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PACING PROFILES OF OLYMPIC AND IAAF WORLD CHAMPIONSHIP LONG
DISTANCE RUNNERS

Running head: Pacing profiles of long distance runners

Luca Filipas^{1*}, Antonio La Torre^{1,2}, Brian Hanley³

¹Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan,
Italy

²IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

³Carnegie School of Sport, Headingley Campus, Leeds Beckett University, United Kingdom

Luca Filipas

Department of Biomedical Sciences for Health, Università degli Studi di Milano

Via Giuseppe Colombo, 71

Milan, Italy

Telephone +39 3408834392

Email: luca.filipas@unimi.it

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ABSTRACT

The aim of this study was to analyze the pacing profiles of Olympic and IAAF World Championship long distance finalists, including the relationship with their recent best times. The times for each 1,000-m split were obtained for 394 men and women in 5,000 m and 10,000 m finals at five championships. Athletes' best times from the previous 32 months were also obtained. Similar pacing profiles were used by athletes grouped by finishing position in 5,000 m races. Women adopted a more even pacing behavior, highlighting a possible sex-based difference over this distance. Pacing behavior over 10,000 m was more similar between men and women compared with over 5,000 m. The main difference between men and women was that in the men's 10,000 m, as in the men's 5,000 m, more athletes were able to follow the leading group until the final stages. There were large or very large correlations between athletes' best times from the previous 32 months and their result; the fastest finishers also ran closer to their previous 32 months' best times. Despite differences in pacing behavior between events, long distance runners should nonetheless stay close to the front from the beginning to win a medal.

Key words: endurance training; fatigue; long-distance events; race tactics; track and field.

INTRODUCTION

The 5,000 and 10,000 m are the longest track races at all major championships. Pacing distribution is one of the most important variables in endurance events (21) as managing one's physiological and psychological efforts is important in reaching the finish in the fastest possible time (2). To do this, it is necessary to regulate exercise intensity in the best way based on perception of effort and the relative remaining distance (6). Even pacing is often considered the best approach for maximizing endurance performance (1); for example, Thiel et al. (19) showed that world record pacing profiles over 5,000 m were even for both men and women (variations of 1.7% and 2.5%, respectively), with similar values found over 10,000 m (1.5% and 2.7% for men and women, respectively). However, in competitive situations where winning is more important than achieving a fast time, small physiological differences between world-class athletes mean strategic pacing is crucial, and collective behavior influences the racing and pacing behavior of opponents (13,16). Therefore, it is possible that pacing strategy in these championship competitions could differ from the ideal one given that race rewards are based on finishing position, rather than time.

The reasons for adopting a championship tactic to vary pace and thus try to challenge the physiological responses of opponents, rather than run it evenly, lie in the large number of athletes with very similar personal and season bests (and similar ambitions) (20), and because of the absence of pacemakers who are commonly used in Grand Prix racing. There is a worsening of performance when running in a leading position because of the absence of drafting, both for physiological and psychological reasons (21). Therefore, athletes of similar ability take advantage of the pack to run within it, or ahead but at a slower pace. On many occasions, most of the top runners form an almost compact group until the last 1,000 m or 400 m, when those who have saved physiological resources are best placed to outsprint their

rivals (3). Previous research on a large number of non-elite 10 km road runners found that men slowed more in the second half and the authors suggested that this difference occurred because of sex-based differences in decision making (5). It is possible that such sex-based differences do not occur in world-class distance runners on the track, as has been found in cross country (12), but if any differences do exist they could inform coaches with regard to adopting different racing approaches for men and women.

Although the very best athletes might vary pace because of tactical reasons, it is more usual for those outside the world's top ranked athletes to start too quickly and slow considerably in the latter stages (10). It may be possible that, despite tactical behavior different from the ideal, time-based ranking of athletes might still be a strong indicator for finishing positions. For example, in the longer racewalking events over 20 and 50 km, it was found that those athletes starting faster than personal best pace were more likely to drop out or slow down than those who didn't (9). Previous best times can therefore be a useful guide to predicting suitable pacing behavior and understand in what group an athlete might likely end up. Prior research has not analyzed the pacing profiles adopted by world-class male and female 5,000 m and 10,000 m competitors during multiple major championships finals. Accordingly, the aim of this study was to describe the pacing profiles of Olympic and International Association of Athletics Federations (IAAF) World Championships long distance finalists, including the relationship with their recent best times in the event. We hypothesized that male and female pacing behavior could differ, and that recent best time could be a strong predictor of pacing behavior and performance in long distance track events. An understanding of the pacing profiles used might provide invaluable information for athletes and coaches in improving their training regimens to win medals in these events (e.g., regarding the importance of improving the final spurt and/or the ability to run in a pack).

METHODS

Experimental Approach to the Problem

All data for this study were obtained from the IAAF's website (www.iaaf.org) and the All-Athletics website (www.all-athletics.com). These data included athlete finishing times, split times, personal best times, and season's best times to date. The five championship editions chosen were the only global championships at which comprehensive split data were available, and allowed for a repeated measures analysis of the pacing profiles of both men and women over the two long distance races held entirely on the track.

Subjects

Race times and 1,000-m split times were obtained from the open-access IAAF website (15) for competitors in the men's and women's 5,000 m and 10,000 m finals at the Olympic Games held in 2008 and 2016 and the IAAF World Championships held in 2013, 2015 and 2017. Institutional review board approval for this study was waived with regard to informed consent because these data are in the public domain. A total of 416 finishers were analyzed (men's 5,000 m: 73; women's 5,000 m: 69; men's 10,000 m: 140; women's 10,000 m: 134). Fourteen men and eight women who did not finish, and one man and three women considered very slow, were excluded. These slow runners were identified as outliers using SPSS Statistics 24 (IBM SPSS, Inc., Chicago, IL), where an outlier was more than 1.5 times the interquartile range from the median of the scores. The total complement of lap times was not available for the women's 5,000 m final in 2008, or for two competitors in the women's 10,000 m in 2017, and these athletes have also been excluded.

Procedures

The study was designed as observational research in describing pacing profiles. Competitors were first divided into groups based on finishing position, and each athlete placed in one group only. For the 5,000 m finals, there were three groups: Medalists (15 men; 12 women); non-Medalists who finished in the top eight ('Top 8': 25 men; 20 women), and those who finished outside the top eight ('Non-Top 8': 33 men; 27 women). Because there were more finalists in the 10,000 m, four groups were analyzed: Medalists (15 men; 15 women); non-Medalists who finished in the top eight ('Top 8': 25 men; 25 women), athletes outside the top eight but within the top 16 ('Top 16': 40 men; 39 women), and athletes outside the top 16 ('Non-Top 16': 56 men; 54 women). Athletes' best times from the previous 32 months were obtained from the All-Athletics website (www.all-athletics.com); for example, for those athletes competing in the 2017 IAAF World Championships, their best time was recorded between January 1st 2015 and the beginning of the championships in August 2017. We chose this time frame because athletes often run their best times some time from the major championships, and because using season's best times could lead to underestimation of ability due to injuries or because of periodization in training (i.e., not peaking until the championships).

Statistical Analysis

Normality was assessed using a Kolmogorov-Smirnov test. Two-way repeated measures ANOVA was conducted on the split time data with repeated contrast tests conducted to identify changes between successive splits and between groups for the same split (7). Greenhouse-Geisser corrections were used if Mauchly's test for sphericity was violated. The split time percentages (of 32 months' best time) were arcsine transformed for the purposes of statistical analysis (11). Statistical significance was accepted as $P < 0.05$. Effect sizes (ES) for

differences between successive splits were calculated using Cohen's d (4) and considered to be either trivial ($ES: < 0.20$), small ($0.21 - 0.60$), moderate ($0.61 - 1.20$), large ($1.21 - 2.00$), or very large ($2.01 - 4.00$) (13). Additionally, Pearson's correlation coefficients (r) were used to quantify associations between race positions and athletes' best times from the previous 32 months, and were considered to be either small ($0.10 - 0.29$), medium ($0.30 - 0.49$), large ($0.50 - 0.69$), or very large ($0.70 - 0.89$) (14). The coefficient of variation was found for 32 months' best times and expressed as a percentage (CV%).

RESULTS

Mean finishing times for each group across all events are shown in Table 1. The mean 32 months' best times and CV% were (respectively): 13:04 ($\pm 0:10$) and 1.2% for the men's 5,000 m; 14:52 ($\pm 0:20$) and 2.2% for the women's 5,000 m; 27:25 ($\pm 0:24$) and 1.5% for the men's 10,000 m; and 31:26 ($\pm 0:42$) and 2.2% for the women's 10,000 m.

*** Table 1 about here ***

The times of the top 3 finishers (gold, silver and bronze medal positions) in each race analyzed are shown in Table 2.

*** Table 2 about here ***

Figure 1 shows the correlation between 32 months' best times and finishing positions in the men's and women's 5,000 m and 10,000 m finals. Overall, the slower the group, the further they finished from their 32 months' best time. In the men and women's 10,000 m, athletes ran nearer to their 32 months' best time than the 5,000 m finalists. Noticeably, the women's

10,000 m medalists group reported the highest percentage of athletes (47%) running quicker than their 32 months' best time.

*** Figure 1 about here ***

Figure 2 shows the mean 1,000 m times for each group competing in the men's 5,000 m. For the Medalists, there was a large effect size for the difference between splits 4 and 5; for the Top 8 group, there was a large effect size for the difference between splits 4 and 5; for the Non-Top 8 group, there was a moderate effect size for the difference between splits 3 and 4. For split 5, there were moderate effect sizes for the differences between the Medalists and Top 8, Top 8 and Non-Top 8, and a large effect size for the difference between Medalists and Non-Top 8. Regarding pacing relative to 32 months' best time, there were no differences between the groups at the first four splits, but during the last 1,000 m the Medalists and Top 8 recorded times lower than the Non-Top 8 ($P < 0.001$, $ES = 1.33$; $P = 0.002$, $ES = 0.99$).

*** Figure 2 about here ***

Figure 3 shows the mean 1,000 m times for each group of athletes competing in the women's 5,000 m. For the Medalists, there were moderate effect sizes for the differences between splits 1 and 2, 2 and 3, and 4 and 5; for the Top 8, there was a moderate effect size for the difference between splits 1 and 2, 2 and 3, and 4 and 5; for the Non-Top 8 group, there was a moderate effect size for the difference between splits 1 and 2 and splits 3 and 4. Most of the effect sizes for differences between groups were trivial, small or moderate. From split 4 onwards, there were large effect sizes for the differences between Medalists and Non-Top 8. For split 5, there were large effect sizes for the differences between Medalists and Top 8, and

Top 8 and Non-Top 8. Regarding pacing relative to 32 months' best time, the Medalists completed the first 1,000 m slower ($108.9\% \pm 4.6$) than the Non-Top 8 ($104.5\% \pm 4.9$) ($P = 0.011$, $ES = 0.91$), and the Medalists and Top 8 ran faster than the Non-Top 8 during both split 4 ($P = 0.007$, $ES = 1.02$; $P = 0.008$, $ES = 0.87$, respectively) and split 5 ($P < 0.001$, $ES = 1.57$; $P < 0.001$, $ES = 1.20$, respectively).

*** Figure 3 about here ***

Figure 4 shows the mean 1,000 m times for each group competing in the men's 10,000 m. For the Medalists, there was a moderate effect size for the difference between splits 1 and 2 and a large effect size for splits 9 and 10. For the Top 8 group, there was a moderate effect size for the difference between splits 1 and 2 and splits 9 and 10. For the Top 16 group, there was a moderate effect size for the difference between splits 1 and 2 and splits 9 and 10, whereas for the Non-Top 16 group, there was a moderate effect size for the difference between splits 1 and 2. Most of the effect sizes for differences between groups were trivial, small or moderate. From split 7 onwards, there were large effect sizes for the differences between Medalists and Non-Top 16 and between Top 8 and Non-Top 16. For splits 8 and 9, there were large effect sizes for the differences between Top 16 and Non-Top 16. From split 9 onwards, there were large effect sizes for the differences between Top 8 and Top 16, and large or very large effect sizes for the differences between Medalists and Top 16. For split 10 only, there were large effect sizes for the differences between Medalists and Top 8.

*** Figure 4 about here ***

Regarding pacing relative to 32 months' best time, there were no differences between the Medalists and Top 8 at any distance, but the Top 16 were faster than the Medalists and Top 8 during split 3 ($P < 0.001$, $ES = 1.45$; $P = 0.001$, $ES = 0.86$), as were the Non-Top 16 group ($P < 0.001$, $ES = 1.16$; $P = 0.004$, $ES = 0.72$). The Medalists and Top 8 were relatively faster than the Top 16 and the Non-Top 16 during splits 9 and 10 (all $P \leq 0.001$, $ES \geq 0.91$); the Top 16 were also faster than the Non-Top 16 during every split from 6,000 m onwards (all $P \leq 0.001$, $ES \geq 0.64$).

Figure 5 shows the mean 1,000 m times for each group of women competing in the 10,000 m. There were moderate and large effect sizes for differences between splits 9 and 10 in all groups. Most of the effect sizes for differences between groups were trivial, small or moderate. From split 4 onwards, there were large effect sizes for the differences between Medalists and Non-Top 16 and between Top 8 and Non-Top 16. From split 6 onwards, there were large effect sizes for the differences between Medalists and Top 16. For split 10 only, there were large effect sizes for the differences between Medalists and Top 8.

*** Figure 5 about here ***

In terms of 32 months' best times, the Medalists were relatively faster than the Non-Top 16 group during every split from 6,000 m onwards (all $P \leq 0.001$, $ES \geq 1.27$). The Medalists were also faster than the Top-16 group during every split from 7,000 m onwards (all $P \leq 0.037$, $ES \geq 0.91$), but were only faster than the Top 8 during split 10 ($P = 0.009$, $ES \geq 1.32$). The Top 8 were never relatively faster than the Top 16 but were faster than the Non-Top 16 during the last five splits (all $P \leq 0.005$, $ES \geq 0.97$). Finally, the Top 16 were relatively faster than the Non-Top 16 only during the final split ($P = 0.001$, $ES = 0.70$).

DISCUSSION

This study examined pacing profiles of Olympic and IAAF World Championship 5,000 m and 10,000 m finalists, including the relationship with their recent best times. To win a medal, the most important factor was to stay close to the front from the beginning, as in other long-distance competitions (9). The Top 8 were close to the Medalists (especially in the men's races) until the latter stages where the differentiation between the top eight positions was decided close to the finish, as found previously in other Olympic track races (19) and the IAAF World Cross Country Championships (10). The results of the present study showed large or very large correlations between athletes' best times from the previous 32 months and the result in the finals. Having a high-ranking best time from the previous 32 months does not guarantee a high finishing position but seems to be a necessary condition to obtain it (i.e., athletes who do not have a high-ranking time entering the competition do not have the requisite ability to finish near the front). In addition, when analyzing the race as a percentage of 32 months' best time, it is evident that the Medalists and Top 8 ran closer to their best time than the Non-Top 8, Top 16 and Non-Top 16. This can be explained from the pacing behavior of the slower finishers being too aggressive to follow the lead group and/or because of less metabolic reserve capacity (as suggested by their lower 32 months' best time). This means that the differences in pacing could appear greater than during Grand Prix meets, where each athlete, less influenced by other athletes and with pacemakers to assist, tries to make the best possible (even) pacing behavior to obtain the best finishing time. The rewards provided by championship racing, such as medals or high-finishing positions, encourage a tactic of matching rivals' behaviors (18). Running with similarly-matched opponents has several advantages; apart from the affordances they present with regard to motivation and drafting (13), they provide external references for pacing (17) that in championship races act as a guide for positioning rather than elapsed time (which is quite easily measured on the

track compared with cross country running, for example (10)). The relatively small number taking part in championship finals means the top athletes are aware of most rivals' abilities and can use these other athletes as pacing guides and/or follow them for a tactical choice even if ultimately their aim is to finish ahead of them (11). In preparation for championship racing, athletes should thus aim to include training sessions and preparatory races that replicate the more variable and tactical aspects of competition.

The top men and women ran the 5,000 m slightly differently. Amongst men, the race was decided in the last 1,000 m, as up to 4,000 m the three groups all adopted the same pacing distribution. In the last split, the Medalists and Top 8 accelerated compared with the previous kilometer, whereas this did not happen for the Non-Top 8. The Medalists and Top 8 did not differ in any split because both groups were still together at the beginning of the last 1,000 m and separated only during it, highlighting the small physiological differences between top athletes and the importance of critical speed (the speed above which finite, predominantly nonoxidative exercise is performed) even in a predominantly aerobic event (3). Amongst women, the difference between the Medalists and Non-Top 8, and between the Top 8 and Non-Top 8, occurred by splits 3 and 4, respectively, with the difference between the Medalists and Top 8 revealed in the last split. The women adopted a more even pacing behavior compared with men (especially in the Non-Top 8), highlighting a sex-based difference over this distance, which has been found previously amongst elite-standard athletes in longer (11) and shorter races (8), and amongst non-elite runners (5). These pacing differences could be due to the different range of abilities in men's and women's 5,000 m finals: amongst men, ability was more similar, making it more difficult to lead from the beginning and reach the finish line alone; amongst women, it was much easier to create such a gap as there were larger variations between athletes' abilities. For example, in both 2015

and 2017 the women winners were out in front at 4,000 m by 15 and 9 s, respectively, whereas in the men's championships considered, the first three athletes were still together at 4,000 m. There were also larger time gaps at the finish line in the women's races; in Table 2 it can be seen that in the men's races, the winning margin was less than 1 s in 7 of the 10 finals, whereas the winners of the women's races finished on average approximately 7 s (5,000 m) and 18 s (10,000) ahead of the silver medalists.

Regarding the 10,000 m, pacing behavior was much more similar between men and women, and in both cases more even than in the 5,000 m. The main difference between men's and women's races was that in the men's 10,000 m, as in the men's 5,000 m, there was a greater number of athletes able to follow the leading group up to the final stages. Indeed, the men's Top 16 slowed compared with the lead pack only from split 8; by contrast, in the women's Top 16, this happened from split 4. In general, the pacing behavior in the 10,000 m was similar for all groups of both sexes and characterized by an even effort distribution and a strong acceleration in the last split. It is important to underline that in the 5,000 m the tactical element was certainly greater, and even pacing behavior therefore assumed a less important role compared with the 10,000 m, where energy management was fundamental. In the longer race, the similar start of all athletes disguised the fact that the groups finishing slower actually started the race quicker relative to 32 months' time than their higher-finishing rivals. The more conservative opening by the Medalists and Top 8 meant they mostly ran a negative split, whereas the Top 16 ran an even split, and the Non-Top 16 a positive split. It should be noted that the limited number of races available for analysis could be a limiting factor in considering of sex differences, and of successful and unsuccessful pacing profiles in general. New data from the next series of major championships could provide more information and confirm (or not confirm) the trends found.

PRACTICAL APPLICATIONS

Previous best times achieved are a strong guide for future performances at major championships. Many long-distance runners achieve their best times in events held with pre-arranged pacemakers where the goal is to achieve a particular time (such as a qualifying time). Championship racing is different in this regard and coaches should include championship-specific race preparation in training once this primary goal has been achieved (or alongside it). Success in the 5,000 and 10,000 m races requires superior aerobic physiology to achieve a fast but even pace for most of the distance, but also the ability to produce very quick finishes where the medal positions are decided. The runners with the best times over the past 32 months run at a lower relative intensity for the major part of the race and are therefore able to increase the speed during the last 1000 m. Athletes should prepare themselves physically and mentally for races that are raced differently from non-championship events. From a tactical point of view, the athletes who are highest ranked should ensure they keep pace with their rivals and save energy until the decisive finishing endspurt.

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Table 1 Finishing time and percentage of 32 months' best time for each group of athletes in the different race categories. Data are presented as means ($\pm$ SD).

		Men's 5,000 m	Women's 5,000 m	Men's 10,000 m	Women's 10,000 m
Medalists	Finishing time	13:24 ($\pm$ 0:19)	14:40 ($\pm$ 0:09)	27:05 ($\pm$ 0:11)	30:34 ($\pm$ 0:48)
	% of 32 months' best time	103.4 ($\pm$ 2.6)	101.6 ($\pm$ 1.4)	100.7 ($\pm$ 1.2)	99.6 ($\pm$ 3.2)
Top 8	Finishing time	13:29 ($\pm$ 0:16)	14:58 ($\pm$ 0:09)	27:16 ($\pm$ 0:15)	31:01 ($\pm$ 0:35)
	% of 32 months' best time	103.5 ($\pm$ 2.2)	101.0 ($\pm$ 1.2)	100.7 ($\pm$ 1.0)	100.3 ($\pm$ 2.4)
Non-Top 8	Finishing time	13:41 ($\pm$ 0:17)	15:21 ($\pm$ 0:17)		
	% of 32 months' best time	104.0 ($\pm$ 2.4)	101.8 ($\pm$ 1.8)		
Top 16	Finishing time			27:41 ($\pm$ 0:19)	31:47 ($\pm$ 0:32)
	% of 32 months' best time			100.9 ($\pm$ 1.7)	101.0 ($\pm$ 1.9)
Non-Top 16	Finishing time			28:28 ($\pm$ 0:31)	32:27 ($\pm$ 0:39)
	% of 32 months' best time			102.8 ($\pm$ 1.8)	101.9 ($\pm$ 1.8)

Table 2 Finishing time (min:s) for each of the medalists in the different race categories.

	Medalist	Men's 5,000 m	Women's 5,000 m	Men's 10,000 m	Women's 10,000 m
Beijing 2008	Gold	12:57.82	15:51.40	27:01.17	29:54.66
	Silver	13:02.80	15:44.12	27:02.77	30:22.22
	Bronze	13:06.22	15:44.96	27:04.11	30:26.50
Moscow 2013	Gold	13:26.98	14:50.19	27:21.71	30:43.35
	Silver	13:27.26	14:51.22	27:22.23	30:45.17
	Bronze	13:27.26	14:51.33	27:22.61	30:46.98
Beijing 2015	Gold	13:50.38	14:26.83	27:01.13	31:41.31
	Silver	13:51.75	14:44.07	27:01.76	31:41.77
	Bronze	13:51.86	14:44.14	27:02.83	31:43.49
Rio 2016	Gold	13:03.30	14:26.17	27:05.17	29:17.45
	Silver	13:03.90	14:29.77	27:05.64	29:32.53
	Bronze	13:04.35	14:33.59	27:06.26	29:42.56
London 2017	Gold	13:32.79	14:34.86	26:49.51	30:16.32
	Silver	13:33.22	14:40.35	26:49.94	31:02.69
	Bronze	13:33.30	14:42.73	26:50.60	31:03.50

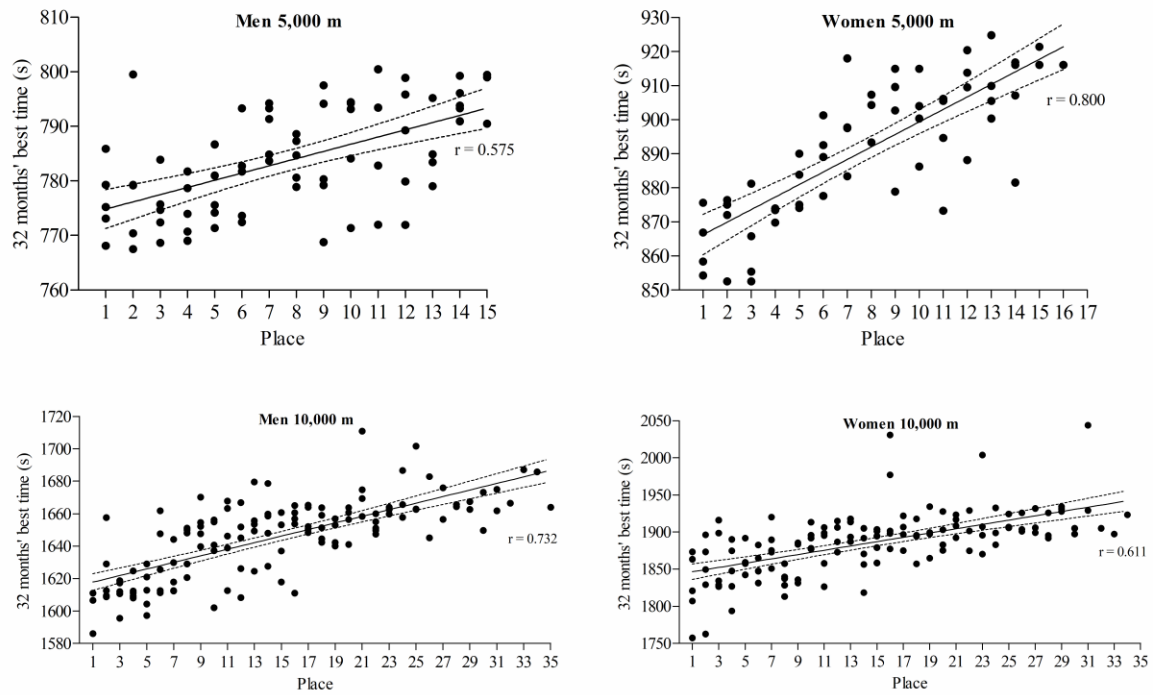


Figure 1. Scatter plots with linear regression lines of positions in the finals and athletes' best times from the previous 32 months with Pearson correlation coefficients. All correlations reported were $P < 0.001$.

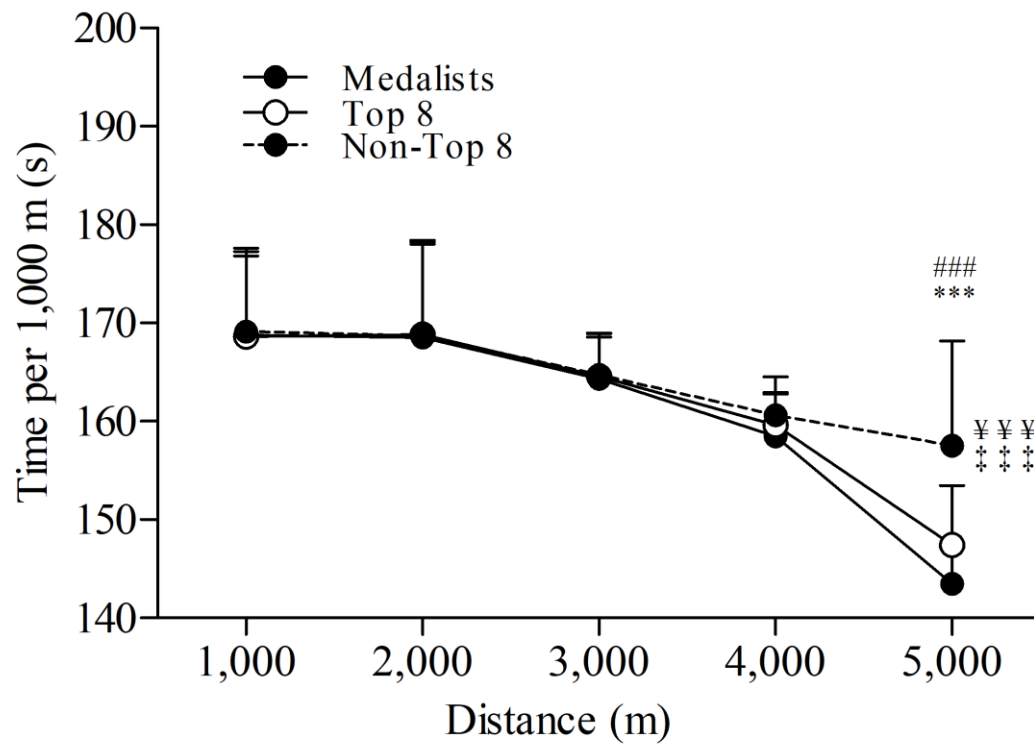


Figure 2. Mean (+ SD) times for each 1,000 m split for athletes competing in the men's 5,000 m. Differences between successive 1,000 m splits are shown as follows: Medalists, *** ($P < 0.001$); Top 8, ### ($P < 0.001$). Differences between groups are shown as follows: group to Medalists, ¥ ¥ ¥ ($P < 0.001$); group to Top 8, ‡ ‡ ‡ ($P < 0.001$).

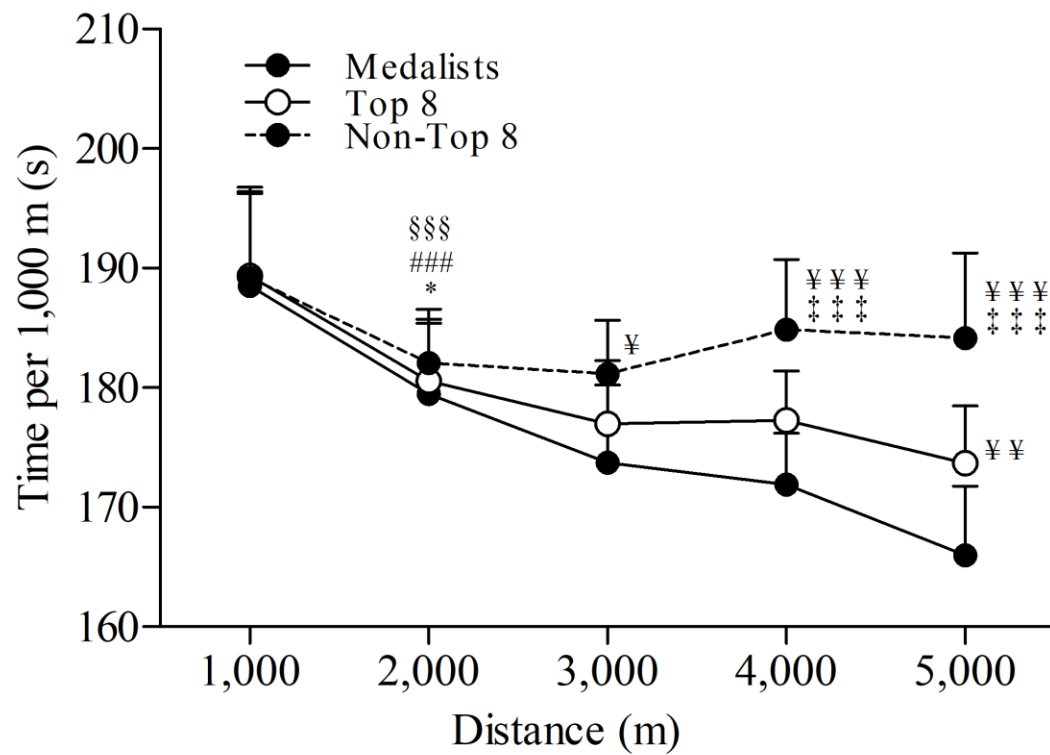


Figure 3. Mean (+ SD) times for each 1,000 m split for athletes competing in the women's 5,000 m. Differences between successive 1,000 m splits are shown as follows: Medalists, * ($P < 0.05$); Top 8, ### ($P < 0.001$); Non-Top 8, §§§ ($P < 0.001$). Differences between groups are shown as follows: group to Medalists, ¥ ($P < 0.05$), ¥ ¥ ($P < 0.01$), ¥ ¥ ¥ ($P < 0.001$); group to Top 8, ‡ ‡ ‡ ($P < 0.001$).

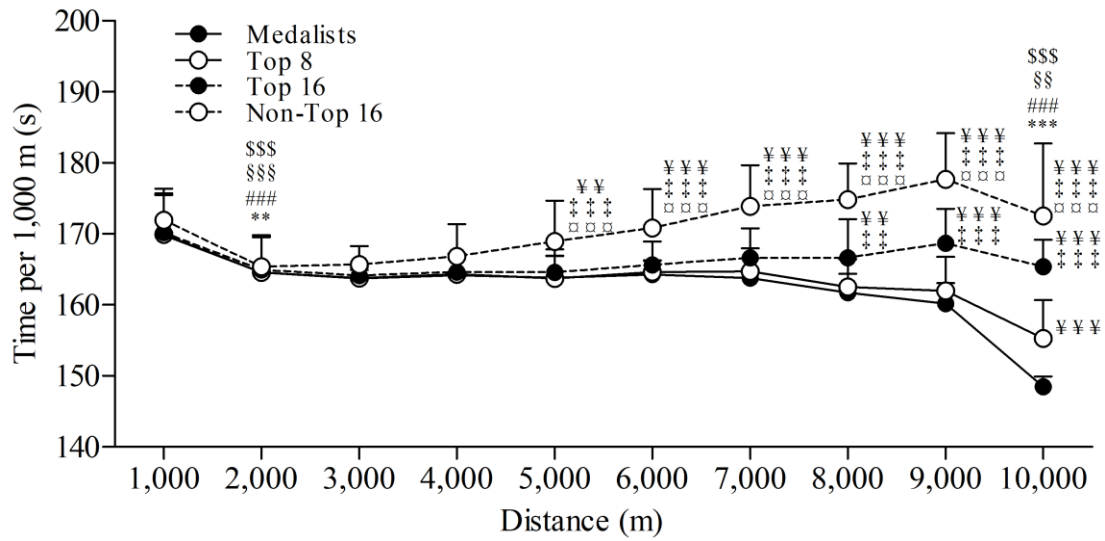


Figure 4. Mean (+ SD) times for each 1,000 m split for athletes competing in the men's 10,000 m. Differences between successive 1,000 m splits are shown as follows: Medalists, ** ($P < 0.01$), *** ($P < 0.001$); Top 8, ### ($P < 0.001$); Top 16, §§ ($P < 0.01$), §§§ ($P < 0.001$); Non-Top 16, \$\$\$ ($P < 0.001$). Differences between groups are shown as follows: group to Medalists, ¥ ¥ ($P < 0.01$), ¥ ¥ ¥ ($P < 0.001$); group to Top 8, ‡ ‡ ($P < 0.01$), ‡ ‡ ‡ ($P < 0.001$); group to Top 16, □ □ □ ($P < 0.001$).

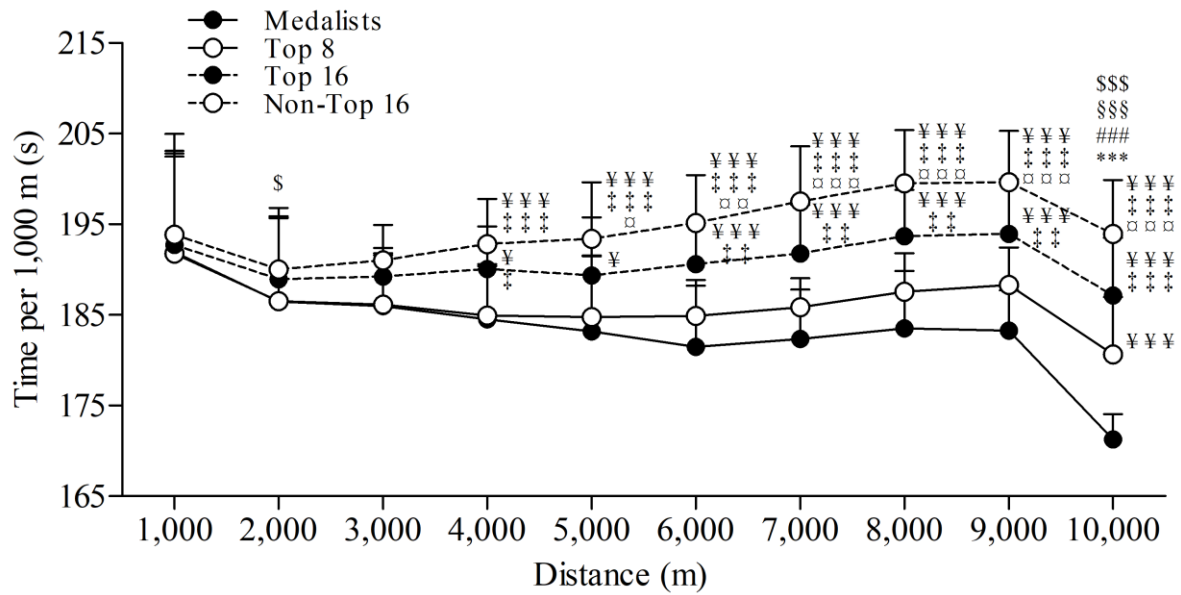


Figure 5. Mean (+ SD) times for each 1,000 m split for athletes competing in the women's 10,000 m. Differences between successive 1,000 m splits are shown as follows: Medalists, *** ($P < 0.001$); Top 8, ### ($P < 0.05$); Top 16, \$\$\$ ($P < 0.001$); Non-Top 16, \$ ($P < 0.05$), \$\$\$ ($P < 0.001$). Differences between groups are shown as follows: group to Medalists, ¥ ($P < 0.05$), ¥ ¥ ¥ ($P < 0.001$); group to Top 8, ‡ ($P < 0.05$), ‡ ‡ ($P < 0.01$), ‡ ‡ ‡ ($P < 0.001$); group to Top 16, □ ($P < 0.05$), □ □ ($P < 0.01$), □ □ □ ($P < 0.001$).