Off seasonal and pre-seasonal assessment of circulating energy sources during prolonged running at the anaerobic threshold in competitive triathletes

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Objectives: To compare changes in circulating energy sources during prolonged exercise in off season (OS) and pre-season (PS) training of triathletes.

Methods: Nine athletes of the Swiss national triathlon team (three female, mean (SD) age 28.7 (4.9) years, height 169.8 (6.0) cm, weight 57.0 (6.2) kg, VO2MAX 66.5 (5.3) ml/min/kg; six male, mean (SD) age 24.0 (4.1) years, height 181.4 (6.9) cm, weight 73.5 (6.0) kg, VO2MAX 75.9 (4.9) ml/min/kg) were tested twice (2.5 months apart) during a 25 km aerobic capacity test run at the end of the OS and just before the season. The average training load during the OS was 9.9 h/week, and this increased to 14.4 h/week in the PS. With heart rates as reference, exercise intensity during the aerobic capacity test was 97.0 (4.9)% of the anaerobic threshold and 91.2 (4.5)% of VO2MAX. Blood samples were collected before, during, and after the aerobic capacity test. Samples were collected every 5 km during three minute rest intervals.

Results: Blood was analysed for triglyceride (TG), free fatty acids, cholesterol, high density lipoprotein cholesterol, glucose, insulin, lactate, and changes in plasma volume. A two factor (season by distance) repeated measures analysis of variance revealed an increase in capacity for prolonged exercise in the PS by a decrease in running intensity during the aerobic capacity test (% of speed at 2.0 mmol/l lactate threshold, p = 0.008), an increase in running speed at the anaerobic threshold (p = 0.003) and at 4.0 and 2.0 mmol/l (p<0.001) of the lactate threshold. A significant season by distance interaction was found for TG (p<0.001). TG concentrations peaked at 5 km and decreased logarithmically throughout the OS (1.48 (0.34) to 0.86 (0.20) mmol/l) and PS (1.90 (0.31) to 0.73 (0.18) mmol/l) tests. From the OS to the PS, there was an increase in the difference in TG at 5–15 km with a concomitant increase at 2.0 mmol/l of the lactate threshold. The peak TG concentrations at 5 km followed by a logarithmic decrease suggest that TG may also provide circulating energy. A greater logarithmic decrease in TG occurred in the PS than in the OS, indicating a higher rate of use. There was an increase in the difference in TG at 5–15 km similar to the increase in the speed at 2.0 mmol/l of the lactate threshold between the two seasons. Glucose, insulin, lactate, and free fatty acids were similar in the two seasons.

Conclusion: Free fatty acid and TG concentrations were much higher than expected, and the two training seasons showed significantly different patterns of TG concentration during prolonged running. These responses may be related to aerobic capacity of prolonged exercise.

Measurements of oxygen consumption (VO2) and lactate concentrations are commonly used to assess aerobic performance. They are regarded as measures of cardiopulmonary and muscle performance. Numerous reference values of maximal oxygen consumption (VO2MAX) are available for the general population, as well as for various sports. It is generally accepted that a VO2MAX value exceeding 70.0 ml/kg/min is a favourable precondition for international competition. Athletes with VO2MAX below 60.0 ml/kg/min may be unable to succeed at this level.1 Previous studies indicate a poor relation between VO2MAX and competitive performance in triathletes2 and long distance runners.3 Performance variables measured at a submaximal intensity—that is, anaerobic or lactate threshold—may provide an indication of competition performance in well trained, long distance athletes.4,5 Other factors such as anaerobic threshold (AT), intracellular substrate availability, circulating energy sources, and economy of motion may be more important in prolonged endurance competitions.

The two primary substrates used by working skeletal muscle are carbohydrates and fats.6,7 It has been suggested that endurance training leads to metabolic and cellular adaptations that allow trained muscle to rely more on fat for oxidation and thus spare carbohydrates.8–11 Some investigators have suggested that this is of major importance in delaying muscle fatigue, as muscle glycogen and blood glucose can be spared. Oberholzer et al12 have calculated that intracellular substrate stores could account for about 50% of the substrate needed in an ultra-long distance event (25% lipids, 22% glycogen). The remaining energy should be provided by the blood and may depend on blood glucose and fat concentrations. Felig et al13 reported depletion of circulating glucose in healthy men engaged in running. Declining glucose concentrations without carbohydrate supplementation have been reported.14–17 Long term training induces a reduction in glucose turnover at moderate exercise intensity14,16 probably due to an increase in fat oxidation.

Abbreviations: ACT, aerobic capacity test; APT, aerobic power test; AT, anaerobic threshold; FFA, free fatty acid; LT, lactate threshold; TG, triglyceride; VO2MAX, maximal oxygen consumption

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utilisation. It is generally accepted that maintenance of carbohydrate availability and oxidation will lead to enhanced endurance exercise performance.\(^{14}\) Therefore, we expected that, during prolonged exercise without carbohydrate supplementation, glucose delivery to contracting muscle would become a limiting factor,\(^{3,5}\) and changes in carbohydrate metabolism would reflect glucose, lactate, and insulin concentration in the serum. These hypotheses may be contrary, as Ahlborg et al\(^{30}\) found only small modifications in circulatory variables. They measured highly significant changes in glycogen stores in the working muscle. Others reported that, during high intensity exercise, the blood glucose concentration does not have any effect on skeletal muscle glycogen metabolism.\(^{20,21}\) Furthermore, without glucose supplementation in most running performance studies, blood glucose concentrations do not decline.\(^{17,22,23}\) Although Knapik et al\(^{18}\) found an increase in blood glucose concentration at 85% of \(V_{\text{O2max}}\). Therefore, the intensity of the exercise and the training state seem to be key factors in this issue.

Prolonged exercise of moderate intensity has been shown to result in a time dependent increase in fat oxidation.\(^{6-8,25}\) After endurance training, the dependence on fat oxidation becomes more pronounced.\(^{3,7,8,15}\) The increased reliance on fat oxidation appears to depend on an increase in intramuscular triglyceride (TG) concentration and the availability of plasma free (non-esterified) fatty acids (FFAs).\(^{5,6,25}\) In an extreme endurance race, it was shown that the volume of intracellular lipid deposits was reduced from 1.3% to 0.3% of the muscle cell’s volume.\(^{16}\) About 50% of the substrate needed could be obtained from the intracellular substrate stores in that particular ultra-long distance event. Therefore the availability of circulating fat sources appears to be important. Oberholzer et al\(^{26}\) support the hypothesis that increasing fat availability immediately before exercise enhances the capacity of trained subjects to perform prolonged exercise.\(^{26}\) It has been suggested that medium chain TG and FFAs may be a readily available energy source for the working muscle.\(^{27-29}\) The pattern of substrate use depends on the interaction between exercise intensity induced response and endurance volume induced response.\(^6\) Carbohydrate availability is regulated by exercise intensity; however, the regulation of lipid metabolism seems to be more complex.\(^6\) Circulating energy sources in well-trained athletes at exercise intensities comparable to competition have been studied. Despite the well known presence of TG in skeletal muscles, regulation of this substrate is not well defined.

Therefore the purpose of this study was to examine changes in circulating energy sources, specifically TG and FFAs, that occur during prolonged exercise at the AT in elite athletes before preparation for the season (off season (OS)) and before the season (pre-season (PS)). From experience, these athletes tend to show better performances in prolonged exercise during their PS than their OS schedule. To assess these performances, specific tests of aerobic capacity were carried out before the trials.

**METHODS**

**Design**

Triathletes were tested twice (2.5 months apart; in an “untrained” state at the end of the OS and in a “trained” state just before the season (PS)) using a maximal aerobic power test (APT) and an aerobic capacity test (ACT, 25 km run). They were invited to participate in identically structured days for both seasons. APT was performed on the first day, and ACT was performed 48 hours later. On test days, training sessions were reduced and food intake was the same. Athletes served as their own controls.

| Subjects |

Data are presented for nine triathletes of the Swiss national team who completed both tests. Table 1 summarises their physical characteristics.

\(V_{\text{O2max}}\) measured on the treadmill was 69.0 (6.2) ml/kg/min and 79.1 (4.8) ml/kg/min in the female and male athletes respectively (mean of the two seasons). Running speed at AT was 16.5 (0.8) km/h and 18.4 (0.9) km/h and represented 84.2 (2.8)% of their maximum speed (mean of the two seasons). During the OS, the average training load was 9.9 (4.9) h/week. During the PS, it increased significantly to 14.4 (4.6) h/week (p = 0.03). The training sessions consisted of 27.6 (19.8)% jogging, 31.4 (15.9)% cycling, 27.2 (16.2)% swimming, and 13.3 (8.0)% other sports. None of the subjects were smokers at the time of the study; however, one had a history of short term smoking. The ethics committee of Davos approved the study protocol. Before participation, all subjects gave written informed consent.

**Nutritional intake and physical activity before testing**

Nutritional intake was quantitatively measured for one week before each ACT using a questionnaire (Nutricare, Basel, Switzerland). Responses were confirmed by personal interviews. An identical breakfast was consumed in the morning of each ACT. Three days before the ACT, all athletes trained below 90% of their AT and exercised no more than 30 min/day except for the APT.

| Aerobic power test (APT) |

Subjects performed a progressive \(V_{\text{O2max}}\) treadmill test protocol with 2.5 minute stages separated by 30 second rest periods. Testing began at 9 km/h at 1.5% grade. Intensity was increased at each stage by 1.8 km/h. Blood samples were taken from the carotid during each rest period. Blood lactate concentration was determined with a YSI model 23L analyser (Yellow Springs Instrument Co, Yellow Springs, Ohio, USA). \(V_{\text{O2}}\) and carbon dioxide production (\(V_{\text{CO2}}\)) were determined (30 second averages) with an open air spirometry system (EOS/Sprint, Jäger, Würzburg, Germany). The test was terminated when the subject could no longer maintain the exercise intensity. At exhaustion, all subjects attained a respiratory exchange ratio of \(\geq 1.1\) and a plateau in \(V_{\text{O2}}\). The AT was determined using the protocol of Lehmann et al,\(^{30}\) which uses the ratio of lactate/\(V_{\text{O2}}\) for each stage of the test. By this method, the minimal workload dependent ratio of lactate/\(V_{\text{O2}}\) (minimum lactate equivalent) determines the optimal working economy. Based on this minimal lactate equivalent, AT was estimated by adding 1.5 mmol/l lactate. Heart rate was determined with an electrocardiograph.

**Aerobic capacity test (ACT)**

The ACT was conducted in the field between 0900 and 1100. The meteorological data during the capacity tests were provided from a measuring station of the Swiss Meteorological Institution stationed in the running field. Air temperature increased during the PS from \(-5.7\)°C to 1.0°C and during the OS from 5.9°C to 9.1°C. The relative humidity was 96% during the first investigation and 95% during the second. The respective barometric pressures were 722 and
The ACT was carried out on flat terrain in a 2.5 km loop. All subjects completed a total distance of 25 km. Athletes were asked to perform at a very high and constant aerobic exercise intensity (97% of their individual AT). The reference intensity was determined by the APT, and the exercise intensity was constantly monitored and controlled by the athletes and the investigators using heart rate (Sport Tester; Polar, Oy, Finland). The ACT was interrupted every 5 km for three minute rest periods. During rest periods, all subjects drank 200 ml water, and blood samples were taken from an indwelling catheter in an antecubital vein.

Laboratory tests
Blood samples were collected nine times: 24 hours before, 30 minutes before, at 5 km, 10 km, 15 km, 20 km, 25 km, and six hours, and 24 hours after the ACT. They were analysed for the variables listed in Table 2. Changes in plasma volume were determined from haemoglobin and packed cell volume measurements by the method of Wright et al. The laboratory methods used for each of the above analyses are given in Table 2.

Statistical analysis
Results were analysed as a comparison of OS vs PS using a two factor (season by distance) repeated measures analysis of variance. A Tukey post hoc test was used when indicated. p<0.05 was considered significant. For comparison, the distance factor included measurements before and after the ACT, unless depicted otherwise.

RESULTS
Table 3 lists energy intake and percentage energy supply from carbohydrates, fats, and proteins before the trial.

ACT was conducted at an almost uniform running intensity averaging 97.0 (4.9)% (OS = 94.6 (5.1)%; PS = 99.7 (4.1)% of the individual AT as assessed from the heart rate. Therefore the group fulfilled the predetermined criteria for running intensity. This corresponded to an average of 91.2 (4.5)% (OS = 92.3 (5.9); PS = 90.1 (2.6)% of the VO2MAX. Mean lactate concentration was 3.0 (1.9) mmol/l (OS = 2.6 (2.2) mmol/l; PS = 3.4 (1.4) mmol/l). Plasma volumes, determined using haemoglobin concentration and packed cell volume, were similar for the two seasons and did not change significantly during the ACT.

Table 4 shows the aerobic power variables measured in the APT and aerobic capacity variables measured in the ACT in the OS and PS. VO2MAX was significantly lower in the PS than in the OS (p<0.05). Speeds at AT, 4.0 mmol/l lactate threshold (LT), and 2.0 mmol/l LT were all significantly greater in the PS than in the OS (p<0.005). Running intensity in the ACT expressed as a percentage of the speed at 2.0 mmol/l LT was significantly lower in the PS than in the OS (p = 0.008). Running speed was not significantly different between the seasons (p>0.05). However, there was a tendency for an increase in running speed in the PS compared with the OS (p = 0.096), although running intensity (expressed as a percentage of the speed at 2 mmol/l LT) was lower. In the ACT, the speed/intensity ratio was significantly (p<0.001) higher during the PS than the OS, indicating increased speed in relation to intensity.

Figure 1 shows serum TG concentrations. TG peaked at 5 km and then decreased logarithmically during the ACT in both trials (p<0.001, for data collapsed across season). A significant season by distance interaction for TG concentrations was found (p<0.001). At 5 km, it was 1.48 (0.34) mmol/l in the OS compared with 1.90 (0.31) mmol/l in the PS (p<0.001). The two curves crossed at 10 km and were 0.98 (0.22) and 0.73 (0.18) mmol/l at 25 km in the OS and PS respectively (p = 0.016). In all athletes, the decrease in TG concentrations between 5 and 15 km was more pronounced in the PS than the OS. The difference in TG concentrations between 5 and 15 km increased from the OS to the PS similarly to the increase in speed at 2.0 mmol/l LT (fig 2). Plasma FFA concentrations were similar in the two seasons before, during, and after the ACT (fig 3). FFA concentrations averaged 0.56 (0.52) mmol/l before the ACT (data collapsed across season and the time before the ACT). At 5 km, values were 0.91 (0.27) mmol/l and increased to 2.66 (0.74) mmol/l by 10 km (p<0.001). At 15 km, they decreased to 1.89 (0.60) mmol/l (p<0.001) and remained unchanged at 20 and 25 km.

Figure 4 shows serum glucose concentrations. They were similar between seasons and increased from 4.5 (0.9) mmol/l (810 (152) mg/l) 30 min before the start to 7.7 (1.4) mmol/l (1386 (233) mg/l) at 5 km (p<0.001). Glucose decreased throughout the ACT to 5.2 (1.2) mmol/l (936 (226) mg/l) by 25 km (p<0.001). One athlete reached hypoglycaemia (glucose concentration below 2.8 mmol/l or 500 mg/l) during the ACT in the PS. Serum insulin concentrations were also similar between seasons and decreased from 13.1 (7.2) μU/ml at 5 km to 7.6 (4.3) μU/ml by 25 km (p<0.001). Blood lactate did not differ between the seasons and decreased from

<table>
<thead>
<tr>
<th>Substance</th>
<th>Medium</th>
<th>Method</th>
<th>Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>Serum</td>
<td>Enzymatic (PAP)</td>
<td>Cobas Mira, Roche</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>Serum</td>
<td>Enzymatic</td>
<td>Cobas Mira, Roche</td>
</tr>
<tr>
<td>Glucose</td>
<td>Serum</td>
<td>Glucose dehydrogenase</td>
<td>ELISA, Boehringer</td>
</tr>
<tr>
<td>Insulin</td>
<td>Serum</td>
<td>Enzymatic</td>
<td>Cobas Mira, Roche</td>
</tr>
<tr>
<td>Lactate</td>
<td>Blood</td>
<td>Enzymatic</td>
<td>K 1000, Digitana (Horgen)</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>Blood</td>
<td>Photometric</td>
<td>K 1000, Digitana (Horgen)</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>Blood</td>
<td>Capillary count</td>
<td>K 1000, Digitana (Horgen)</td>
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</table>

PAP, Peroxidase-antiperoxidase smear test; ELISA, enzyme linked immunosorbent assay.

Table 3

<table>
<thead>
<tr>
<th>Energy intake in the off season and pre-season before the trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off season</td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>2956 (750)</td>
</tr>
<tr>
<td>3270 (856)</td>
</tr>
</tbody>
</table>

Data are mean (SD) (n = 9). There were no significant differences between the seasons.
Table 4 Aerobic power and capacity variables in the off season (OS) and pre-season (PS)

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 3)</th>
<th>Men (n = 6)</th>
<th>Total (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OS</td>
<td>PS</td>
<td>OS</td>
</tr>
<tr>
<td>APT V˙O₂MAX (ml/min/kg)</td>
<td>66.5 (5.3)</td>
<td>63.1 (6.9)</td>
<td>75.9 (4.9)</td>
</tr>
<tr>
<td>Speed at AT (km/h)</td>
<td>16.6 (0.6)</td>
<td>16.5 (1.0)</td>
<td>17.8 (0.6)**</td>
</tr>
<tr>
<td>Speed at 4 mmol/l LT (km/h)</td>
<td>17.2 (0.3)</td>
<td>17.7 (0.2)</td>
<td>19.2 (0.5)**</td>
</tr>
<tr>
<td>Speed at 2 mmol/l LT (km/h)</td>
<td>15.1 (1.3)**</td>
<td>16.2 (0.9)</td>
<td>16.8 (0.5)**</td>
</tr>
<tr>
<td>ACT Speed (km/h)</td>
<td>14.3 (1.4)</td>
<td>15.0 (0.7)</td>
<td>16.9 (0.8)</td>
</tr>
<tr>
<td>Intensity (% of speed at 2 mmol/l LT)</td>
<td>95.2 (5.9)</td>
<td>92.7 (3.6)</td>
<td>100.8 (3.8)**</td>
</tr>
</tbody>
</table>

Data are mean (SD).

*p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001, significantly different from the PS.

ACT, aerobic capacity test; APT, aerobic power test; AT, anaerobic threshold; LT, lactate threshold; V˙O₂MAX, maximal oxygen consumption.

3.4 (1.0) mmol/l at 5 km to 2.8 (1.9) mmol/l by 25 km (p = 0.034). Plasma volume did not change significantly in the two seasons or during the ACT.

DISCUSSION
When comparing different training seasons with changes in circulating energy sources that occur during prolonged exercise, our study is in the forefront of assessing elite athletes at the AT (aerobic high intensity performance) during 25 km running. In this study, FFA and TG concentrations were much higher than expected, yielding interesting results. (a) During prolonged exercise, FFA and TG concentrations peaked at 5 and 10 km followed by a logarithmic decrease. (b) The two training seasons (OS and PS) had significantly different patterns of TG concentrations during prolonged and high intensity running. (c) In all athletes, the decrease in TG concentration between 5 and 15 km was more pronounced in the OS than the PS, and the difference increased similarly in the 2.0 mmol/l LT. (d) During prolonged exercise, the pattern of glucose, insulin, and FFA concentration did not differ between the two seasons.

It is generally accepted that TG is the main intracellular substrate, along with FFAs from adipose tissue. Our findings suggest that circulating TG may have a role in energy provision also. In our athletes, the serum TG concentrations peaked at 5 km and subsequently decreased logarithmically during the ACT. We observed a high initial peak and a subsequent decrease in circulating TG. This may be due to the subjects being well trained and the high exercise intensity in this study. When the two training seasons are compared, in the PS the initial TG concentration was significantly higher. The two curves crossed at 10 km, and the TG concentrations were lower in the PS than the OS at 15, 20, and 25 km. The resting concentrations of TG before and after the ACT did not differ between the two training seasons. As our athletes increased their performance concentration for prolonged exercise, a training effect possibly reflects this occurrence. In the PS, the better trained athletes may show increased availability of serum TG at the beginning of the run and improved delivery to the working tissue during the ACT. Moreover, the difference in TG concentration at 5–15 km increased similarly to the speed at 2.0 mmol/l LT (fig 2). FFAs showed an initial increase (2.66 mmol/l) at 10 km and stabilised at about 2.0 mmol/l. The maximal FFA concentrations observed were twice as high (or more) as the values found in other studies carried out at moderate intensity.5 8 26 29 The pattern with an initial increase in FFAs during the first 30 minutes is in line with the findings of Romijn et al and depends on the exercise intensity. Furthermore, the studies conducted in untrained subjects showed a continuous increase in plasma FFAs during prolonged exercise.1 4 21 In well trained athletes, however, there is evidence that FFA concentration peaks at the beginning of an intensive workload and decreases after about 30 minutes.5 27 28 Although we did not find significant differences between the different training seasons, we noted a similar or delayed pattern in TG concentration. Other studies5 26 32 show diminished circulating plasma FFA concentrations during prolonged exercise as a result of training.

![Figure 1](https://www.bjsportmed.com)

**Figure 1** Triglyceride concentration before, during, and after the aerobic capacity test (ACT) in the off season (OS) and pre-season (PS) (n = 9). Values are mean (SD). – 24 h, – 0.5 h, + 6 h, and + 24 h indicate 24 hours before, 0.5 hours before, 6 hours after, and 24 hours after the start of the ACT. Season by distance interaction p < 0.001; no season main effect; *p < 0.05 and **p < 0.001 respectively; †p < 0.05 and ††p < 0.001 respectively).

![Figure 2](https://www.bjsportmed.com)

**Figure 2** Running speed at the 2.0 mmol/l lactate threshold (LT) compared with the decrease (∆) in serum triglyceride concentration between 5 and 15 km during the aerobic capacity test (n = 9).
not find any differences in glucose, insulin, and lactate between the two training seasons during the ACT. The ACT was performed at an intensity similar to that of competition. A limitation of this study was the acceptance of individual training schedules. Nevertheless, all athletes performed their training with the goal of improving aerobic capacity for prolonged exercise between the OS and PS. In accordance with the usual training studies, this should occur mainly through an increase in training volume. Our subjects increased their training volume by 46% from the OS to PS. The hypothetical improvement in aerobic capacity for prolonged exercise in the PS compared with the OS is documented by a significantly increased speed in the PS at AT, 4.0 mmol/l LT, and 2.0 mmol/l LT (table 4). The assessments of AT and LT are considered to be predictive of competition performance in well trained, long distance athletes. Nevertheless, there was a decrease in VO2\text{MAX} (table 4). However, this is in line with the knowledge that the assessment of VO2\text{MAX} is not a valid indicator of aerobic capacity for prolonged exercise in elite triathletes and long distance athletes.

A logarithmic decrease in TG concentrations occurred during the first 20 km of prolonged running. Significantly different patterns of TG concentration during prolonged running were found in the OS and PS, indicating an effect of the training status with respect to the aerobic capacity of prolonged exercise on the amount of logarithmic decrease in TG concentration.

Notably, in our well trained subjects during prolonged exercise near the AT, the pattern of glucose and insulin concentrations did not differ between the two seasons.

Muscle glycogen concentration has been suggested to be the key factor contributing to aerobic capacity in prolonged high intensity exercise. Performance time during a given workload can be influenced by diet, and there is a strong correlation between initial muscle glycogen concentration and exercise time. Although we did not measure glycogen concentrations in the athletes in our study, we attempted to standardise nutritional intake and training before the test to reduce modulation in glycogen concentrations. We found a continuous and significant decrease in serum glucose during exercise. However, the athletes (except one) did not show hypoglycaemic concentrations (<2.8 mmol/l). In the PS, hypoglycaemia occurred in one athlete at 15 km, but he recovered fully at 20 and 25 km by adjusting his running speed. As previously discussed, differences in circulating glucose concentrations during prolonged exercise between trained and untrained subjects may depend mainly on exercise intensity and the subject’s performance. We did determine from haemoglobin concentration and packed cell volume, were minimal during the ACT and similar in both seasons. However, to be really sure that shifts in plasma...
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volume were not affecting TG or FFA measurements, calculation of plasma volume from total proteins or glycerol would have given additional information. Athletes performed in the ACT at a similar speed and near their individual AT (table 4).

At an exercise intensity comparable to competition, the availability and use of circulating substrate sources is controversial. Our study was performed with elite athletes at an aerobic high intensity exercise (near the AT) during a 25 km run comparing different training seasons with changes in circulating energy sources that occurred during prolonged exercise.

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