

Energy Expenditure during Extreme Endurance Exercise

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Energy Expenditure during Extreme Endurance Exercise: The Giro d'Italia

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¹Department of Nutrition and Movement Sciences, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre, Maastricht, THE NETHERLANDS; and ²Department of Human Physiology and Sports Medicine, Vrije Universiteit Brussel, Brussels, BELGIUM

ABSTRACT

PLASQUI, G., G. RIETJENS, L. LAMBRIKS, L. WOUTERS, and W. H. M. SARIS. Energy Expenditure during Extreme Endurance Exercise: The Giro d'Italia. *Med. Sci. Sports Exerc.*, Vol. 51, No. 3, pp. 568–574, 2019. **Purpose:** Little data are available on doubly labeled water (DLW) assessed total daily energy expenditure (TDEE) during extreme endurance exercise. Doubly labeled water is considered the gold standard to measure TDEE, but different calculations are being used, which may have a large impact on the results. The aim of the current study was to measure TDEE during the Giro d'Italia and apply two different calculation methods. **Methods:** Seven male cyclists (age, 28 ± 5 yr; body mass index, 22.1 ± 2.1 kg·m⁻²) completed the 24-d professional cycling race “Giro d'Italia” in which a total distance of 3445 km was covered, including 10 mountain stages. Total daily energy expenditure was measured over the entire duration of the race, with the ingestion of DLW at three different time points. To calculate TDEE and body composition, the isotope dilution space was calculated using two different techniques, the “plateau” and “intercept” technique. **Results:** The %fat mass at baseline was 7.8% and 16.8% with the plateau and intercept technique respectively and did not significantly change over the course of the race. Total daily energy expenditure was on average 32.3 ± 3.4 MJ·d⁻¹ using the plateau technique versus 28.9 ± 3.2 using the intercept technique, resulting in an average physical activity level (PAL) of 4.37 ± 0.43 versus 3.91 ± 0.39 , respectively. The dilution space ratio was on average 1.030 with the plateau and 1.060 with the intercept technique. **Conclusions:** Given that the observed dilution space ratio with the plateau technique is similar as the expected ratio from literature and the % fat mass of 7.8% is more realistic for the athletes being studied, we propose the application of the plateau rather than the intercept method, when using DLW during extreme endurance exercise. **Key Words:** DOUBLY LABELED WATER, CYCLING, PROFESSIONAL ATHLETES, PLATEAU VERSUS INTERCEPT

Total daily energy expenditure (TDEE) of the general population has been found to range between 1.2 and 2.5 times basal metabolic rate (BMR) (1). However, physical activity levels above 2.5 have been found for soldiers in training (2,3) and endurance athletes (4,5). At these high rates of energy turnover, maintaining energy balance is challenging as it is hard to match energy expenditure with sufficient energy intake (2,3).

Energy expenditure during competitive endurance events. Given the difficulty of accurately obtaining data on energy expenditure during professional competitive events, such as multiple stage cycling races, not many studies are available providing data on energy turnover in professional endurance athletes during the race. During the 6-d Tour of

Southland total energy expenditure was found to be 27.4 MJ·d⁻¹, which was adequately compensated with an average energy intake of 27.3 MJ·d⁻¹ (ie, mean PAL 2.4) (6). Sjodin et al. (4) measured energy expenditure over 1 wk in professional cross-country skiers and found mean values of 18.3 and 30.3 MJ·d⁻¹ (corresponding PAL, 3.4 and 4.0) for females and males, respectively. Also, these athletes were able to maintain energy balance as no changes in body mass occurred. In cyclists competing in the Tour de France, measured PAL were as high as 4.3 to 5.3 (5). During this cycling race, athletes covered over 4000 km and about 30 mountain passes in 3 wk time. For optimal performance, it is essential that during such race, energy requirements are still met. The PAL values of 4.3 to 5.3 corresponded to a TDEE of 29.4 to 36.0 MJ·d⁻¹ (5) and corresponding energy intake was found to be 24.7 MJ·d⁻¹ on average over the entire race (5,7). Although these data suggest that the cyclists in the study were not able to compensate energy loss, given that no weight loss occurred, energy intake may have been underestimated because of self-reporting methods (5,8).

Using doubly labeled water during extreme endurance events. Using doubly labeled water (DLW) under conditions with such high energy turnover is challenging. One of the debated issues with DLW is the calculation of CO₂ production from the isotope elimination curves, that is, whether to use the plateau or intercept method. To calculate the isotope distribution space, the plateau method uses the isotopic enrichment of the urine samples, taken after equilibration of the

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isotopes has taken place. This means that a small part of the isotopes has already been washed out during equilibration. The dilution space can also be calculated from the final (at the end of the observation period) and initial urine samples by extrapolating back to time 0. Then an error could be made because the decline in isotopic enrichment during equilibration (for example, with equilibration overnight) is smaller than the decline over the entire observation period (i.e., 1–2 wk), resulting in an overestimation of the initial isotopic enrichments and consequent underestimation of the dilution spaces. This problem that was already indicated by Schoeller et al. (9) may be a lot larger during extreme endurance exercise due to the high isotope turnover. To our knowledge, this has not been studied.

Study aims. Given the scarcity of DLW measured energy expenditure during extreme endurance exercise and the possible impact of choosing the plateau versus intercept technique to calculate CO₂ production, the primary aims of this study were: 1) to measure energy expenditure using DLW during the entire 3-wk cycling race, the Giro d'Italia and 2) to compare the effect of using the plateau or intercept calculation technique to measure energy expenditure. We hypothesize that the intercept method will lead to an underestimation of the dilution space and hence underestimation of TDEE. Given that we could collect the data during the actual race, a secondary aim was to study the relationship between energy expenditure and race duration.

METHODS

Subjects. Subjects were eight male cyclists from a professional UCI World Tour Team. Because seven of eight cyclists completed the entire 3-wk race, data presented here are for these seven subjects only. Subjects were on average 28 ± 5 yr old with a mean body mass index (BMI) of 22.1 ± 2.1 kg·m⁻². Subject characteristics are presented in Table 1. All subjects were fully informed about the study, and informed consent was obtained from all participants before the collection of any urine samples.

Race characteristics. The study was performed during the Giro d'Italia, which is one of the three “grand tours” or major European professional cycling stage races, the other two being the “Tour de France” and “Vuelta a España.” This 24-d stage race covered 18 mass start stages (eight flat, five medium mountain, five high mountain), two individual time trials, one team time trial, and three resting days (see Table, Supplemental Digital Content 1, overview of the different race stages, times of dosing and urine

sampling, <http://links.lww.com/MSS/B422>). A total distance of 3445.4 km was covered, with the best overall time being 88 h, 14 min, and 32 s.

Energy expenditure and body composition. Energy expenditure over the entire 24-d race was measured using the DLW technique. Given the high turnover rates of the isotopes during extreme endurance exercise, subjects received a dose of DLW at three time points, that is, the day before the start of the race (day 0), on day 10, and day 17. Baseline urine samples were always collected in the evening, just before dosing. After overnight equilibration, the second urine sample was collected from the second morning urine. Subsequent urine samples were collected approximately in the middle and at the end of each observation period. For energy expenditure calculations, the entire race was subdivided in three observation periods, that is, days 1 to 10, days 11 to 17, and days 18 to 24 (see Table, Supplemental Digital Content 1, overview of the different race stages, times of dosing and urine sampling, <http://links.lww.com/MSS/B422>).

The given dose was calculated based on the subjects' total body water (TBW), which was estimated based on BMI, age and sex (10). Subjects received a first dose of 2.5 g·L⁻¹ TBW of a water mixture containing 9.8% enriched H₂¹⁸O and 6.5% enriched ²H₂O, followed by a second dosing of approximately 80% of the initial dose and third dosing that was 74% of the initial dosing. These numbers were based on the doses used in the Tour de France study by Westerterp et al. (5). This resulted in an average amount of 112.6, 90.1, and 82.9 g of labeled water mixture given for the first, second, and third doses, respectively, and resulted in an initial excess enrichment of TBW with ~135 ppm for ²H and ~200 ppm for ¹⁸O.

Urine samples were collected by the team's physician and immediately transferred to 2 × 2 mL airtight glass vials and frozen at -20°C. At the end of the race, all urine samples were transported to Maastricht University where they were stored until analysis.

For the analysis of deuterium concentrations in urine, a 2-mL glass vial containing 300 μL of urine was filled with hydrogen gas and equilibration occurred for 3 d at room temperature with a catalyst (5% platinum-on alumina, 325 mesh; Aldrich Chemical Company Ltd) placed in an insert. For measuring ¹⁸O in urine, again 300 μL of urine was put in a glass vial, which was then filled with CO₂. Equilibration then took place for 4 h at 40°C. The relative amounts of deuterium in hydrogen gas and ¹⁸O in CO₂ were then determined using isotope ratio mass spectrometry (Micromass Optima, Manchester, UK).

The isotope dilution spaces were calculated using two different methods, that is, the plateau method and the intercept method. Using the plateau method, the isotopic dilution spaces for ²H and ¹⁸O were calculated from the background enrichment in the baseline urine samples and the urine sample from the second voiding in the morning after dosing (T1), allowing overnight equilibration. Using the intercept method, the dilution space was calculated from the final (at the end of the observation period) and initial urine samples

TABLE 1. Subject characteristics at the start of the race (n = 7).

	Mean ± SD	(Range)
Age (yr)	28 ± 5	(23–37)
Weight (kg)	76.4 ± 8.4	(64.2–86.7)
Height (m)	1.86 ± 0.04	(1.79–1.93)
BMI (kg·m ⁻²)	22.1 ± 2.1	(19.3–24.8)
*%FM	7.8 ± 2.0	(4.7–10.2)

*%FM at the start of the race as calculated using the plateau method.

TABLE 2. Body mass and body composition over the course of the race (mean ± SD).

	Day 1	Day 5	Day 10	Day 11	Day 15	Day 17	Day 18	Day 24
BM	76.4 ± 8.4	75.7 ± 7.9	76.2 ± 8.1	76.2 ± 8.0	76.1 ± 8.6	75.7 ± 8.1	76.1 ± 8.3	76.3 ± 8.2
TBW _p	51.3 ± 4.7*	—	—	52.7 ± 5.0*	—	—	52.6 ± 5.3*	—
TBW _i	46.4 ± 4.9	—	—	45.9 ± 4.4	—	—	47.3 ± 5.0	—
%FM _p	7.8 ± 2.0**	—	—	5.1 ± 3.0**	—	—	5.2 ± 3.3**	—
%FM _i	16.8 ± 2.6	—	—	16.9 ± 2.1	—	—	17.0 ± 2.6	—

Body composition was calculated using the plateau method (T1, i.e., morning urine sample after overnight equilibration) and from the intercept method (T0, i.e., backward extrapolation). BM, body mass; TBW_p, TBW from plateau method; TBW_i, TBW from intercept method; %FM_p, %fat mass from plateau method; %FM_i, %fat mass from intercept method.

*Significant difference between intercept and plateau method ($P < 0.05$).

**Significant difference between intercept and plateau method ($P < 0.001$).

by extrapolating back to time 0 (T0). Calculated energy expenditure using both the morning samples (further referred to as T1) and from backward extrapolation (further referred to as T0) are reported. Total body water was calculated from the dilution spaces of ²H (N_d) and ¹⁸O (N_o), assuming the dilution space of ²H is 3% larger than the ¹⁸O dilution space, and the latter is 1% larger than TBW.

CO₂ production was calculated from the difference in elimination rates of ²H and ¹⁸O using the equation derived from Schoeller et al. (equation A6) (9). CO₂ production was converted to energy expenditure by assuming a respiratory quotient (RQ) of 0.90 taking into account the higher carbohydrate oxidation in elite athletes compared to the general population. Westerterp et al. (5) found an average RQ of 0.90 from food records in four athletes during the Tour de France.

Body mass was measured on the mornings of days 1, 5, 10, 11, 15, 17, 18, and 24. At the start of each observation period, percentage body fat was calculated from TBW assuming a hydration fraction of fat-free mass (FFM) of 0.73 (11,12).

Statistics and calculations. Subject characteristics, body composition and energy expenditure values are expressed as mean ± SD. Basal metabolic rate was calculated using the Oxford equation based on body mass (on day1) and height (13). Consequently, the PAL was calculated by dividing TDEE by BMR.

Results for body composition and energy expenditure are reported using both the dilution space calculated from the morning urine sample (T1, i.e., plateau) and from using backward extrapolation (T0, i.e., intercept). Differences between both methods were assessed using repeated-measures ANOVA with the three observation periods as “within” subjects effect and plateau versus intercept as “between” effect.

The relation between TDEE from both methods was investigated by plotting TDEE from the plateau method

against TDEE from the intercept method, including the regression line and line of identity. In addition, bias and limits of agreement between both methods is shown by a Bland–Altman plot.

To statistically investigate the relation between TDEE and total race duration, multiple linear regression analysis was used with FFM and race duration as independent variables. In addition, energy expenditure divided by body mass was plotted against total race duration.

Statistical analyses were performed with SPSS 23.0 for Mac (IBM Corp., Armonk, NY). The statistical significance level was set at $P < 0.05$.

RESULTS

Body composition. Body mass did not significantly change over the course of the race (Table 2). Total body water and %FM calculated using both the plateau and intercept method did not change over the duration of the race. Using the intercept method, TBW was consistently lower ($P < 0.05$) and %FM consistently higher ($P < 0.001$) compared with the plateau method (Table 2).

Energy expenditure, PAL, and dilution space ratio. Using the plateau method, energy expenditure was on average 32.3 ± 3.2 MJ·d⁻¹ corresponding to a PAL of 4.37 ± 0.40 (range, 3.95–5.05) (Table 3). The highest energy expenditures were achieved during the third period (days 18–24 of the race), corresponding to an average PAL of 4.70 ± 0.35 . The average dilution space ratio (N_d/N_o) was 1.030 ± 0.003 (Table 3).

Using the intercept method, energy expenditure and PAL for the three periods were consistently lower ($P < 0.05$) with an average energy expenditure of 28.9 ± 3.1 and PAL of 3.91 ± 0.37 (Table 3). The average dilution space ratio (N_d/N_o) was then 1.060 ± 0.004 (Table 3).

TABLE 3. Total daily energy expenditure, physical activity level and the dilution space ratio over the three observation periods calculated using the plateau method (T1, i.e., morning urine sample after overnight equilibration) and from the intercept method (T0, i.e., backward extrapolation).

	Period 1 (10 d)	Period 2 (7 d)	Period 3 (7 d)	Total (24 d)
TDEE (MJ·d ⁻¹) plateau	31.1 ± 4.5*	31.6 ± 2.5*	34.8 ± 3.2*	32.3 ± 3.4*
TDEE (MJ·d ⁻¹) intercept	28.3 ± 4.3	27.5 ± 2.3	31.2 ± 3.1	28.9 ± 3.2
PAL plateau	4.21 ± 0.62*	4.27 ± 0.27*	4.70 ± 0.35*	4.37 ± 0.43*
PAL intercept	3.82 ± 0.57	3.72 ± 0.24	4.22 ± 0.32	3.91 ± 0.39
N _d /N _o plateau	1.029 ± 0.003*	1.028 ± 0.006*	1.033 ± 0.008*	1.030 ± 0.002*
N _d /N _o intercept	1.063 ± 0.011	1.057 ± 0.008	1.058 ± 0.003	1.060 ± 0.006

“Total” represents the weighted average of the three periods.

*Significant difference between intercept and plateau method ($P < 0.05$).

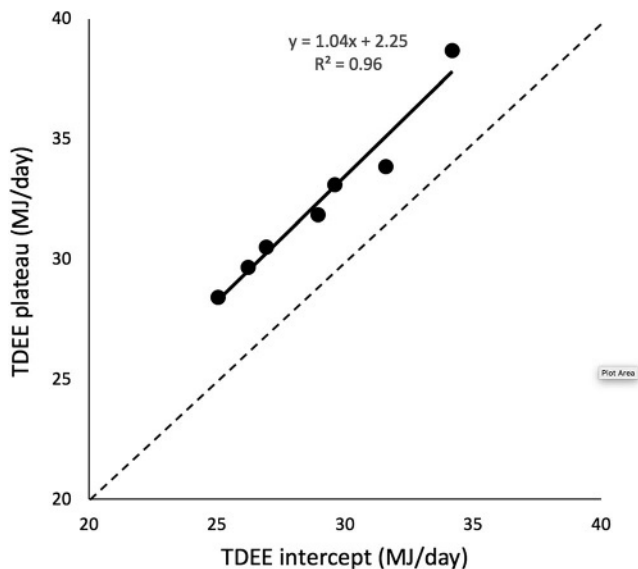


FIGURE 1—Scatterplot of TDEE according to the two calculation methods, plateau vs intercept. The full line indicates the regression line, the dashed line indicates the line of identity.

Regression analysis showed a strong relation between both methods ($R^2 = 0.96$, $P < 0.001$; Fig. 1) and Bland–Altman analysis showed a mean ($\pm 2SD$) bias of 3.4 (± 1.3) $\text{MJ}\cdot\text{d}^{-1}$ (Fig. 2).

Determinants of total energy expenditure. The fastest subject of the team finished the entire race in 88 h 25 min 32 s corresponding to an average speed of $39 \text{ km}\cdot\text{h}^{-1}$ ($10.8 \text{ m}\cdot\text{s}^{-1}$). There was a strong negative correlation between energy expenditure over the entire race ($\text{MJ}\cdot\text{d}^{-1}\cdot\text{kg}^{-1}$) and race duration ($B = -2.83$, $R^2 = 0.72$, $P = 0.01$; Fig. 3). Multiple linear regression analysis showed that both FFM ($B = 0.79$, $P < 0.05$) and total race duration (in hours)

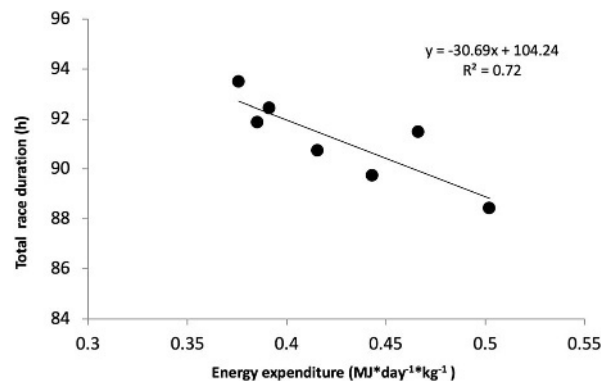


FIGURE 3—Relation between energy expenditure (expressed as $\text{MJ}\cdot\text{day}^{-1}\cdot\text{kg}^{-1}$) and total time needed to complete the 3-wk race.

($B = -2.4$, $P < 0.05$) significantly added to the explained variation in TDEE (model $R^2 = 0.79$, $P < 0.05$).

DISCUSSION

Using DLW during the entire 3 wk multistage cycling race Giro d’Italia, we have shown that energy expenditure values were as high $32.3 \text{ MJ}\cdot\text{d}^{-1}$ corresponding to an average PAL of 4.37. When backward extrapolation was used to determine the dilution space, the observed PAL was considerably lower (3.91). There was a strong negative correlation between total race time and energy expenditure (per kg body mass).

Limits of human energy expenditure. Energy expenditure values measured here are amongst the highest reported in literature. To our knowledge, the only other study measuring during similar conditions was the Tour de France study, that is, 3-wk multistage professional cycling race with a total distance covered of 3820 km including nine

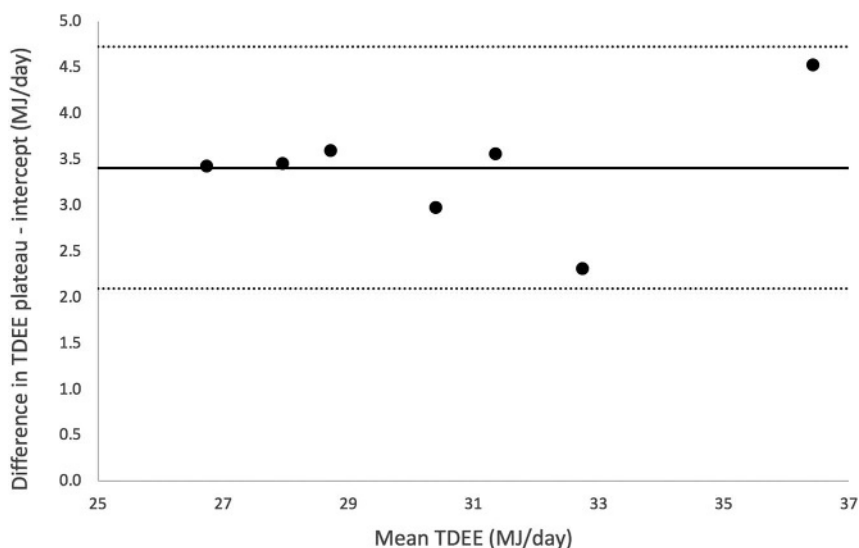


FIGURE 2—Bland–Altman plot of TDEE calculated according to both calculation methods, plateau and intercept. The solid line indicates the mean bias, the dashed lines indicate the limits of agreement (mean $\pm 2SD$).

mountain stages (5). In that study, there was a large discrepancy between EE and EI intake (from diaries) as well as between EE from DLW and EE recalculated by Saris et al. (7). However, in concordance to the current study, there were also no changes in body mass, indicating that energy balance was maintained and hence energy requirements were met with sufficient energy intake. Also, the measured PAL values in the current study ranged from 3.95 to 5.05 and confirmed the high values obtained by Westerterp et al. (range, 4.3–5.3), even though the equations used to calculate CO₂ production differed between the Tour de France study and the current study. Other data from multistage cycling races is scarce. Rehrer et al. (6) found TDEE (from DLW) to be 27.4 MJ·d⁻¹ during the 6-d Tour of Southland, resulting in an average PAL of 2.4. That PAL value is surprisingly low given the high TDEE, and a closer look at the measured resting metabolic rate (RMR) showed surprisingly high values. When mean body mass and height obtained from that study was filled-out in the same BMR equation as used for the current study, the resulting BMR was 8.00 MJ·d⁻¹ instead of 11.5 MJ·d⁻¹ reported by Rehrer et al. This observation was also made and discussed by the researchers, and they speculate that previous day exercise may have increased measured resting metabolic rate. So it is plausible to assume that true BMR was lower and hence the resulting PAL in that study would be considerably higher (ie, PAL = 27.4/8.00 = 3.4).

Our energy expenditure values can be put further in perspective by comparing them with other extreme endurance exercise events. During the race, athletes were on average cycling for a little over 4 h·d⁻¹. A well-known example of extreme energy expenditure values was for two men crossing Antarctica for 2300 km pulling sledges with a starting mass of 222 kg, exercising for approximately 10 h d for 95 d with temperatures ranging from -45°C to -10°C (14). No PAL were reported in that study, but on average, TDEE for these two men was 32.3 MJ·d⁻¹ for the first 50 d (mean starting body mass was 85.2 kg), that is, comparable to that in the current study. However, between days 20 and 30, values as high as 46.7 MJ·d⁻¹ were measured, which would roughly correspond with a PAL ~6.3. These men were, however, unable to maintain energy balance, leading to considerable weight loss (~20 kg in 50 d) (14).

DLW during extreme endurance. During extreme endurance exercise, measuring EE using DLW is a challenge. The technique is based on the fact that after a subject is given a dose of DLW, containing deuterium and oxygen-18, deuterium is eliminated from the body as water and oxygen-18 is eliminated as both water and CO₂. The difference in disappearance rates between the deuterium and oxygen-18 isotopes is hence a measure of CO₂ production. To calculate CO₂ production, the distribution space needs to be measured and this is often done by using backward extrapolation also known as the “slope/intercept” method, based on the final (at the end of the observation period) and initial urine samples (samples taken after equilibration has

taken place). This is done because some isotopic loss already occurs during equilibration, and also the analytical error decreases when the slope/intercept approach is used (15). However, using backward extrapolation, the isotopic loss is measured over several 24 h periods whereas the equilibration period was only one night. As mean energy expenditure and hence isotopic loss is much smaller during the night than over 24 h, the slope/intercept approach is not entirely correct. In the case of extreme endurance exercise, this problem is even amplified due to the very high-energy turnovers and leads to incorrect results. To make this apparent, we have reported both body composition and EE data using the dilution space calculated from backward extrapolation and from using the morning sample after overnight equilibration. The latter is in this case more correct. This is confirmed by the dilution space ratio (N_d/N_o), which was on average 1.060 using backward extrapolation but 1.030 using the morning samples. The latter is exactly the expected value as reported by Schoeller et al. (9) and close to the expected value of 1.034 reported by Racette et al. (15) and 1.037 reported by Westerterp et al. (16). This is in our opinion an important issue to consider as the differences in the measured body composition and EE values are significant. During extreme endurance exercise as in the current study, the resulting values for %FM were ~17% versus ~8% and PAL values were 3.92 versus 4.39 when using the intercept versus plateau technique, respectively.

An important consideration is that for the slope–intercept method to be used, it is assumed that the isotopic depletion is uniform over the entire observation period. It can be argued that this is not the case in the current study as for the three observation intervals, the first day was either a team time trial (period 1) or a rest day (period 2 and 3). It is therefore conceivable that EE on the first day of each observation interval was lower than on the following days. However, we argue that the rate of isotope depletion over the entire measurement period is too steep compared with the rate of isotope depletion during the first night, when isotope equilibration is taking place. So regardless of some variation in EE over the observation interval, we believe the slope–intercept method will always underestimate the dilution space and hence TDEE when isotopic decline is so steep because of the extreme high energy expenditures.

Determinants of race duration. The between subject variability in PAL is considerable, even though all subjects covered the same distance and all finished the race in a time between 88.5 and 93.5 h in total. Expressing this as a relative number, one could say that the slowest subject is only 5.6% slower (average speed 36.9 km·h⁻¹) than the fastest (average speed 39 km·h⁻¹). Yet, looking at this from an energy expenditure perspective, the fastest member of the team needed to spend on average 33% more energy to cover the same distance than the slowest (ie, 0.50 vs 0.38 MJ·d⁻¹·kg⁻¹; Fig. 3). Many factors determine the energetic cost of cycling and the ability of an athlete to sustain high levels of oxygen consumption over time. As wind resistance increases

proportionally to the square of the speed, the cost of transport also increases with increasing speed (17). Also, the faster cyclist, to achieve a better racing time, will spend less time in the peloton, especially during high mountain stages, therefore, gaining less wind protection. On the other hand, this rider may be more sheltered in the peloton during other stages. McCole et al. have shown that $\dot{V}O_2$ ($L \cdot \text{min}^{-1}$) may be reduced up to 39% by riding at the back of an eight-rider pack formation at $40 \text{ km} \cdot \text{h}^{-1}$ (18). From a physiological point of view, cycling performance will be determined by maximal oxygen uptake capacity of the body ($\dot{V}O_{2\text{max}}$), the percentage of $\dot{V}O_{2\text{max}}$ where the lactate threshold occurs and cycling economy. Even with the same $\dot{V}O_{2\text{max}}$, subjects with a higher lactate threshold can longer maintain a high power output (19). It is also known that differences in energetic efficiency (economy) exist between cyclists (20). Unfortunately, we were unable to measure any of these factors in the current study, but it is clear from the data provided that the fastest subject is not just being more energy efficient but is able to maintain considerably higher levels of energy turnover (on average 32% more) over long periods than the slowest.

Limitations. We only measured one professional team consisting of eight cyclists of which seven finished the race. Despite the small sample size, we believe the sample is a good representation of cyclists participating in these ‘grand tours’ with the fastest rider of the team finishing in the top 10 and the slowest finishing with the last 10.

For the current study, we could not collect any data on energy intake. However, given that neither body mass nor body composition changed over the course of the race, energy balance was maintained and energy intake should therefore match energy expenditure.

Unfortunately, data on actual mechanical power performed is lacking. Even though an attempt was made to collect this data from the power meters fitted to the bicycles, the amount of missing data was so large that none could be presented here. In future work, we aim to collect both power and energy expenditure data simultaneously.

As BMR could not be measured in this study, the calculated PAL is also dependent on the choice of the formula used to calculate BMR. We chose the Oxford equations as they were developed on a large database with a differentiation for sex and age (13). It can be debated that for athletes it would be better to use an equation based on body composition rather than body mass and height. Therefore, PAL was also calculated using a BMR equation based on age, FFM and FM (Sabounchi et al., equation 11) (21). The resulting PAL was then 4.18 instead of 4.37.

In conclusion, during the Giro d’Italia, measured PAL values were on average 4.37, confirming the high levels measured previously during the Tour de France. When applying DLW during such extreme endurance exercise, the “plateau” rather than the “intercept” method should be used to calculate the dilution space.

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The results of the present study do not constitute endorsement by ACSM.

The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

REFERENCES

- Black AE, Coward WA, Cole TJ, Prentice AM. Human energy expenditure in affluent societies: an analysis of 574 doubly-labelled water measurements. *Eur J Clin Nutr.* 1996;50(2):72–92.
- Forbes-Ewan CH, Morrissey BL, Gregg GC, Waters DR. Use of doubly labeled water technique in soldiers training for jungle warfare. *J Appl Physiol (1985).* 1989;67(1):14–8.
- Hoyt RW, Jones TE, Stein TP, et al. Doubly labeled water measurement of human energy expenditure during strenuous exercise. *J Appl Physiol (1985).* 1991;71(1):16–22.
- Sjodin AM, Andersson AB, Hogberg JM, Westerterp KR. Energy balance in cross-country skiers: a study using doubly labeled water. *Med Sci Sports Exerc.* 1994;26(6):720–4.
- Westerterp KR, Saris WH, van Es M, ten Hoor F. Use of the doubly labeled water technique in humans during heavy sustained exercise. *J Appl Physiol.* 1986;61(6):2162–7.
- Rehrer NJ, Hellemans IJ, Rolleston AK, Rush E, Miller BF. Energy intake and expenditure during a 6-day cycling stage race. *Scand J Med Sci Sports.* 2010;20(4):609–18.
- Saris WH, van Erp-Baart MA, Brouns F, Westerterp KR, ten Hoor F. Study on food intake and energy expenditure during extreme sustained exercise: the tour de France. *Int J Sports Med.* 1989;10(1 Suppl):S26–31.
- Westerterp KR, Goris AH. Validity of the assessment of dietary intake: problems of misreporting. *Curr Opin Clin Nutr Metab Care.* 2002;5(5):489–93.
- Schoeller DA, Ravussin E, Schutz Y, Acheson KJ, Baertschi P, Jequier E. Energy expenditure by doubly labeled water: validation in humans and proposed calculation. *Am J Physiol.* 1986;250(5 Pt 2):R823–30.
- Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *Br J Nutr.* 1991;65(2):105–14.
- Pace N, Rathbun EN. Studies on body composition III: the body water and chemically combined nitrogen content in relation to fat content. *J Biol Chem.* 1945;158:685–91.
- Wang Z, Deurenberg P, Wang W, Pietrobelli A, Baumgartner RN, Heymsfield SB. Hydration of fat-free body mass: review and critique of a classic body-composition constant. *Am J Clin Nutr.* 1999;69(5):833–41.
- Henry CJ. Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutr.* 2005;8(7A):1133–52.
- Stroud MA, Ritz P, Coward WA, et al. Energy expenditure using isotope-labelled water ($2\text{H}_2^{18}\text{O}$), exercise performance, skeletal muscle enzyme activities and plasma biochemical parameters

in humans during 95 days of endurance exercise with in adequate energy intake. *Eur J Appl Physiol Occup Physiol*. 1997; 76(3):243–52.

15. Racette SB, Schoeller DA, Luke AH, Shay K, Hnilicka J, Kushner RF. Relative dilution spaces of 2H- and 18O-labeled water in humans. *Am J Physiol*. 1994;267(4 Pt 1):E585–90.
16. Westerterp KR, Wouters L, van Marken Lichtenbelt WD. The Maastricht protocol for the measurement of body composition and energy expenditure with labeled water. *Obes Res*. 1995;3(1 Suppl):49–57.
17. Burke ER. Physiology of Cycling. In: Garrett WEJ, Kirkendall DT, editors. *Exercise and Sport Science*. Philadelphia: Lippincott Williams and Wilkins; 2000. pp. 759–70.
18. McCole SD, Clancy K, Conte JC, Anderson R, Hagberg JM. Energy expenditure during bicycling. *J Appl Physiol (1985)*. 1990;68(2): 748–53.
19. Joyner MJ, Coyle EF. Endurance exercise performance: the physiology of champions. *J Physiol*. 2008;586(1):35–44.
20. Coyle EF, Feltner ME, Kautz SA, et al. Physiological and biomechanical factors associated with elite endurance cycling performance. *Med Sci Sports Exerc*. 1991;23(1):93–107.
21. Sabouchi NS, Rahmandad H, Ammerman A. Best-fitting prediction equations for basal metabolic rate: informing obesity interventions in diverse populations. *Int J Obes (Lond)*. 2013; 37(10):1364–70.