J SPORTS MED PHYS FITNESS 2007;47:491-5

Ketone production in ultra marathon runners

J. WEIBEL [1], T. GLONEK [2]

Aim. **The aim of this study was to determine the magnitude of ketone production in ultra marathon runners and what affect if any this has on performance.**
Methods. **Participants in the Cliff Young Australian Six Day Race (n=31) provided a prerace urine sample and, then, random urine samples throughout the duration of the event, ranging from 4-20 samples each. Based on urinalysis results, participants were divided into two groups: those who formed ketones (ketone group), and those who did not form ketones or formed ketones only once during a race at the lowest recordable value (non-ketone group).**
Results. **The average ketone level of the 22 athletes in the ketone group (value±standard deviation: 5.67±5.59 mg/dL) was statistically different from 9 athletes who were in the non-ketone group (0.18±0.14 mg/dL) (P<0.05). The average distances run for the two groups were 498.09±153.99 and 535.6±181.08 km, respectively (P=0.56). When average ketone value was compared, excluding runners who did not complete the race, the ketone group (5.88±1.37) remained statistically different from the non-ketone group (0.2±0.45) (P<0.05). The average distances for those athletes who completed the race were 583.9±116.09 and 557.8±85.82 km, respectively (P=0.52).**
Conclusion. **We conclude that although two runner sub-populations were revealed, runners who produce ketones and runners who do not make ketones, the level of ketones produced did not affect overall distance run, which is the performance criterion of the race. The nature of this extreme event has illuminated a physiologic difference among ultra marathon runners, and although this difference does not appear to affect race performance, the long-term health consequences are unknown and additional rigorous research is warranted.**

KEY WORDS: Ketones - Ketosis - Athletes - Urinalysis - Exercise.

[1]Department of Family Medicine
University of Wisconsin, Appleton, WI, USA
[2]Department of Osteopathic Manipulative Medicine
Midwestern University/Chicago
College of Osteopathic Medicine, Downers Grove, IL, USA

Funding.—This study was supported by the Andrew S. Makenzie Research Fund.

Acknowledgements.—We thank Drs K. E. Nelson and K. Heinking for professional guidance and mentorship in medicine, and B. Sutcliffe and M. Matthews of the Cliff Young Australian Six Day Race for their co-operation and encouragement.

Received on November 10, 2006.
Accepted for publication on March 27, 2007.

Address reprint requests to: Dr. J. Weibel, University of Wisconsin Department of Family Medicine, 229 South Morrison Street, Appleton, WI, United States 54911-5725. E-mail: Jennifer.Weibel@fammed.wisc.edu

Ketogenesis occurs when carbohydrates are metabolically unavailable for oxidation in the tricarboxylic acid cycle (TCA) and is seen primarily in such states as uncontrolled diabetes,[1] starvation,[1, 2] diets entirely composed of fat,[1] and in association with periods of prolonged, intense exercise.[2-4] The effect of exercise on ketone body production has been studied for nearly a century; much of this literature focuses on low carbohydrate intake prior to exercise, postexercise ketosis, and the utilization of free fatty acids (FFA) as energy. Previous studies involve a fasting or carbohydrate loading protocol prior to an exercise regime that is performed by fit and unfit subjects.[4-6]

Blood samples and/or muscle biopsies have been tested for ketones,[2] glucose,[5-10] FFA, [6, 8-10] glycerol,[8, 10] and β-hydroxybutyrate.[8] Some protocols failed to elicit ketone body production.[3, 11] In general, it is untrained or sedentary subjects with a lower carbohydrate intake, who are model ketone producers, compared to trained athletes consuming a high carbohydrate diet.[4] Although several studies set up endurance protocols spanning up to several hours, no studies were found specifically looking at the effect of ketone production on performance during an ultra-endurance athletic event or in the presence of carbohydrate ingestion.[6, 10-12]

The ultra marathon is the ultimate test of human endurance: athletes run for days. It is defined as any running event that is longer than 42 km (26.2 miles), the standard marathon distance, and provides a unique venue for studying the effects of prolonged exercise on metabolism. Participants in the Australian Six Day Race run for a continuous 144 h, stopping only for short naps and quick meals. Thought to be in a self-imposed state of starvation, a pilot study was conducted which found that ultra marathon participants were forming urine ketones. Based on these initial results and on what is already known about the physiology of glucose metabolism and storage, all athletes participating in an ultra marathon could be assumed to be forming ketones due to depleted glycogen stores and increased FFA oxidation. Given this, two contradictory hypotheses were formed: 1) athletes in ultra marathons oxidize FFA for energy. Those who produce ketones are dumping valuable energy in the form of 4 carbon butyric acid chains and, thus, will not be able to run as far or as fast as those who are utilizing the available energy (*i.e.* ketone producers perform poorly) or 2) athletes who are running fastest and, therefore, are exercising at a maximum threshold level of glucose sequestration are utilizing FFA for energy at a rate greater than the peripheral tissue can oxidize all of the by-products, thus produce ketones (*i.e.* ketone producers perform well). This study was designed to determine to what extent ultra marathon participants form ketones and what affect this has on overall race performance.

This type of event is of particular importance in the study of human physiology, primarily in the areas of carbohydrate and fatty acid metabolism, and ketone formation. It is reasonable to assume that athletes will exercise harder and longer during a competitive event, rather than while participating in a laboratory study, making them ideal subjects for this type of research. During the course of two 6-day events, we measured urine ketones from ultra marathon runners.

Materials and methods

Site

The Cliff Young Australian Six Day Race was the site of the study: an annual international invitational held at Colac (Melbourne). The races were held in Memorial Square on a 400-m oval grass/dirt track. Runners tested their endurance over a consecutive 144 h, changing the direction of running every 4 h to distribute stresses evenly throughout the body. Participants were required to complete a minimum of 65 km (40.6 miles) daily to prevent disqualification. Each athlete ran, ate, and slept on their own schedule.

Subject recruitment

Participants in the Australian Six Day Race (November 16-22, 2003), renamed the Cliff Young Australian Six Day Race (November 21-27, 2004), were recruited, and all signed a Midwestern University, institutionally-approved informed consent form. Of 39 registered athletes, 31 agreed to participate in the study. The age of the participants ranged from 33 to 78 years, with an average age of 53.8 years. Twenty-nine had participated in at least one ultra marathon prior to this event and 5 runners had competed in the Colac race at least 10 times. Veteran runners had each completed a minimum of ten 6-day events and had an average of over 20 years running experience. Nine runners participated in both events. A complete record of participant characteristics is listed in Table I.

Data collection

Each athlete provided a prerace urine sample and, then, random urine samples throughout the duration of the event, ranging from 4-20 samples each. Urinalysis was done in the field using Bayer Multistyx®. The degree of ketogenesis was graded as 0, 5, 15, 40, 80, and 160 mg/dL, according to the instructions provided. Urine specific gravity (g/mL) also was recorded. Individual lap-by-lap timing data were provided by the race co-ordinators. Five athletes did not complete the race for reasons unrelated to the study.

TABLE I.—*Characteristics of participants in the Cliff Young Australian Six Day Race.*

Gender	Age group (years)	# Ultra races
M	30-35	3-5
M	30-35	5-10
M	30-35	1
M	30-35	>10
M	35-40	5-10
M	35-40	5-10
F	35-40	1
M	40-45	>10
M	40-45	>10
M	45-50	>10
M	45-50	>10
M	45-50	3-5
F	50-55	>10
F	50-55	>10
M	55-60	>10
M	55-60	3-5
M	55-60	>10
M	60-65	>10
M	60-65	5-10
M	60-65	5-10
M	60-65	3-5
M	60-65	>10
M	60-65	>10
M	60-65	5-10
M	65-70	>10
M	65-70	>10
M	65-70	>10
M	65-70	3-5
M	65-70	>10
M	75-80	>10
M	75-80	>10

M: male; F: female.

TABLE II.—*Average ketone levels* vs *distance run for all 31 ultra marathon participants.*

	All runners	
Runner group	Average ketones (mg/dL)	Distance (km)
Ketone group	5.67±5.59	498.1±153.99
Non-ketone group	0.18±0.14	535.6±181.08
Significance	P<0.05	P=0.56

Values are expressed as mean±standard deviation.

Statistical analysis

Data were analysed using an independent samples t-test, except when ketone levels and specific gravity were correlated using the Pearson correlation (two-tailed significance). Statistical significance was accepted at P<0.05. Values are presented as means ± standard deviation (SD).

Results

Performance, according to the race criterion, was measured as overall distance covered during the 144-h race. Based on urinalysis results, participants were divided into two groups: those who formed ketones (ketone group), and those who did not form ketones or formed ketones only once during a race at the lowest recordable value (non-ketone group). The average ketone level of the 22 athletes in the ketone group (value±SD: 5.67±5.59 mg/dL), was statistically different from that of the 9 athletes who were in the non-ketone group (0.18±0.14 mg/dL) (P<0.05). The average distances run for the two groups were 498.09±153.99 km and 535.6±181.08 km, respectively (P=0.56) (Table II). No participants formed ketones prior to the race.

The performance for each runner in the study is presented in Figure 1. Note that each bar in the figure corresponds to a single runner. In Figure 1, the minimum distance corresponding to finishing the race is 390 km. Of the 5 runners who did not complete the race, one was in the low-ketone group and 4 produced ketones at the same levels as runners who finished the race. The horizontal line at 0.5 mg/dL indicates the division between the ketone group and the non-ketone group. Overall distance run appears to have little effect on ketone production. Note for the winners of the two events (746 km, 2003; 756 km, 2004), one was in the non-ketone group and the other produced ketones at a low level.

When average ketone values were compared, excluding runners who did not complete the race, the ketone group (5.88±1.37) remained statistically different from the non-ketone group (0.2±0.45) (P<0.05). The average distances for those athletes who completed the race were 583.9±116.09 km and 557.8±85.82 km, respectively (P=0.52) (Table III).

The specific gravity means between the two groups were compared. No significant difference was found: non-ketone group had 10.25±0.073 g/mL, and ketone group 10.25±0.07 g/mL (equal variance not assumed; P=0.96). Correlations for each group were computed between ketone level and urine specific gravity. For the non-ketone group no correlation was found (Pearson correlation: 0.1; two-tailed significance: 0.541). For the ketone group, a marginal correlation was computed that was significant (Pearson correlation: 0.22; two-

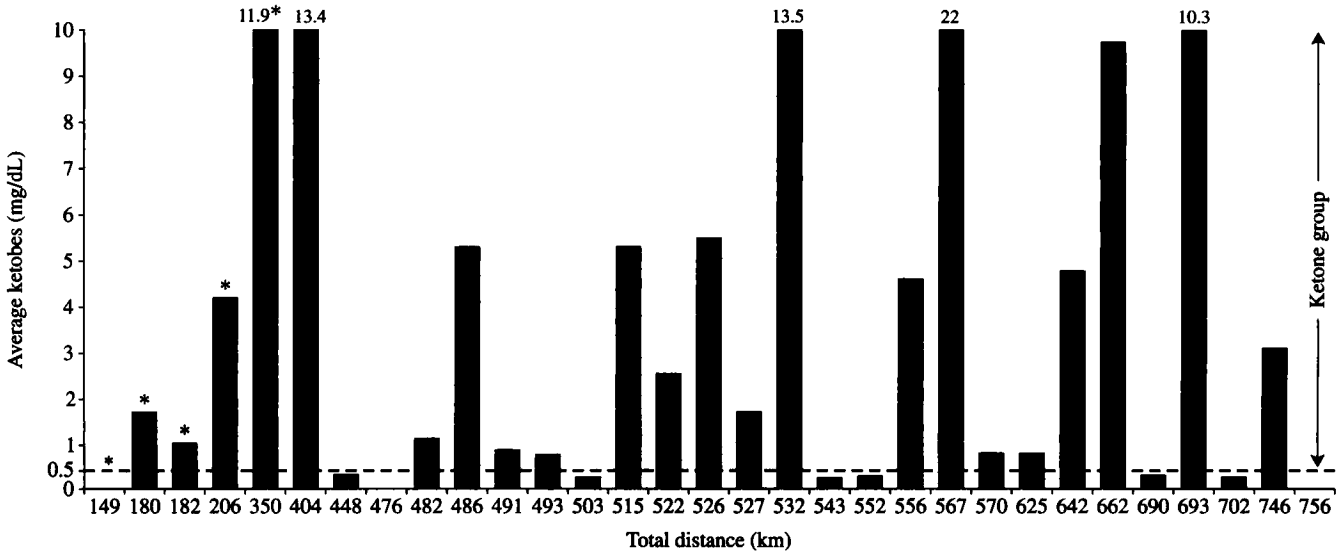

Figure 1.—Results for each individual runner is given in kilometers on the x-axis. The bar indicates average ketone formation for each runner. The minimum distance corresponding to finishing the race is 390 km. *Runners who did not complete the race (n=5). Of the 5 runners who did not complete the race, one was in the low-ketone group and 4 produced ketones at the same levels as runners who finished the race. The horizontal line at 0.5 mg/dL indicates the division between the ketone group and the non-ketone group. (In order to display small values, large values exceed the graph area: their numeric values of ketone production are indicated for these runners).

TABLE III.—*Average ketone levels* vs *distance run for all 26 ultra marathon participants who completed the events.*

	Finishers only	
Runner group	Average ketones (mg/dL)	Distance (km)
Ketone group	5.88±1.37	583.9±116.09
Non-ketone group	0.2±0.45	557.8±85.82
Significance	P<0.05	P=0.52

Values are expressed as mean±standard deviation.

tailed significance: 0.002). Although a small significant correlation was found between specific gravity and urine ketone concentration, this correlation is considered marginal at best and was not investigated further.

Discussion

The accumulation of ketone bodies (acetoacetate and D-β-hydroxybuterate) in the blood generally occurs from the synthesis of excess acetyl-CoA. Most acetoacetate is formed in the liver from a process in which acetoacetyl-CoA is derived from two acetyl-CoA moieties and then undergoes deacylation to yield free acetoacetate. However, some acetoacetyl-CoA is in the chemical form of the 4-carbon butyric acid by-product resulting from the β oxidation of long chain fatty acid molecules. The free acetoacate so produced is enzymatically reduced to β-hydroxybutyrate. It is a mixture of acetoacetate and β-hydroxybuterate that diffuses out of liver cells into the bloodstream and is transported to peripheral tissues. Ordinarily, these ketone bodies are rather low in blood, but in fasting, or diabetes, and in this case, extreme energy consumption, ketosis arises because the rate of formation by the liver and from FFA oxidation exceeds the capacity of utilization in the peripheral tissues. Hence, their accumulation in the blood.[13]

A factor that will accelerate ketosis is the lack of oxaloacetate (an essential TCA intermediate) from carbohydrate metabolism.[1] Oxaloacetate is required to bind with acetyl-CoA before it can be processed in the TCA. It is also a required substrate for gluconeogenesis (which is occurring simultaneously with FFA oxidation during low carbohydrate states). When glucose levels are low, the oxidation of the TCA will be inhibited due to reallocation of these intermediates. The result of too little glucose in the system is: 1) the inability to utilize the available ketone bodies, which will be lost through excretion, and 2) impaired func-

tion of the TCA and, thus, the availability of energy. This raises the question of glucose availability in these extreme events. Koeslag *et al.*,[2] in their study on post-exercise ketosis and starvation, demonstrated that ketone formation from exercise and from starvation are *via* the same mechanism. Subjects in this study were not diet-restricted, indicating that those in the ketone group consumed inadequate carbohydrates in relation to physiologic requirements.

The results from this study are inconclusive. Apparently, in an ultra marathon event, an alternate pathway is operant, because the performance in the race does not appear to be related to ketone production, *i.e.*, some performers do not make ketones, some make ketones consistently, and still others make ketones, but then return to normal levels as the race progresses. However, these levels were not related to race performance. In addition, there are multiple factors unrelated to ketone production which may have affected the race performance of an individual athlete, including resistance to fatigue, diet, injury, liver size, and overall conditioning.

Although the renal threshold for ketones is quite low, making qualitative measurements readily available from urine, urinalysis is not the most accurate way to measure ketones and is limited in that it reveals nothing about concentration of glucose, FFA, or the predominant type of ketone body in the blood. Ideally, the measurement of FFA degradation and ketone production would be from liver biopsy and serum samples. However, this was not practical due to the competitive nature and the duration of the event. Recently, finger-prick,[14] breath acetone,[15] and automated urine test strip readers[16] have become available for rapid quantitative ketone body testing, which would be useful in future studies. Also interesting, although only observed during this study, is ketone production during moderate to high carbohydrate consumption. Future studies might also include dietary logs as a factor in ketone levels produced.

Conclusions

The mechanism by which athletes participating in a 144-h event would develop ketones in their urine is explained by existing knowledge of human physiology. What remains intriguing is that, while consuming carbohydrates, some athletes produce ketones at different levels and times during the race, while others produce no ketones at all. Moreover, what are the unknown affects these differences have on performance? These questions necessitate continued research on the physiology of endurance athletes and make ultra marathon runners a unique model for studying metabolism under extreme athletic conditions.

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