementation

190   strategy has required the use of a parallel group design [7,8]. A major strength of the current study is,

191   therefore, the ability to implement a randomised crossover design as this enabled each participant to act

192   as their own control, minimising the potential impact subtle differences in participant characteristics

193   and individual responses to astaxanthin could have upon the outcome variable [12,13]. This would have

194   improved the statistical power of the study and may have increased the ability to detect subtle

195   differences in substrate utilisation during exercise.

196   The current study also measured substrate utilisation during the completion of an ecologically valid

197   performance event and not during a single-intensity, steady-state preload [7,8]. Therefore, the metabolic

198   measures obtained during the 40 km TT may have more accurately reflected the ergogenic mechanism

199   by which astaxanthin is purported to improve performance during self-paced, best effort endurance

200   events. Conversely, the change in FATox and RER reported between 39-40 km may be attributable to

201   an increased utilisation of carbohydrates in the placebo condition, with a seemingly greater increase in

power (+6.6%) observed from 20-30 km to 30-40 km when compared to the astaxanthin condition (+3.0%). No differences were, however, reported in the general pacing profile of the TT between conditions, with indices of CHox, blood glucose and/or lactate also not different between conditions at any time point. Furthermore, the reported change in FATox between 39-40 km also occurred at the same relative exercise intensity (astaxanthin: 46.3 ± 8.6 mL·kg^{-1}·min^{-1} vs. placebo: 45.2 ± 7.4 mL·kg^{-1}·min^{-1}), providing further evidence to an increased utilisation of fat at this time point.

Possible explanations for the metabolic effect of astaxanthin are received from previous exploratory research. Astaxanthin, for example, accumulates in the mitochondrial membrane following consumption where it is suggested to indirectly enhance FATox through protecting CPT1 from oxidative modifications during exercise [5,26,27]. The expression of AMPK is also reported to be upregulated following astaxanthin intake [6]. As a key enzyme in skeletal muscle metabolism, AMPK is implicated in the stimulation of fatty acid oxidation; the transportation of fatty acids into the mitochondria, potentially through the intercalation of CPT1 and fatty acid translocase/CD36; as well as the upregulation of transcription factors, such as peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), that are known to promote mitochondrial biogenesis and control mitochondrial oxidative capacity [28]. As this mechanistic insight is exclusively from mice-models, future exploratory research is necessary to elucidate similar mechanistic information in exercising humans.

Finally, as astaxanthin uptake was not quantified in the current investigation, the 7 day supplementation strategy was informed by previous literature [11]. Nevertheless, an ergogenic and metabolic effect of astaxanthin was demonstrated following this 7-day strategy, thus an exploration of the human pharmacokinetics of astaxanthin is clearly required so that an optimal supplementation strategy can be designed and implemented for future practice within this research area. Another potential limitation is that intra-individual variation in performance was also inferred from previous literature that investigated the reproducibility of the 40 km TT in trained cyclists $(0.9 \pm 0.7\%)$ [15]. Although greater intra-individual variations of 3.4% are reported following repeated TTs of a similar duration (~ 1.0 h) [29], it should be noted that caution is suggested when comparing pacing and performance between time- and distance-based TTs [30]. As such, the intra-individual variation of $0.9 \pm 0.7\%$ may be more appropriate for the

current study. To ensure that changes in performance ($1.2 \pm 1.7\%$ in the current study) can be confirmed as meaningful, future research should seek to calculate intra-individual variation within the actual sample recruited.

Conclusion

Supplementation with 12 mg·day^{-1} astaxanthin for 7 days provided an ergogenic benefit to 40 km cycling TT performance in recreationally trained male cyclists and enhanced whole-body fat oxidation in the final stages of this endurance-type performance event. Future research should seek to determine an optimal supplementation strategy for astaxanthin intake based on pharmacokinetics, while exploring the underlying mechanistic factors by which astaxanthin is purported to exert its ergogenic effect in exercising humans.

Practical Implications

- The ergogenic potential of astaxanthin may be elicited following a shorter duration intake than previously advocated.
- The outcomes of this study suggest that 12 mg·day^{-1} astaxanthin may provide an ergogenic benefit and promote fat oxidation during endurance-type cycling TTs.
- To enable the successful application of astaxanthin in sport nutrition future investigations should aim to determine an optimal supplementation strategy for astaxanthin intake in exercising humans.

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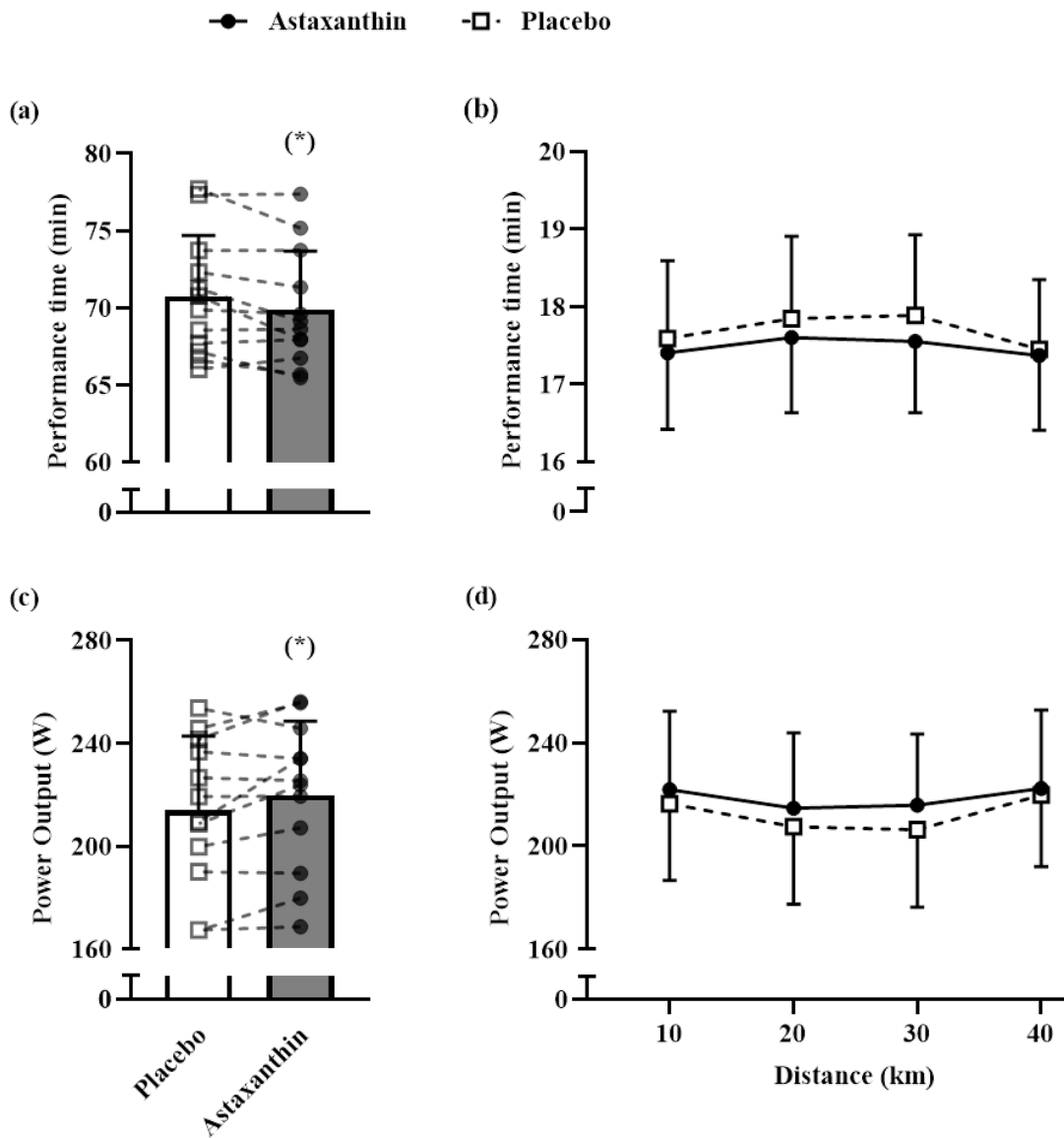
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315 **Tables**

316 **Table 1.** Mean ± SD. Physiological and perceptual results. δ denotes a significant difference to the previous time point, † denotes a significant difference to all

317 previous time points ($p < 0.05$).

Variable	Astaxanthin				Placebo			
	10 km	20 km	30 km	40 km	10 km	20 km	30 km	40 km
VO_2 ($mL·kg^{-1}·min^{-1}$)	41.0 ± 7.6	40.1 ± 7.6	40.6 ± 8.2 [δ]	46.3 ± 8.6 [†]	39.6 ± 5.8	39.2 ± 6.9	41.1 ± 6.6 [δ]	45.2 ± 7.4 [†]
HR ($beats·min^{-1}$)	153 ± 10	155 ± 9	156 ± 10	171 ± 10 [†]	153 ± 13	154 ± 11	156 ± 11	171 ± 9 [†]
ROF	3.7 ± 1.5	5.2 ± 1.3 [δ]	6.6 ± 1.3 [δ]	8.1 ± 1.6 [†]	3.2 ± 1.2	5.0 ± 1.5 [δ]	5.8 ± 1.7 [δ]	7.6 ± 1.8 [†]
RPE_O	13.8 ± 1.4	14.9 ± 1.4 [δ]	16.3 ± 1.4 [δ]	18.1 ± 1.3 [†]	13.3 ± 1.7	14.8 ± 1.5 [δ]	16.2 ± 1.5 [δ]	18.3 ± 1.8 [†]
RPE_L	14.9 ± 1.7	16.0 ± 1.2 [δ]	17.3 ± 1.2 [δ]	18.8 ± 0.8 [†]	14.8 ± 1.9	16.3 ± 1.5 [δ]	17.0 ± 1.3 [δ]	18.8 ± 0.8 [†]

318

321   **Figure 1.** Mean ± SD. Individual values for performance time (a) and power output (c) during the 40
322   km time trial following each condition. Data for 10 km quartile performance times (b) and power
323   outputs (d) are also displayed as mean (± SD) for each condition. * denotes a significant difference
324   between conditions ($p < 0.05$).
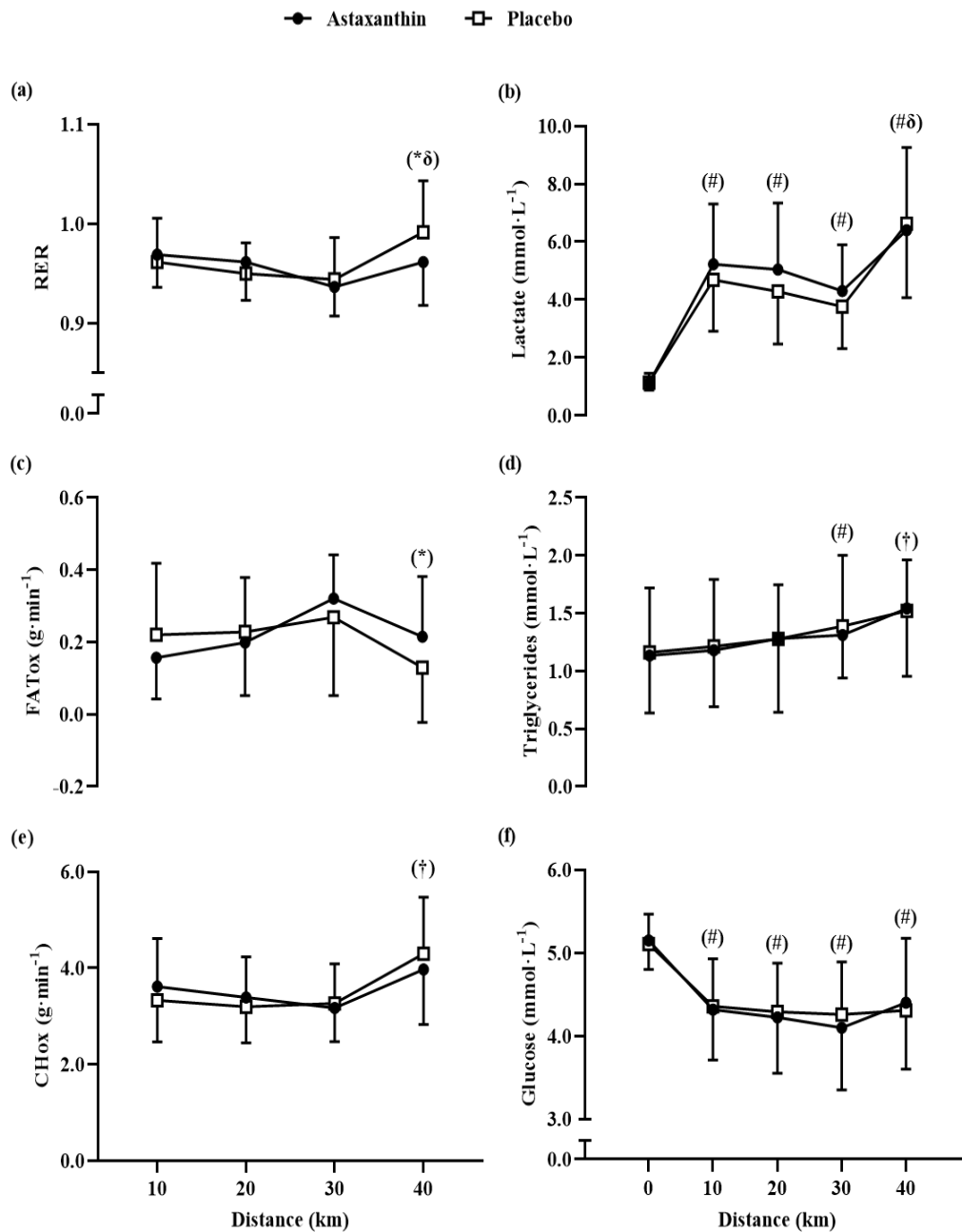
Figure 2. Mean ± SD. Respiratory measures of the respiratory exchange ratio (RER) (a), whole-body fat oxidation rates (FATox) (c), whole-body carbohydrate oxidation rates (CHox) (e), and blood metabolites lactate (b), triglycerides (d) and glucose (f) obtained over the duration of each 40 km time trial. * denotes a significant difference between conditions, # denotes a significant difference to baseline, δ denotes a significant difference to the previous time point, † denotes significant difference to all previous time points ($p < 0.05$).

333 **Acknowledgements**