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

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Word count (excluding abstract and references): 2914

Abstract word count: 234

Number of Tables: 1

Number of Figures: 2

Abstract

Objectives

This study aimed to investigate whether supplementation with 12 mg·day^{-1} astaxanthin for 7 days can improve exercise performance and metabolism during a 40 km cycling time trial.

Design

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

Methods

Twelve recreationally trained male cyclists (VO_{2peak}: 56.5 ± 5.5 mL·kg^{-1}·min^{-1}, W_{max}: 346.8 ± 38.4 W) were recruited. Prior to each experimental trial, participants were supplemented with either 12 mg·day^{-1} astaxanthin or an appearance-matched placebo for 7 days (separated by 14 days of washout). On day 7 of supplementation, participants completed a 40 km cycling time trial on a cycle ergometer, with indices of exercise metabolism measured throughout.

Results

Time to complete the 40 km cycling time trial was improved by 1.2 ± 1.7% following astaxanthin supplementation, from 70.76 ± 3.93 min in the placebo condition to 69.90 ± 3.78 min in the astaxanthin condition (mean improvement = 51 ± 71 s, p = 0.029, *g* = 0.21). Whole-body fat oxidation rates were also greater (+0.09 ± 0.13 g·min^{-1}, p = 0.044, *g* = 0.52), and the respiratory exchange ratio lower (-0.03 ± 0.04, p = 0.024, *g* = 0.60) between 39-40 km in the astaxanthin condition.

Conclusion

Supplementation with 12 mg·day^{-1} astaxanthin for 7 days provided an ergogenic benefit to 40 km cycling time trial performance in recreationally trained male cyclists and enhanced whole-body fat oxidation rates in the final stages of this endurance-type performance event.

Key words

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.

As such, the current study implements a 7 day supplementation period to ensure that participants could act as their own control, mitigating the potential impact inter-individual differences could have upon the outcome variable [12,13]. A 40 km cycling TT was used as a reliable measure of endurance performance obtained during a distance that is common in competitive cycling events [14–16]. Therefore, the aim of the current study was to investigate whether supplementation with 12 mg·day^{-1} astaxanthin for 7 days can improve exercise performance and metabolism during a 40 km cycling TT using a randomised crossover design. It was hypothesised that astaxanthin supplementation would improve cycling TT performance, an ergogenic effect underpinned by the ability of astaxanthin to enhance fat oxidation during exercise.

Methods

Twelve recreationally trained male cyclists (age: 27.5 ± 5.7 years, height: 1.78 ± 0.07 m, body mass: 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) volunteered to participate in the study, with prior ethical approval attained from the institutional ethics committee (SPA-REC-2017-323). The term "recreationally trained cyclist" was deemed most appropriate for the sample recruited, as although performance criteria for a "trained cyclist" was met (VO_{2peak}: 55-64.9 mL·kg^{-1}·min^{-1}; W_{max}: 320-379 W), training load criteria was not (distance covered: 60-290 km·week^{-1}; cycling frequency ≥ 3 times·week^{-1}) [17].

Supplementation with additional antioxidants/vitamins was not permitted alongside those provided in the current study, with a list of astaxanthin-rich foods to avoid also provided to limit the additional dietary intake of astaxanthin. Participants refrained from strenuous exercise and the consumption of alcohol and caffeine in the 24 h preceding each visit [18,19]. Habitual dietary intake was maintained; however, participants entered the laboratory in a 4 h postprandial state, except for the ingestion of water to ensure euhydration. Compliance with the above procedures was checked via 24 h dietary recall, with dietary intake replicated prior to each trial. All participants visited the laboratory (temperature: 18.0 ± 1.2 °C; pressure: 754.4 ± 8.8 mmHg, humidity 44.7 ± 3.5%) on four occasions (two preliminary trials and two experimental trials) at a similar time of day (± 1.0 h). A randomised, double-blind, crossover design was employed.

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

84 Prior to each experimental trial, participants supplemented with one of two randomly assigned
85 supplements for 7 days, with supplementation separated by a 14-day washout period. Estimations were
86 made based upon calculations that > 99.9% of a treatment is eliminated after a time period equivalent
87 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
88 consumed would be eliminated following ~ 7 days of washout. As this was an estimation, a more
89 conservative 14-day washout period was decided upon in the current study. Supplementation consisted
90 of either 12 $mg \cdot day^{-1}$ astaxanthin (AstaReal®, Sweden) or an appearance-matched placebo with no
91 viable constituents (AstaReal®, Sweden). Participants ingested two capsules daily (one morning and
92 one evening), with compliance ensured via daily text message reminders and a pill count post-ingestion.
93 To ensure the study remained double-blind, each supplement was assigned a randomised
94 alphanumerical code until after data analysis was complete.

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

at rest and every 10 km during the TT to determine blood lactate (Lactate Pro 2, Japan), glucose (Hemocue, Sweden) and triglycerides (Reflotron, USA). Breath-by-breath expired air was obtained during the 10^{th}, 20^{th}, 30^{th} and 40^{th} km of the TT. Respiratory gas data were then used to calculate whole-body fat and carbohydrate oxidation rates (FATox and CHox, respectively) using the method of Jeukendrup and Wallis [24].

As assumptions of normality and homogeneity were met, a paired *t*-test was used to compare differences in performance time and mean power between conditions, and to determine whether a trial order effect was present. A two-way [condition x time] analysis of variance (ANOVA) was used to determine differences in performance, respiratory and perceptual variables, blood metabolites and HR. *Post-hoc* analysis was performed with a Bonferroni adjustment. Effect sizes were calculated using Hedge's *g* and were interpreted as trivial (< 0.20), small (0.20-0.49), moderate (0.50-0.79) or large (≥ 0.80) [25]. Confidence intervals (CI) (± 95.0%) were also calculated and are reported where necessary. Descriptive data are displayed as mean ± standard deviation (SD). Statistical analysis was conducted using a statistical software package (SPSS, Version 25, USA), with significance accepted at $p < 0.05$.

Results

Time to complete the 40 km TT (Figure 1a) was improved from 70.76 ± 3.93 min in the placebo condition to 69.90 ± 3.78 min in the astaxanthin condition, which equates to a 1.2 ± 1.7% improvement (MI = 51 ± 71 s, 95.0% CI = 6-96 s, p = 0.029, *g* = 0.21). Mean power (Figure 1c) was also improved from 213.8 ± 29.0 W in the placebo condition to 219.9 ± 28.7 W in the astaxanthin condition, which equates to a 2.8 ± 4.1% improvement (MI = 6.1 ± 9.5 W, 95.0% CI = 0.1-12.1 W, p = 0.047, *g* = 0.20). No trial order was present for performance time (p = 0.993, *g* = 0.04) or mean power (p = 0.996, *g* = 0.02). There was also no [condition x time] interaction observed across each 10 km quartile for either performance time (p = 0.158; Figure 1b) or mean power (p = 0.242; Figure 1d), suggesting that the general pacing profile of the 40 km TT was similar across conditions.

A [condition x time] interaction was observed for FATox (p = 0.037), whereby FATox was greater between 39-40 km following astaxanthin supplementation (Figure 2c), increasing from 0.13 ± 0.04

130 g·min^{-1} in the placebo condition to 0.22 ± 0.05 g·min^{-1} in the astaxanthin condition (+0.09 ± 0.13
131 g·min^{-1}, 95.0% CI = 0.00-0.17 g·min^{-1}, p = 0.044, *g* = 0.52). A similar [condition x time] interaction
132 was also observed for the respiratory exchange ratio (RER) (p = 0.007), whereby RER was lower
133 between 39-40 km following astaxanthin supplementation (Figure 2a), decreasing from 0.99 ± 0.02 in
134 the placebo condition to 0.96 ± 0.01 in the astaxanthin condition (-0.03 ± 0.04, 95.0% CI = -0.01 to -
135 0.06, p = 0.024, *g* = 0.60). For CHox a [condition x time] interaction was present (p = 0.037), with
136 CHox greater at 39-40 km in both conditions (p < 0.045). There were, however, no differences reported
137 between conditions for CHox at any time point during the TT (p ≥ 0.118; Figure 2e).

138 Lactate (Figure 2b) was increased above baseline throughout the TT (p ≤ 0.001) and was greater at 40
139 km compared to 30 km (p = 0.002). Glucose (Figure 2f) was lower throughout the TT when compared
140 to baseline (p ≤ 0.003), and triglycerides (Figure 2d) were increased above baseline at 30 km (p = 0.027)
141 and 40 km (p = 0.002), as well as being greater at 40 km than at any other time point (p ≤ 0.003). There
142 were no differences between conditions for each of these blood metabolites (p ≥ 0.346).

143 Ratings of fatigue (p < 0.001), RPE_O (p < 0.001) and RPE_L (p < 0.001) all increased progressively over
144 time with no effect of condition (p ≥ 0.131). A main effect of time was also present for HR (p < 0.001)
145 and VO_2 (p < 0.001) in both conditions (p ≥ 0.338), with HR greater at 40 km than at each previous time
146 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
147 each previous time point (p ≤ 0.002) (Table 1).

148 **Discussion**

149 The current investigation is the first to demonstrate an increase in whole-body fat oxidation (FATox)
150 and a corresponding reduction in RER during endurance exercise in humans supplementing with
151 astaxanthin. This study also reports a small, yet significant, ergogenic benefit from 12 mg·day^{-1}
152 astaxanthin supplementation for 7 days in recreationally trained male cyclists completing a 40 km
153 cycling TT. This equates to a mean 51 s (1.2%) time improvement when compared to the placebo.

154 The performance findings of this study are, therefore, consistent with those reported by Earnest et al. [7],
155 as 4 weeks of 4 mg·day^{-1} astaxanthin improved 20 km cycling TT performance in trained male cyclists

[7]. Furthermore, the 121 s time improvement (5.1%) reported in the astaxanthin group was greater than the corresponding 18 s improvement (0.8%) reported in the placebo, suggesting a treatment effect was present [7]. In contrast, an ergogenic benefit was not reported during a 1.0 h cycling TT in trained male cyclists or triathletes following 4 weeks of supplementation with either 20 mg·day^{-1} astaxanthin (MI = 74 s (2.1%)) or a placebo (MI = 52 s (1.4%)) [8]. Although there is no clear explanation for the disparity between the two studies [7,8], neither Earnest et al. [7] nor Res et al. [8] reported differences in substrate utilisation during exercise. Four weeks of 4 mg·day^{-1} astaxanthin supplementation, for example, did not influence measures of RER, CHox or FATox obtained during a 2 h submaximal cycle at 5.0% below the lactate threshold [7]. Likewise, 20 mg·day^{-1} astaxanthin for 4 weeks did not influence measures of RER, CHox or FATox obtained during the completion of a 1.0 h steady-state cycle at 50.0% W_{max} [8]. As such, the increase in FATox and the decrease in RER reported in the latter stages of exercise in the current study are in contrast with previous research [7,8].

The shorter 7-day supplementation strategy implemented in the current study, which enabled the use of a randomised crossover design, may provide a methodological insight as to why a metabolic effect of astaxanthin has been observed. In previous research the application of a prolonged supplementation strategy has required the use of a parallel group design [7,8]. A major strength of the current study is, therefore, the ability to implement a randomised crossover design as this enabled each participant to act as their own control, minimising the potential impact subtle differences in participant characteristics and individual responses to astaxanthin could have upon the outcome variable [12,13]. This would have improved the statistical power of the study and may have increased the ability to detect subtle differences in substrate utilisation during exercise.

The current study also measured substrate utilisation during the completion of an ecologically valid performance event and not during a single-intensity, steady-state preload [7,8]. Therefore, the metabolic measures obtained during the 40 km TT may have more accurately reflected the ergogenic mechanism by which astaxanthin is purported to improve performance during self-paced, best effort endurance events. Conversely, the change in FATox and RER reported between 39-40 km may be attributable to 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 $mL \cdot kg^{-1} \cdot min^{-1}$ vs. placebo: 45.2 ± 7.4 $mL \cdot kg^{-1} \cdot min^{-1}$), 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 ± 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 ± 0.7% may be more appropriate for the

current study. To ensure that changes in performance (1.2 ± 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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Tables

Table 1. Mean ± SD. Physiological and perceptual results. δ denotes a significant difference to the previous time point, † denotes a significant difference to all 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 ($mL \cdot kg^{-1} \cdot min^{-1}$)	41.0 ± 7.6	40.1 ± 7.6	40.6 ± 8.2 $^{\delta}$	46.3 ± 8.6 †	39.6 ± 5.8	39.2 ± 6.9	41.1 ± 6.6 $^{\delta}$	45.2 ± 7.4 †
HR ($beats \cdot min^{-1}$)	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 $^{\delta}$	6.6 ± 1.3 $^{\delta}$	8.1 ± 1.6 †	3.2 ± 1.2	5.0 ± 1.5 $^{\delta}$	5.8 ± 1.7 $^{\delta}$	7.6 ± 1.8 †
RPE_O	13.8 ± 1.4	14.9 ± 1.4 $^{\delta}$	16.3 ± 1.4 $^{\delta}$	18.1 ± 1.3 †	13.3 ± 1.7	14.8 ± 1.5 $^{\delta}$	16.2 ± 1.5 $^{\delta}$	18.3 ± 1.8 †
RPE_L	14.9 ± 1.7	16.0 ± 1.2 $^{\delta}$	17.3 ± 1.2 $^{\delta}$	18.8 ± 0.8 †	14.8 ± 1.9	16.3 ± 1.5 $^{\delta}$	17.0 ± 1.3 $^{\delta}$	18.8 ± 0.8 †

Figures

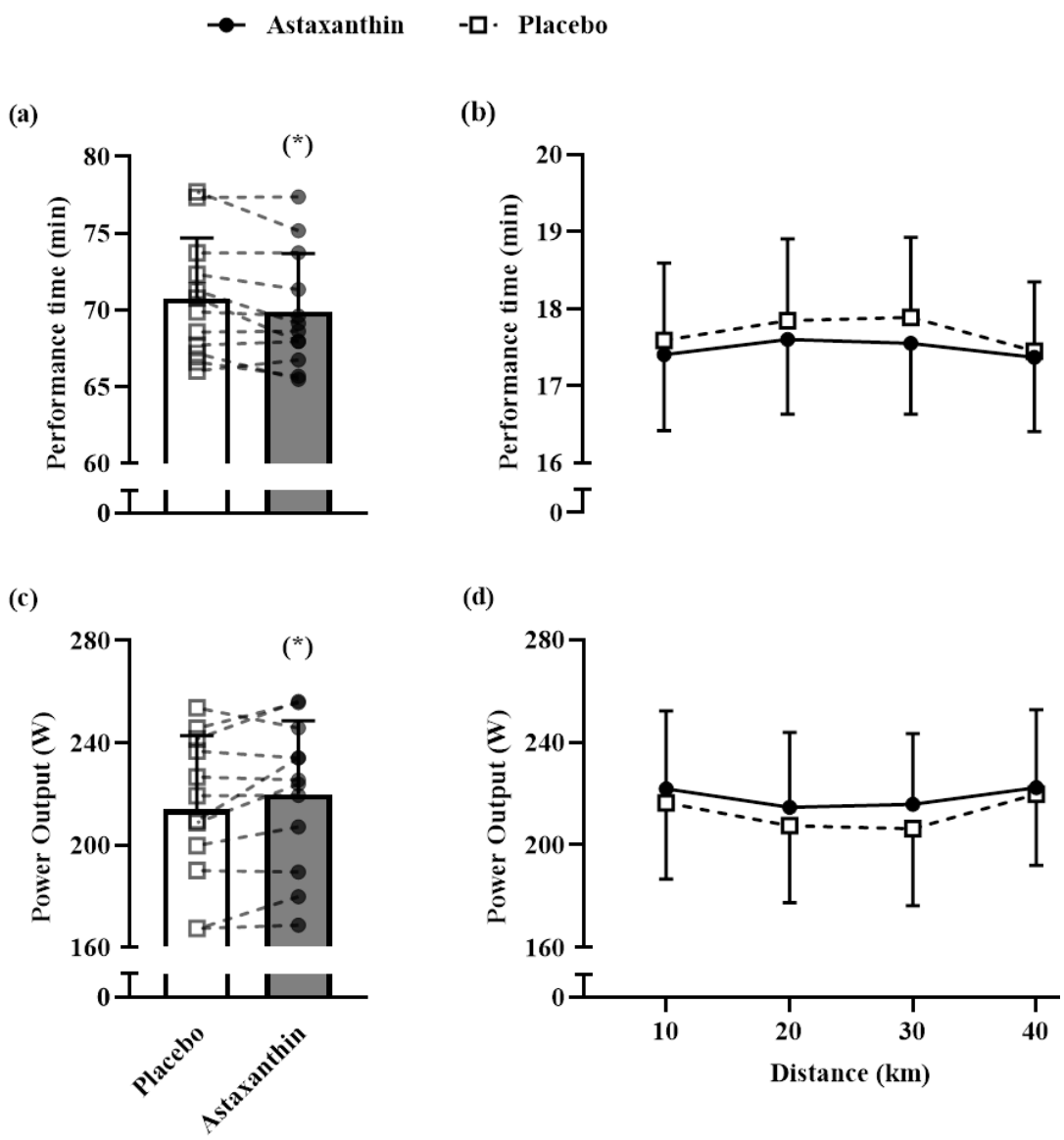
Figure 1. Mean ± SD. Individual values for performance time (a) and power output (c) during the 40 km time trial following each condition. Data for 10 km quartile performance times (b) and power outputs (d) are also displayed as mean (± SD) for each condition. * denotes a significant difference between 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 ($p < 0.05$).

Acknowledgements

DB has received external research funding from AstaReal® (Sweden) to complete the current project as part of his doctoral thesis. AS and LM have a professional relationship with AstaReal®. AW, SD and LG have no professional relationship with AstaReal® and have no conflict of interest.

1 **Figures**



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3 **Figure 1.** Mean ± SD. Individual values for performance time (a) and power output (c) during the 40
4 km time trial following each condition. Data for 10 km quartile performance times (b) and power
5 outputs (d) are also displayed as mean (± SD) for each condition. * denotes a significant difference
6 between conditions ($p < 0.05$).

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Figure 2

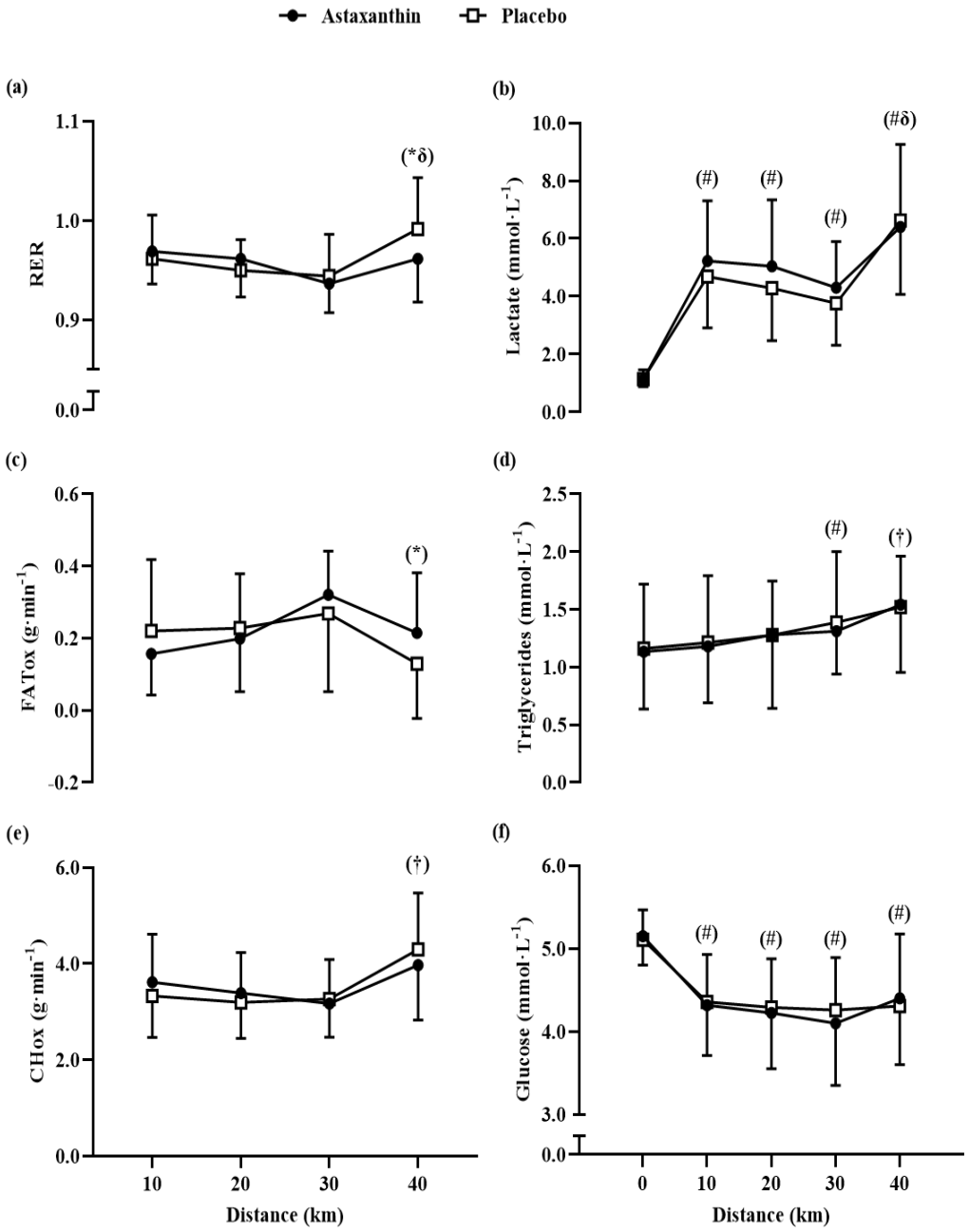

Figure 2. Mean ± SD. Respiratory measures of the respiratory exchange ratio (RER) (a), whole-body fat oxidation rates (FATox) (c), whole-body carbohydrate oxidation rates (CHox) (e), and blood metabolites lactate (b), triglycerides (d) and glucose (f) obtained over the duration of each 40 km time trial. * 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 ($p < 0.05$).