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Association Between Energy Balance and Metabolic Hormone Suppression During Ultra-Endurance Exercise

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Abstract

Ultra-endurance athletes often accumulate an energy deficit when engaging in ultra-endurance exercise, and upon completion of the exercise, they exhibit endocrine changes that are reminiscent of starvation. However, it remains unclear whether these endocrine changes are a result of the exercise *per se* or secondary to the energy deficit, and more importantly, whether these changes can be attenuated by increased dietary intake. Our goal was to assess the relationship between changes in key metabolic hormones following ultra-endurance exercise and measures of energy balance. Metabolic hormones as well as energy intake and expenditure were assessed in 14 well-trained male cyclists who completed a 1,230-km ultra-endurance cycling event. After completion of the event, serum testosterone (-67±18%), insulin-like growth factor-1 (IGF-1) (-45±8%), and leptin (-79±9%) were significantly suppressed ($p<0.001$), and remained suppressed after a 12-h recovery period ($p<0.001$). Changes in IGF-1 were positively correlated with energy balance over the course of the event ($r=0.65$, $p=0.037$), which ranged from a 11,859 kcal-deficit to a 3,593 kcal-surplus. The marked suppression of testosterone, IGF-1, and leptin following ultra-endurance exercise is comparable to changes occurring during acute starvation. The suppression of IGF-1, but not that of other metabolic hormones, was strongly associated with the magnitude of the energy deficit, indicating that athletes who attained a greater energy deficit exhibited a more pronounced drop in IGF-1. Future studies are needed to determine whether increased dietary intake can attenuate the endocrine response to ultra-endurance exercise.

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1. Introduction

Ultra-endurance cycling events such as *Paris-Brest-Paris*, the *Race Across America*, or *Trondheim-Oslo* have become increasingly popular in recent years. Ultra-endurance exercise is considered as one of the greatest physiological challenges,[1] and adequate dietary strategies are essential for successful participation in these events.[2] Continuous aerobic exercise for multiple hours requires the availability of metabolic fuels to sustain a high level of energy expenditure, and ultra-endurance athletes are advised to consume adequate amounts of food, and particularly energy dense foods, to remain in energy balance and to not compromise their performance and health.[2] However, available literature suggests that most athletes participating in prolonged endurance events fail to match the energy expended during continuous exercise and subsequently attain an energy deficit while exercising. Even though there is only limited data available for ultra-endurance cycling, previously reported energy expenditure (6,420-17,965 kcal/d) consistently exceeded dietary energy intake (4,918-9,612 kcal/d), thus resulting in energy deficits ranging from 1,000 to 8,352 kcal/d.[3,4] Reasons why endurance athletes fail to eat adequately include satiety and gastrointestinal discomfort while exercising, limited gastrointestinal capacity to absorb nutrients, and difficulties in balancing food and fluid consumption to prevent dehydration.[2,5,6] We recently reported that energy balance can vary considerably among ultra-endurance cyclists, even when they competed at similar exercise intensities, and that this large variation in energy balance is predominantly a result of the highly varying intake of solid foods.[5]

Previous literature suggests that prolonged endurance exercise is also associated with the suppression of key metabolic hormones, including leptin and testosterone;[7-10] whereas, the effect of prolonged endurance exercise on other metabolic hormones, such as IGF-1[11,12] and ghrelin remain oblique. However, it is only poorly understood whether these endocrine changes are a result

of prolonged exercise *per se* or secondary to the energy deficit that frequently occurs during ultra-endurance exercise. It has been well established in the literature that leptin, testosterone, and insulin-like growth factor 1 (IGF-1) serve as biochemical markers of starvation,[13-15] and the suppression of these metabolic hormones is related to the magnitude of the energy deficit.[14] It is well recognized in the literature that the suppression of metabolic hormones secondary to prolonged energy deficiency is associated with unfavorable health outcomes, such as impaired reproductive, bone, and cardiovascular health.[16-19] The suppression of testosterone and IGF-1, which both possess strong anabolic properties, may further result in unfavorable alterations in fuel utilization and more importantly, in the long-term loss of muscle mass and muscle function.[20,21] Therefore, it remains unclear whether strategies to increase dietary energy intake during prolonged ultra-endurance exercise in efforts to prevent energy deficiency could also attenuate the suppression of metabolic hormones, and minimize the detrimental effects of energy deficiency in the long-term.

The purpose of the present study was to assess changes in key metabolic hormones during ultra-endurance bicycling and to determine the relationship between these changes and measured energy balance. We hypothesized that serum concentrations of leptin, testosterone, and IGF-1 would be significantly suppressed following the cycling event, and that changes in these parameters would be significantly correlated with energy balance such that athletes who accumulated a larger energy deficit would exhibit a more pronounced suppression when compared to athletes who accumulated a smaller energy deficit or remained in energy balance.

2. Methods

Study design

The present study is a secondary analysis of previously unpublished changes in metabolic hormones following an ultra-endurance cycling event. Data were collected in ultra-endurance cyclists who participated in the bicycle event *Paris-Brest-Paris* (PBP) in August 2011. *Paris-Brest-Paris* is a so-called *Brevet*, and cyclists must finish the distance within a prescribed time limit. Support vehicles were not permitted on the course, but cyclists were allowed to stock up on food, beverages, and clothing at various time stations throughout the course. The participants in our study completed the 1,230-km event in 54 h, which was well below the time limit of 80 h. Participants did not ride for a total of 11:12 h due to intentional breaks to refill foods and beverages, change clothes, and rest as well as unintended breaks due to mechanical issues and severe weather conditions, resulting in a net cycling time of 42:48 h and an average speed of 28.7 km/h. We have previously reported dietary intake, energy expenditure, and hydration status from the same data set.[5] We chose this group of athletes because even though the athletes completed the event as a group, their energy intake, and consequently their energy balance varied considerably, [5] whereas environmental factors were identical among all participants.

Participants

Well-trained male amateur cyclists who were enrolled in a commercial training program for ultra-endurance cyclists were recruited to participate in this study. Prior to the start of the study, written consent was obtained from all participants. The study was approved by our University's institutional review board. All participants were healthy and did not take any medication at the time of the study. Of the 18 participants who were initially enrolled, 4 cyclists did not complete the event because of issues unrelated to the investigation and were excluded from the present

analysis. The 14 remaining participants were on average 43.6 (SD: 7.8) years old, 180.1 (SD: 6.2) cm tall, weighed 74.1 (SD: 6.8) kg and had a maximal oxygen uptake (VO_{2max}) of 63.2 (SD: 3.3) ml/min/kg.

Preliminary Visit

Between 3-6 weeks prior to the event, participants completed a screening visit, which included anthropometric assessments and a graded exercise performance test to assess VO_{2max} and submaximal energy expenditure, which served to quantify energy expenditure during the event. The test was conducted on a stationary bicycle ergometer (SRM, Jülich, Germany), and participants cycled at intensities of 100 W, 150 W, 200 W, and 250 W for 4 minutes each, before exercise intensity was increased by 25 W every 30 seconds until VO_{2max} was reached. Oxygen uptake and carbon dioxide production were measured in breath-by-breath mode (ZAN 600, nSpire Health, Inc., Colorado, USA). Submaximal oxygen uptake and carbon dioxide production were converted to energy expenditure using the Weir equation.[22]

Metabolic hormone and metabolite assessment

Venous blood samples were collected by a trained phlebotomist between 06:00 and 09:00 on the morning before the start of the event following an overnight fast (pre), within 120 minutes after the finish (22:00-23:59; post), and again following an overnight recovery period of approximately 12 h prior to breakfast (09:00-12:00; post+12h). Blood samples were obtained from all 14 participants before the start of the event and following the overnight recovery period. Unfortunately, no blood samples could be obtained within 120 minutes after the finish from three participants; consequently, hormone and metabolite data were only available for 11 participants at this time point. Blood samples were processed to obtain serum, and serum aliquots were stored at

-20C within 1 h of collection. Following completion of the event, samples were shipped to our laboratory and stored at -80C until analysis. Serum leptin was assessed using a sensitive immunoassay (Mediagnost, Reutlingen, Germany) with an analytical range of 0.05-5 ng/mL and a precision of ≤7.5%. Serum IGF-1 was assessed on an Immulite 2000 (Siemens Healthcare, Eschborn, Germany) with a sensitivity of 20 ng/mL and a precision ≤8.1%. Serum concentrations of total testosterone, and insulin were assessed on a fully automated chemiluminescent immunoassay system (Advia Centaur, Siemens Healthcare, Eschborn, Germany). Analytical sensitivity were 0.1 ng/mL (testosterone) and 10 µU/mL (insulin) and total precision (intra- and inter-assay coefficients of variation) was ≤7.6% (testosterone) and ≤7.5% (insulin). Ghrelin was assessed using a radioimmunoassay (Merck Chemicals, Schwalbach, Germany) with a sensitivity of 100 pg/mL and inter- and intra-assay precision ≤17.8%. Serum glucose was measured on an automated system (Advia, Siemens Healthcare, Eschborn, Germany, sensitivity: 4 mg/dL, precision ≤1.9%) and glycerol (Sigma, St. Louis, USA, sensitivity: 0.07 mmol/L, precision: ≤11%) and free fatty acid (FFA) (Wako, Neuss, Germany, sensitivity: 0.07 mmol/L, precision: ≤1.5%) were assessed photometrically. Assay results below the limit of detection were adjusted to the lower end of the analytical range, and serum concentrations were adjusted for changes in plasma volume.[23]

Energy Balance

Energy balance was calculated as the difference between dietary energy intake and energy expenditure. Because each athlete consumed food and beverages *ad libitum*, dietary intake was obtained by direct observation for each individual athlete.[5] Participants received all food and beverages from supporting staff. All food and beverages were packed individually for each athlete and were weighed to the nearest gram prior to loading the support vehicles before the start of the

event. Because supporting staff were not allowed on the route, participants received food and beverages only at the start and the 14 time stations along the route and had to carry all items in their jersey pockets or in bike bottles. Not consumed food and beverages were returned to support staff. After each time station and upon completion of event, all food and beverages remaining in the support vehicles as well as returned items were weighed again and recorded. Food and beverage intake between the finish and the next morning was not recorded. Energy expenditure was computed from power output, which was recorded continuously using power meters that were mounted to each participant's bottom bracket (SRM, Jülich, Germany). Power output was converted to energy expenditure utilizing each participant's relationship between power output and submaximal energy expenditure, as determined during the preliminary visit.[5]

Statistical analysis

Statistical analyses were performed with R (version 2.14.1). If not stated otherwise, data were reported as mean ± standard deviation. Data were tested for normality using the Kolmogorov-Smirnov test. To assess changes between time points (pre, post, post+12h), one-way analysis of variance with repeated measures was applied for normally distributed data, and the Kruskal-Wallis test was chosen for not normally distributed data. The level of significance was set at $p<0.05$, and was adjusted for multiple comparisons using Holm's correction. Spearman's correlation coefficient was used to determine the association between changes in endocrine and metabolic outcomes and energy balance. Based on previously published changes in metabolic hormones during prolonged strenuous exercise as well as previous findings from our laboratory, a sample size of n=7 was deemed sufficient to detect changes in leptin, IGF-1, and testosterone of 32%, 57%, and 53%, respectively[9-11,24] at $p<0.05$ at a power of 0.8, and a sample size of 11 was deemed

sufficient to detect a correlation of $\rho>0.6$ between changes in metabolic hormones and measures of energy balance at $p<0.05$ at a power of 0.8.

3. Results

Changes in Metabolic Hormones and Metabolites

Over the course of the cycling event, serum leptin (Figure 1a) dropped by 68 ± 35%, (-1.0 ± 0.6 ng/mL, $p<0.001$ vs. pre), and remained suppressed following the 12-h recovery period (-36 ± 138%; -0.8 ± 0.7 ng/mL; $p<0.001$ vs. pre). Likewise, IGF-1 (Figure 1b) dropped by 45 ± 8 % (-76 ± 18 ng/mL, $p<0.001$ vs. pre), and remained suppressed following the 12-h recovery period (-30 ± 18%; -55 ± 40 ng/mL; $p<0.001$ vs. pre). At the start of the event, no athlete exhibited serum IGF-1 concentrations below 2.5th percentile for male adults (25-45 years, 95 ng/mL)[25], but IGF-1 dropped below 95 ng/mL in 7 out of 11 athletes (64%) after completion of the event, and remained below that threshold in 5 athletes (36%) after 12 hours of recovery. Serum testosterone (Figure 1c) was suppressed by 67 ± 18% (-2.3 ± 1.0 ng/dL; $p<0.001$ vs. pre) following completion of the event. Even though testosterone increased during the 12-h recovery period ($p=0.019$), it remained 45 ± 14% lower than before the event (-1.8 ± 1.0 ng/dL; $p<0.001$ vs. pre). Only one athlete (7%) had a serum testosterone below the lower end of the clinical range of 230 ng/dl[26] prior to the event. Following completion of the event, serum testosterone was below the clinical range in all 11 athletes (100%) and remained below the clinical range after the 12-h recovery period in 10 of 14 athletes (71%). There were no significant alterations in insulin and ghrelin at any time point (Table 1). Serum concentrations of FFA and glycerol were significantly elevated after completion of the event when compared to the start, but returned to baseline concentrations after the 12-h recovery period. Changes in glucose following completion of the event failed to reach statistical significance ($p=0.069$).

Correlation between Metabolic Hormones and Energy Balance

As previously reported,[5] energy balance over the course of the event ranged from a 11,859 kcal-deficit (-5,271 kcal/24h) to a 3,593 kcal-surplus (+1,597 kcal/24h), with an average energy deficit of 5,554 ± 4,567 kcal (2,468 ± 2,030 kcal/24h). Energy intake during the 54-h event ranged from 13,657 kcal (6,070 kcal/24h) to 28,395 kcal (12,620 kcal/24h) and energy expenditure ranged from 21,801 kcal (9,689 kcal/24h) to 30,567 kcal (13,585 kcal/24 h). There was a strong positive correlation between energy balance and changes in IGF-1 (ρ=0.65, p=0.034), indicating that athletes who were exposed to a greater energy deficit throughout the event exhibited greater reductions in IGF-1 when compared to athletes who were exposed to a smaller energy deficit or who remained in energy balance. There was further a trend suggesting that changes in IGF-1 were negatively correlated with energy intake (ρ=0.50, p=0.09). There were no significant correlations between changes in other metabolic hormones and measures of energy balance (Table 2).

4. Discussion

This study is the first of its kind to integrate measurements of metabolic hormones and energy balance in the same individuals such that endocrine changes could be directly related to the actual energy deficit. This approach is needed to understand whether the suppression of metabolic hormones occurs in response to ultra-endurance exercise *per se* or secondary to the energy deficit that is often accumulated during such exercise. To this end, we were able to demonstrate that the suppression of IGF-1, but not that of leptin or testosterone, was highly correlated with the magnitude of the energy deficit such that those athletes who were in larger energy deficit demonstrated a greater suppression of IGF-1 than those who attained a smaller energy deficit or remained in energy balance.

Consequently, strategies to minimize the energy deficit during ultra-endurance exercise may help to attenuate the suppression of metabolic hormones, particularly that of IGF-1. Even though our participants completed the event as a group, energy balance was highly variable and ranged between an 11,859-kcal deficit and a 3,593-kcal surplus. As we have reported previously, energy balance in this group was largely driven by *ad libitum* caloric intake. We previously identified caloric intake from solid foods as an important predictor of energy intake, since those athletes who managed to consume more energy from solid foods were more likely to remain in energy balance.[5] Further the decline in caloric intake occurred primarily during the second half of the event, when most participants were unable to maintain their caloric intake at levels sufficient to prevent an energy deficit.[5] Unfortunately, we were not able to collect venous blood samples throughout the event to determine whether the drop in metabolic hormones coincided with the reduction in caloric intake. It is further noteworthy that the reduction in caloric intake throughout the second half of the event was largely the result of a reduced carbohydrate intake.[5] Given that not only energy but also carbohydrate availability may modulate metabolic function,[14] future research is needed to determine whether carbohydrate or energy intake should be prioritized in order to prevent the observed metabolic changes.

The present study further underlines the extreme physiological challenges of ultra-endurance exercise. Completion of a 1,230-km ultra-endurance cycling event resulted in a marked suppression of key metabolic hormones such as leptin, IGF-1, and testosterone. The severity of these endocrine aberrations is highlighted by the fact that testosterone dropped to levels typically observed in hypogonadism,[26] while IGF-1 reached the 2.5th percentile for male adults.[25] Our findings are in agreement with previous studies reporting that athletes participating in ultra-endurance exercise demonstrate metabolic hormone suppression following exercise completion.[7-

[10] Our results further indicate that 12 hours of recovery including *ad libitum* food intake are not sufficient for metabolic hormones to return to baseline concentrations. These findings are consistent with previous studies according to which IGF-1 remained suppressed for at least 3 days following marathon running[11] and testosterone remained suppressed for at least 1 day following Ironman distance triathlon.[7] Further, the fact that all three metabolic hormones were suppressed in recovery samples, which were collected at approximately the same time of day as the baseline samples, allowed us to ascertain that the observed changes were not an artifact due to circadian differences in circulating hormone concentrations,[27,28] but in fact due to participation in ultra-endurance exercise.

It is important to understand whether these endocrine effects of prolonged endurance exercise can be modulated by energy balance. The suppression of metabolic hormones, particularly if it persists over a prolonged time, may put ultra-endurance athletes at increased health risks. Unfavorable health outcomes associated with the suppression of metabolic functions secondary to energy deficiency include impaired reproductive, bone, and cardiovascular health and have been reported primarily in female athletes.[16] However, recent evidence suggests that energy deficiency may also impair bone and reproductive health in male athletes.[17-19] Even though men appear to be protected from reductions in IGF-1 and testosterone following short-term, moderate energy deficiency,[29] larger energy deficits of 1,000 to 4,000 kcal/d have been associated with changes in IGF-1 and testosterone comparable to our study.[21,24,30,31] Further research is needed to determine potential sex differences with regards to the magnitude of the energy deficit that is needed to perturb metabolic hormones.

Limitations

All data were collected in the field. Because *Paris-Brest-Paris* is an officially sanctioned event and participants were exposed to race-like conditions, we made every attempt to minimize the participants' burden before, during, and after the event. As a consequence, we were unable to collect venous blood samples during the race, and in efforts to honor the recovery of each individual athlete, food intake following the event was not assessed, and no additional blood samples were collected following the 12-h recovery period. Dietary information were collected through indirect observation by trained staff. Although staff were not present when food and beverages were ingested, they were able to track all food that the athletes were handed as well as all food that was returned. Dietary observation has previously been reported to yield highly reliable results in athletic settings[32] and is considered superior to other dietary assessment methods that rely on self-reported data.[33] For the assessment of energy expenditure, an alternative method would have been the doubly labelled water (DLW) method, which is often considered as the gold-standard method for the assessment of energy expenditure in the field.[34] However, our assessment period of approximately 2.5 days was considerably shorter than the duration of at least 7-10 days typically recommended for the DLW method.[35] Therefore, we chose to assess energy expenditure using a combination of indirect calorimetry and continuously measured power output, an approach that has previously been shown to provide valid energy expenditure data.[36]

5. Practical Applications

Our results suggest that some of the severe endocrine alterations that occur during ultra-endurance exercise may be attenuated by increased dietary energy. Considering that overall food intake dropped during the later stages of the ultra-endurance exercise,[5] athletes as well as their

supporting staff should attempt to develop a dietary regimen that allows the athletes to maintain their food intake at high levels throughout the whole event. Further, athletes should attempt to emphasize the consumption of solid foods, which appears to contribute to overall energy balance to a much greater extent than liquid foods.[5] However, future controlled experiments are needed to confirm that strategies of increased caloric intake, and particularly the ingestion of solid foods, can diminish the endocrine effects of ultra-endurance exercise.

6. Conclusion

The present study confirms that key metabolic hormones such as leptin, IGF-1, and testosterone are markedly suppressed following prolonged ultra-endurance exercise and that the direction and magnitude of these endocrine suppressions is reminiscent of severe starvation. At least for IGF-1, the suppression was found to be proportional to the energy deficit accumulated during the exercise. Future studies are needed to determine whether strategies to increase caloric intake can attenuate the suppression of key metabolic hormones following ultra-endurance exercise.

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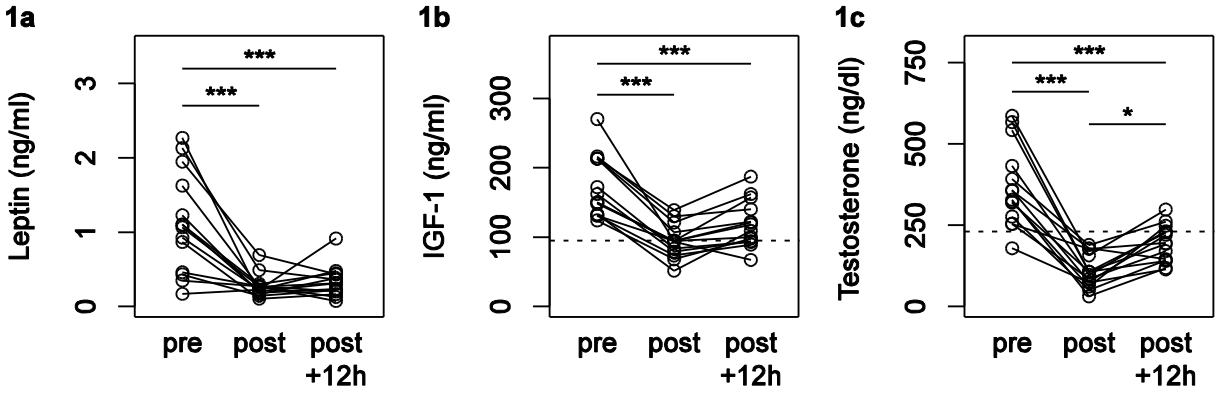

Figure 1. Changes in leptin (1a), IGF-1 (1b), and testosterone (1c) across the 1,230-km event and 12 h of recovery.*, ***: significantly different from each other ($p<0.05$, $p<0.001$). Dashed lines indicate the 2.5th percentile for male adults between 25-45 years (95 ng/mL)[23] for IGF-1 and the lower end of the clinical range (230 ng/dL)[24] for testosterone.

"Association Between Energy Balance and Metabolic Hormone Suppression During Ultra-Endurance Exercise "
by Geesmann B, Gibbs JC, Mester J, Koehler K
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Table 1. Changes in metabolic hormones and metabolites across the 1,230-km cycling event and 12 h of recovery.

	pre	post	post+12h
Insulin (µU/mL)	6.7 ± 2.9	11.0 ± 7.9	14.9 ± 15.1
Ghrelin (pg/mL)	1042 ± 377	1057 ± 339	1243 ± 385
Glucose (mg/dL)	89.6 ± 10.3	111.6 ± 26.7	96.3 ± 13.2
Free Fatty Acids (mmol/L)	0.20 ± 0.01	**0.65 ± 0.57***	0.41 ± 0.36
Glycerol (mmol/L)	0.07 ± 0.00	**0.14 ± 0.07****	0.1 ± 0.03

pre: before the event, post: after event completion, post+12h: approximately 12 h after event completion; *,**: different from pre ($p<0.05$, $p<0.01$)

Table 2. Spearman's correlation coefficients (ρ) between changes in metabolic hormones and metabolites across the 1,230-km cycling event and measures of energy balance.

	Energy Intake	Energy Expenditure	Energy Balance
Leptin	0.21	0.26	-0.01
IGF-1	0.50†	-0.3	**0.65***
Testosterone	0.28	-0.52†	0.45
Free Fatty Acids	0.31	-0.35	0.13
Glycerol	0.41	-0.06	0.28

*$p<0.05$; †:$p<0.1$