

Inability to match energy intake with energy expenditure at sustained near-maximal rates of energy expenditure in older men during a 14-d cycling expedition

Citation for published version (APA):

Rosenkilde, M., Morville, T., Riis Andersen, P., Kjaer, K., Rasmussen, H., Holst, J. J., Dela, F., Westerterp, K., Sjodin, A., & Helge, J. W. (2015). Inability to match energy intake with energy expenditure at sustained near-maximal rates of energy expenditure in older men during a 14-d cycling expedition. *American Journal of Clinical Nutrition*, 102(6), 1398-1405. <https://doi.org/10.3945/ajcn.115.109918>

Document status and date:

Published: 01/01/2015

DOI:

[10.3945/ajcn.115.109918](https://doi.org/10.3945/ajcn.115.109918)

Document Version:

Publisher's PDF, also known as Version of record

Document license:

Taverne

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 28 Apr. 2024

Inability to match energy intake with energy expenditure at sustained near-maximal rates of energy expenditure in older men during a 14-d cycling expedition¹

Mads Rosenkilde,^{2*} Thomas Morville,^{2,3} Peter Riis Andersen,^{2,3} Katja Kjær,^{2,3} Hanne Rasmusen,⁶ Jens Juul Holst,^{2,4} Flemming Dela,^{2,3} Klaas Westerterp,⁷ Anders Sjödén,⁵ and Jørn W Helge^{2,3}

²Department of Biomedical Sciences, ³Center of Healthy Aging, ⁴Novo Nordisk Foundation Center for Basic Metabolic Research, and ⁵Department of Nutrition, Exercise, and Sports, University of Copenhagen, Copenhagen, Denmark; ⁶Institute of Sports Medicine Copenhagen, Bispebjerg Hospital, Copenhagen, Denmark; and ⁷Department of Human Biology, Maastricht University, Maastricht, Netherlands

ABSTRACT

Background: The upper rates of energy expenditure (EE) and the corresponding regulation of energy intake (EI), as described in younger trained subjects, are not well elucidated in older subjects.

Objectives: The aim was to investigate EE in older men during prolonged cycling and determine whether it is sufficiently matched by EI to maintain energy balance. In addition, we investigated appetite ratings and concentrations of appetite-regulating hormones.

Design: Six men (mean \pm SE age: 61 \pm 3 y) completed 2706 km of cycling, from Copenhagen to Nordkapp, in 14 d. EE was measured by using doubly labeled water, and food and drink intake was recorded by the accompanying scientific staff. Energy balance was calculated as the discrepancy between EI and EE and from changes in body energy stores as derived from deuterium dilution. Fasting hormones were measured before and after cycling, and appetite ratings were recorded twice daily.

Results: EE (\pm SE) increased from 17 \pm 1 MJ/d before to 30 \pm 2 MJ/d during the cycling trip ($P < 0.001$), which is equivalent to 4.0 \pm 0.1 times the basal metabolic rate. Although body weight remained stable during the 14 d of cycling, body fat decreased (-2.2 ± 0.7 kg; $P = 0.02$) and fat-free mass increased (2.5 ± 0.6 kg; $P = 0.01$). EI was 25 \pm 1 MJ/d during cycling, resulting in a negative energy balance calculated by the EE $-$ EI gap (-5.2 ± 1.2 MJ/d). Calculated from changes in body energy stores, energy balance was also negative (-4.8 ± 2.0 MJ/d) during the first week. In the morning and evening, hunger ratings increased (both $P = 0.02$), whereas ratings of fullness decreased in the evening ($P = 0.04$). Fasting plasma concentrations of insulin increased by 120% \pm 15% ($P = 0.02$), glucagon-like peptide 1 (GLP-1) by 60% \pm 20% ($P < 0.01$), and Polypeptide YY₃₋₃₆ by 80% \pm 30% ($P < 0.02$) after cycling.

Conclusions: Older male cyclists sustained near-maximal rates of EE during prolonged cycling but were unable to upregulate EI to maintain energy balance. Despite the presence of increased motivation to eat, a more profound counteracting physiologic stimulus inhibiting increases in EI was present. This trial was registered at clinicaltrials.gov as NCT02353624. *Am J Clin Nutr* 2015;102:1398–405.

Keywords: appetite, energy balance, energy expenditure, energy intake, exercise

INTRODUCTION

More than 50 y ago, Jean Mayer hypothesized that an increase in energy expenditure (EE)⁸ (within normal ranges) is followed by a concomitant increase in energy intake (EI) to maintain energy homeostasis (1, 2). This concept has recently been extended such that the regulation of energy balance (i.e., weight stability) requires a certain amount of physical activity beyond sedentarism (3). In a modern society, energy homeostasis is “unregulated” (i.e., overweight and obesity) due to low levels of physical activity (4). However, at upper limits of energy production, the capacity to maintain energy homeostasis and match EI with EE is challenged and may result in an insufficient caloric intake to cover the energy demand (1, 2). In the literature, the upper limits of sustained EE have mostly been investigated in field studies in younger, well-trained athletes, soldiers, and adventurers who, in many ways (e.g., motivational and metabolic), are adapted and entrained to these extreme workloads (5). Despite very high rates of EE (~ 35 MJ/d) as determined by the doubly labeled water (DLW) technique, professional Tour de France cyclists are able to maintain energy balance during the first 2 wk of cycling, except for a minimal decline in body fat mass (-0.4 kg) (6). These endurance athletes reach a maximal sustained EE that is equal to 4.3 times the basal metabolic rate (BMR), similar to that in wild animals (7). A matching of EI to high rates of EE as well as maintenance of body weight and composition were also observed in elite cross-country skiers (EE: ~ 18 MJ/d for women and 30 MJ/d for men) during an

¹MR’s salary was funded by the University of Copenhagen Excellence Program for Interdisciplinary Research. Materials for the bicycle trip were supported by Maribo Medico A/S, Toms Gruppen A/S, and Orkla Health.

*To whom correspondence should be addressed. E-mail: madsrl@sund.ku.dk.

⁸ Abbreviations used: BMR, basal metabolic rate; DLW, doubly labeled water; DXA, dual-energy X-ray absorptiometry; EE, energy expenditure; EI, energy intake; FFM, fat-free mass; GLP-1, glucagon-like peptide 1; PYY₃₋₃₆, polypeptide YY₃₋₃₆; TBW, total body water; $\dot{V}O_2$, oxygen uptake; $\dot{V}O_{2max}$, maximal oxygen uptake.

Received February 25, 2015. Accepted for publication September 10, 2015.

First published online October 21, 2015; doi: 10.3945/ajcn.115.109918.

intense training period (8). In a study of prolonged hill walking for 10 d in mountainous terrain (~21 MJ/d), Ainslie et al. (9) showed that older subjects (~56 y) reported a lower EI and experienced a greater loss of body fat (i.e., body energy storage; -1.9 compared with -0.3 kg) than younger subjects (~24 y) completing the same trip. Although most studies examined upper limits of EE in young subjects, no studies have investigated upper limits of EE in older recreational athletes, as well as the subsequent EI and ratings of appetite. Furthermore, we found no studies that measured hormones implicated in the regulation of long-term energy homeostasis and food intake (i.e., leptin and insulin) and more short-term appetite regulation [i.e., ghrelin, glucagon-like peptide 1 (GLP-1), and polypeptide YY₃₋₃₆ (PYY₃₋₃₆)] at high rates of EE.

The main aim of the present study was to measure total EE and EI in 6 older men during 14 d of prolonged strenuous cycling. Furthermore, we wanted to investigate whether EI could sufficiently match high rates of EE to maintain energy balance and to assess the regulation of EI from appetite ratings and appetite-regulating hormones.

METHODS

Six older (aged 61.3 ± 3.8 y) (Table 1) male recreational cyclists [maximal oxygen uptake (V̇O₂max): 48 ± 2 mL/(kg · min)] (Table 2) participated in the study. This amount of oxygen uptake is similar to previous values in athletes with the same age (10, 11). On their own initiative, to win a bet the subjects organized the 14-d cycling trip with a distance of 2706 km from the Town Hall Square in Copenhagen, Denmark, to North Cape in Norway (Nordkapp), as far north as possible on the European continent (Figure 1). All of the subjects had previously participated in numerous demanding recreational cycling activities, and they volunteered to participate in a scientific experiment planned around the 14 d of cycling. They were informed about the possible risks and discomfort involved before written consent to participate was obtained. The study was performed according to the Declaration of Helsinki and was approved by the Science Ethical Committee of the Copenhagen Region (H3-2011-008) and registered at clinicaltrials.gov (NCT02353624).

Experimental conditions

At 2 to 5 d before leaving and again 28–33 h after arrival at Nordkapp, the subjects were tested under standardized conditions in the morning after an overnight fast. A month before departure, 5 of 6 subjects participated in an optional test day to be familiarized with the test procedures. One subject was unavailable due to job constraints.

During the 14 d of cycling the subjects' own support team handled all of the practical logistics, providing transport of extra gear and maintenance of bikes and supplying, cooking, and preparing all food and beverages before, during, and after cycling. The subjects knew they should be conscientious about eating and drinking sufficient amounts to ensure an adequate EI and hydration to maintain their capacity to perform the required prolonged cycling. The entire cycling trip was supervised by accompanying scientific staff who obtained the relevant measures described below.

TABLE 1
Physical characteristics and blood values of subjects¹

	Baseline	Day 8	Change
Age, y	61.3 ± 3.4	—	—
Height, cm	178 ± 3	—	—
Body weight, kg	77.4 ± 4.2	77.8 ± 4.2	0.4 ± 1.0
TBW, ² L	46.3 ± 2.3	48.1 ± 2.6	1.8 ± 0.5*
Fat-free mass, ² kg	63.4 ± 3.2	65.9 ± 3.5	2.5 ± 0.6*
Fat mass, ² kg	14.0 ± 1.5	11.8 ± 1.1	-2.2 ± 0.7*
Fat mass (DXA), kg	16.3 ± 4.5	—	—

¹Values are means ± SEs, n = 6. *Difference between baseline and after cycling, P < 0.05 (paired t test). DXA, dual-energy X-ray absorptiometry; TBW, total body water.

²Estimated at day 8 of cycling by using isotope dilution.

Tests

The testing procedure before departure and on arrival at Nordkapp was identical, except for the location. In the morning after an overnight fast and voiding, body weight was measured on an electronic scale (Seca) and venous blood was sampled from an antecubital vein. Then, V̇O₂max was measured on an electronically braked bike (Monark 839E, Monark Exercise AB) starting with a 100-W workload for 3 min followed by 40-W increments every minute until attainment of V̇O₂max by using the following criteria: a leveling off of oxygen uptake (V̇O₂) and a respiratory exchange ratio >1.15. Respiratory gas exchange was analyzed with an online system (Quark b²; CosMed).

Total EE

Total EE was measured weekly in the week before departure and throughout the 14 d of cycling by applying the DLW technique according to the Maastricht protocol (12). The protocol was modified such that a dosage of labeled water was applied weekly instead of biweekly because of the expected high levels of EE (13). Briefly, every dosage of labeled water was ingested in the evening, shortly after the collection of a background urine sample. Subjects drank an amount of ²H₂¹⁸O to ensure that baseline amounts were increased by 100–150 parts per million for ²H and by 200–250 parts per million for ¹⁸O. A second voiding was collected in the morning on the following day, and subsequently urine samples were collected twice in the morning on days 3 and 7 and twice in the evening on days 2 and 7 during each of the three 7-d dosage intervals. All of the morning samples were obtained before engagement in any activity. Carbon dioxide production, as calculated from the difference in ¹⁸O and ²H elimination rates as part of the DLW technique, was converted to EE according to the metabolic fuel quotient calculated from the macronutrient composition of the diet as measured with dietary records and the use of body energy stores (14). Because of potential changes in background water sources (primarily tap water) over the course of cycling, 3 noncyclist subjects, who consumed the same background water, also gave urine samples at the same time points to adjust for a potential variability in water sources. However, we did not observe any changes in ¹⁸O and ²H in the urine samples of the 3 noncyclists and thus did not have to make adjustments for background changes due to changes in water sources.

TABLE 2
Physical characteristics and blood values of the subjects¹

	Baseline	Day 14	Change
Body weight, kg	77.4 ± 4.2	77.7 ± 4.3	0.3 ± 1.0
BMI, kg/m ²	24.4 ± 0.9	24.5 ± 0.8	0.1 ± 0.2
$\dot{V}O_2$ max, mL/(kg · min)	48.3 ± 2.3	45.1 ± 2.4	-3.1 ± 1.7 *
$\dot{V}O_2$ max, mL/min	3705 ± 165	3485 ± 195	-220 ± 65 *
Plasma glucose, mmol/L	5.9 ± 0.2	6.0 ± 0.3	0.1 ± 0.2
Plasma insulin, pg/mL	15.5 (13.8–18.2)	23.2 (19.6–42.1)	—*
Plasma leptin, pg/mL	1257 ± 201	1354 ± 177	97 ± 80
Plasma ghrelin, pg/mL	867 ± 516	824 ± 497	-43 ± 18
Plasma GLP-1, pmol/mL	12.7 ± 2.1	19.5 ± 4.4	6.8 ± 4.5 *
Plasma PYY _{3–36} , pg/mL	60 ± 14	104 ± 28	44 ± 30 *
Plasma citrulline, μmol/L	31.9 ± 6.6	41.5 ± 9.6	9.5 ± 10.2

¹Values are means ± SEs, except for plasma insulin values, which are shown as medians with 25–75% confidence limits in parentheses. *n* = 6. *Difference between baseline and after cycling, *P* < 0.05. Differences are compared using a paired *t* test. GLP-1, glucagon-like peptide 1; PYY_{3–36}, polypeptide YY_{3–36}; $\dot{V}O_2$ max, maximal oxygen uptake.

Body composition

Body composition was estimated from total body water (TBW) calculated from ²H isotope dilution according to the Maastricht protocol (12), at departure and after 8 d of cycling. This measurement was derived after the ingestion of labeled water. We were primarily interested in measuring total EE with the DLW technique, and because there was no ingestion of ²H at day 14, body composition could be determined only at baseline and day 8. Fat-free mass (FFM) was calculated as FFM = TBW ÷ 0.73 (15), and fat mass was subsequently calculated as the difference between TBW and FFM. In addition, fat mass and FFM were measured by using dual-energy X-ray absorptiometry (DXA; Lunar iDXA Series; GE Medical Systems) at the familiarization test and 2–5 d before departure to calculate energy balance from changes in body composition in the period leading up to the cycling trip; however, DXA was unavailable at Nordkapp because of impossible logistics.

EI

Total EI was assessed via all foods and drinks consumed by the subjects during cycling. These food items and beverages were observed, recorded, and weighed by the scientific staff at 3 periods (days 2–3, 5 and 7, and 12–13). All drinking bottles were weighed before and after cycling, and the wrapping and packing of food and supplements consumed during cycling were collected such that individual consumption could be established. We had access to all of the recipes of the foods and meals eaten, and the recordings were processed by using appropriate software (Dankost 3000; Dansk Catering Service) to calculate total EI and the macronutrient composition of the diet.

Exercise heart rate and EE

Heart rate during exercise and total cycling duration was obtained by using heart rate monitors (Polar RS800, Polar Electro), and relative exercise intensity maximum during cycling was expressed as % $\dot{V}O_2$ max and estimated via the heart rate– $\dot{V}O_2$ relation for each subject as an average of the pre- and post-relations. For all 14 d, the relative exercise intensity was obtained from measured heart rate and calculated for every 10-min period during cycling. $\dot{V}O_2$ during cycling was calculated as the

product of exercise intensity and average $\dot{V}O_2$ max (pre-post). Subsequently, exercise EE (in kJ) was estimated by using the following equation: average $\dot{V}O_2$ max (L O₂/min) × exercise intensity (expressed as % $\dot{V}O_2$ max) × exercise duration (min) × 20 (kJ/L O₂) with the assumption that the energy equivalent of oxygen is 20 kJ/L (16).

Physical activity and sleep

Physical activity and sleep were monitored by using the Sensewear Armband (Bodymedia) and divided into time spent sleeping, lying down, and different physical activity levels as well as nonwear time were determined by using software specifications. Physical activity was classified as sedentary and light or

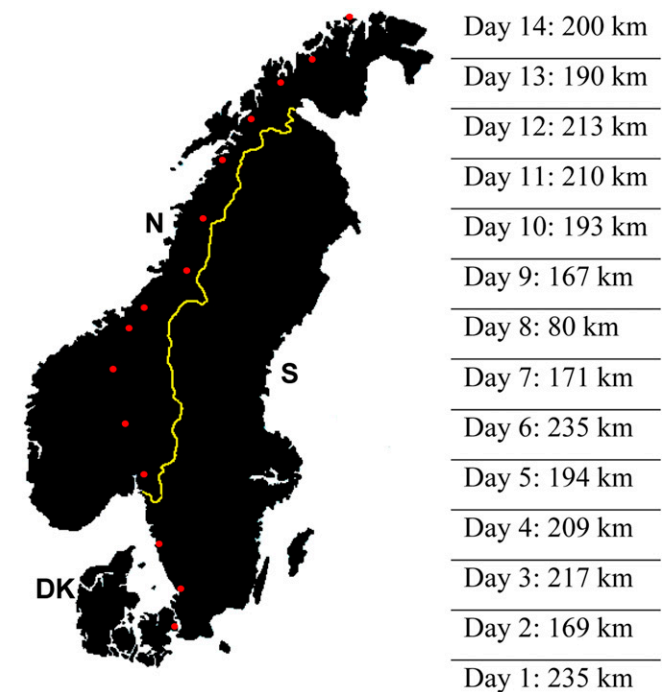


FIGURE 1 Geographical depiction of the 2706-km route from Copenhagen, Denmark, to Nordkapp, Norway, via Sweden. The red dots depict departure and arrival points of daily stages throughout the trip. Daily stage distances in kilometers are shown from days 1 to 14. DK, Denmark; N, Norway; S, Sweden.

vigorous activity. A threshold physical activity level of 3.0 was used to dissociate vigorous from sedentary and light activity, implying that vigorous activity was 3 times resting levels. Dissociation of lying down and sleeping was assessed from software specifications. All subjects wore the armband for 4 d (3 weekdays and 1 weekend day) in the week before departure and during 4 specified time periods during cycling (days 2–3, 5 and 7, and 12–13); days 5 and 7 were subsequently combined.

Appetite ratings

Subjective sensations of appetite and thirst were subsequently rated by using visual analog scales for hunger, satiety, fullness, and prospective food intake as well as thirst (17) immediately before breakfast and the evening meal. Ratings were obtained for 4 d before departure and daily from day 2 to 14 during cycling. Ratings were later averaged from the 4 d before departure and in 3 intervals during cycling (days 2–5, 6–9, and 10–14).

Analytic procedures

Fasting blood samples were collected in tubes containing 0.3 ml EDTA/L (10 μL/mL blood) and immediately centrifuged at 4°C for 10 min at 23,000 × g. The plasma was stored at –80°C until analysis. Plasma glucose was analyzed on an automatic analyzer (Cobas Fara; Roche). Concentrations of plasma insulin were determined by using a commercially available ELISA kit (Dako) and read on a Labsystems Multiskan MS (Thermo Electron Oy). Total ghrelin, GLP-1, and PYY_{3–36} were measured by using radioimmunoassay with commercially available kits (Linco Research). Plasma leptin was measured by using a specific high-sensitivity human ELISA kit (Human Leptin Immunoassay; R&D Systems). Plasma citrulline, a marker of enterocyte capacity and mass, was measured to provide a possible mechanistic explanation of changes in hormones from the gut by using ultra-performance liquid chromatography hydrophilic interaction–tandem mass spectrometry of acetonitrile-derived supernatants with the use of a method that has been validated and described in detail elsewhere (18).

Calculations and statistical analysis

Energy balance, in essence the discrepancy between EI and EE (3), was calculated during cycling as EI (observations) minus total

EE (DLW). In addition, we calculated net energy balance from changes in body composition using the chemical energy equivalents for changes in fat mass (39.5 MJ/kg) and FFM (7.6 MJ/kg) (19) from the assumption that changes in body energy stores reflect energy balance over this period (16). To validate the accuracy of the 6-d recordings of EI during cycling, a calculated EI (DLW) was obtained by using the intake–balance method [difference between total EE (DLW) and net energy balance] (20). Differences between methods are presented as the mean difference with 95% CIs. BMR was calculated by using a standard equation (21), and to quantify the increase in total EE above baseline levels, total EE from DLW was divided by the calculated BMR.

All of the data were tested for normality and equal variance. Parametric data are calculated as means ± SEs; significant changes from baseline to after cycling were evaluated with paired *t* tests, whereas appetite ratings were analyzed by using a 1-factor repeated-measures ANOVA. Nonparametric data were tabulated as medians with 25–75% percentiles, and differences were evaluated with ranked statistical tests (Mann-Whitney for pre-post measures and Shapiro-Wilks for repeated measures). Correlations were assessed by using simple linear regression. All statistical analysis was conducted in SAS enterprise guide, version 6.1 (SAS Institute), or in Sigmaplot, version 11 (Systat).

RESULTS

All 6 subjects completed the full distance of 2706 km in 14 d (Figure 1). The average daily distance covered by the subjects was 193 ± 10 km/d and with the seventh day as a “resting” day (80 km) (Figure 1). More than 90% of the exercise heart rate was retrieved. However, because all 6 subjects cycled together, there were sufficient data to determine that the full exercise time amounted to 631 ± 37 min/d. On the basis of the sampled data, the average daily intensity was 53.1% ± 1.1% of $\dot{V}O_{2max}$, of which ~198 ± 58 min/d were spent at an intensity >60% of $\dot{V}O_{2max}$. $\dot{V}O_{2max}$ decreased by 6% ± 2% (*P* = 0.04) after cycling, as well as when expressed as absolute $\dot{V}O_{2max}$ (*P* < 0.01; Table 2).

EE

A remarkable 83% ± 13% increase in total EE was observed during cycling (30.1 ± 1.5 MJ/d) compared with the week

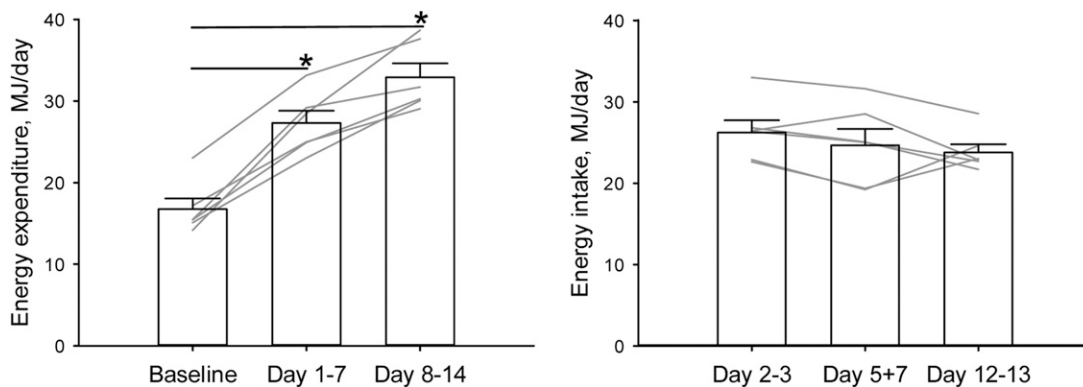


FIGURE 2 Energy expenditure at baseline (before cycling) and during days 1–7 and 8–14 and observations (left panel) and recordings of energy intake during cycling on days 2–3, 5 and 7, and 12–13 (right panel) during a 2706-km bicycle trip from Copenhagen, Denmark to Nordkapp, Norway in 6 older male recreational cyclists. Values are means ± SEs. Lines depict individual values. *Different from baseline, *P* < 0.05 (paired *t* test).

before cycling (16.7 ± 1.3 MJ/d; $P < 0.001$) (Figure 2). During the first week, total daily EE was 27.3 ± 1.5 MJ/d, increasing to 32.9 ± 1.7 MJ/d ($P < 0.01$) in the second week (Figure 2). Calculated exercise EE during cycling contributed to 22.1 ± 2.1 MJ/d, which corresponded to $73\% \pm 4\%$ of the total EE on average. Calculated BMR at baseline was 7.7 ± 0.3 kJ/d, and hence total EE was 4.0 ± 0.1 times the BMR.

EI

Observations on EI as well as dietary macronutrients during cycling are presented in Figure 2 and Table 3. The recordings of EI (24.9 ± 1.3 MJ/d) and the calculation of EI (25.3 ± 2.3 MJ/d) from the intake-balance method (DLW minus net energy balance) provided similar estimates on a group level (difference: 0.4 MJ; 95% CI: $-3.7, 2.9$ MJ; $P = 0.74$) and these estimates correlated well on an individual level ($R^2 = 0.87, P = 0.03$), supporting that registrations of EI provided a robust estimate of total EI during cycling. All of the subjects consumed supplementary foods (bars, gels, and drinks) during cycling, which accounted for $17\% \pm 2\%$ of total EI. Without supplementation, EI was 20.7 ± 1.3 MJ/d per kilogram of body weight, carbohydrate intake was 12.1 ± 0.8 g/kg per day, and protein intake was 2.1 ± 0.2 g/kg per day (Table 3).

Body composition

Over the course of these 14 d of cycling, body weight remained unchanged ($P = 0.8$), but after 8 d body fat content decreased by $15\% \pm 4\%$ ($P = 0.02$), whereas TBW ($P = 0.01$) and hence FFM ($P = 0.01$) increased by $4\% \pm 1\%$ (Table 1). There were no changes from the familiarization test to the test before departure in body weight (-1.3 ± 1.8 kg; $P = 0.87$), fat mass (-1.1 ± 2.1 kg; $P = 0.76$), or FFM (-0.2 ± 1.6 kg; $P = 0.97$). Fat mass assessed by DXA at pretesting or by isotope dilution at baseline was highly correlated ($R^2 = 0.97, P < 0.001$) and not significantly different ($P = 0.31$).

Energy balance

Because of a larger total EE relative to EI, there was a discrepancy between energy spent and consumed (-5.2 ± 1.2 MJ/d) (Figure 3). As calculated from changes in body composition (i.e., energy stores) after 8 d, net energy balance was also negative (-4.8 ± 2.0 MJ/d) (Figure 3). The 2 methods used to calculate energy balance provided similar results on a group level (differ-

ence: 0.4 ± 1.3 MJ/d; $P = 0.74$). However, they tended to correlate only on an individual level ($R^2 = 0.78, P = 0.065$). In the 5 subjects who participated in the familiarization test 1 mo before departure, net energy balance was calculated (-1.7 ± 1.2 MJ) from body-composition changes according to DXA.

Physical activity and sleep

Because of the nature of the experiment, movement behaviors changed: time spent in vigorous activities increased markedly (baseline compared with cycling: $03:14 \pm 00:29$ compared with $10:28 \pm 00:23$ h:min; $P < 0.001$) and time spent in sedentary and light activities was reduced (baseline compared with cycling: $12:41 \pm 00:37$ compared with $06:04 \pm 00:21$ h:min; $P < 0.001$) (Figure 4). Sleeping time was reduced at all 3 measurement periods by ~ 50 min/d ($05:41 \pm 00:14$ h:min; $P < 0.04$) compared with baseline ($06:31 \pm 00:09$ h:min) (Figure 4). However, the time spent lying down without sleeping and the amount of steps taken daily ($P = 0.26$) remained unchanged (Figure 4). Total wear time of the monitor did not differ (data not shown; $P = 0.89$).

Blood samples

Fasting plasma glucose was unchanged ($P = 0.53$), but there was an increase in fasting concentrations of insulin ($P = 0.02$), GLP-1 (difference: 6.8 ± 4.5 pmol/L; $P < 0.01$), and PYY₃₋₃₆ ($P < 0.02$) (Table 2). Fasting leptin ($P = 0.66$) and ghrelin ($P = 0.38$) remained unchanged, although fasting ghrelin concentrations were lower in 5 of 6 subjects after cycling (Table 2). Plasma citrulline did not increase significantly, although a tendency was apparent ($P = 0.07$) (Table 2).

Appetite ratings

Before cycling only 5 of the 6 subjects provided valid ratings because one subject erroneously filled out the recordings after food consumption. His data were omitted from these analyses. There was a significant overall effect of cycling in which ratings of hunger increased in the evening (baseline compared with days

TABLE 3
Macronutrient composition of the diet during 14 d of cycling¹

	Value
Energy intake, MJ/d	24.9 ± 1.3
Carbohydrate, g/d	931 ± 58
Total energy intake, %	62.8 ± 1.0
Fat, g/d	161 ± 11
Total energy intake, %	23.7 ± 0.7
Protein, g/d	165 ± 9
Total energy intake, %	11.1 ± 0.4
Alcohol, g/d	20 ± 9
Total energy intake, %	2.5 ± 1.2

¹Values are means \pm SEs, $n = 6$.

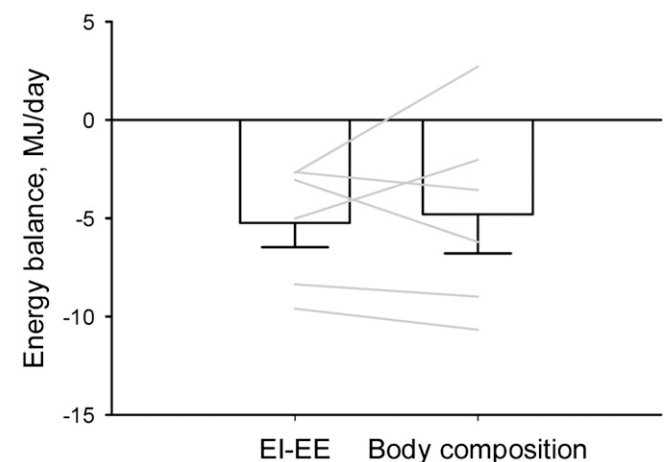


FIGURE 3 Energy balance during a 2706-km bicycle trip from Copenhagen, Denmark to Nordkapp, Norway in 6 older male recreational cyclists. Energy balance is calculated as EI - EE or from changes in body energy stores (body composition). Values are means \pm SEs. Lines depict individual values. EE, energy expenditure; EI, energy intake.

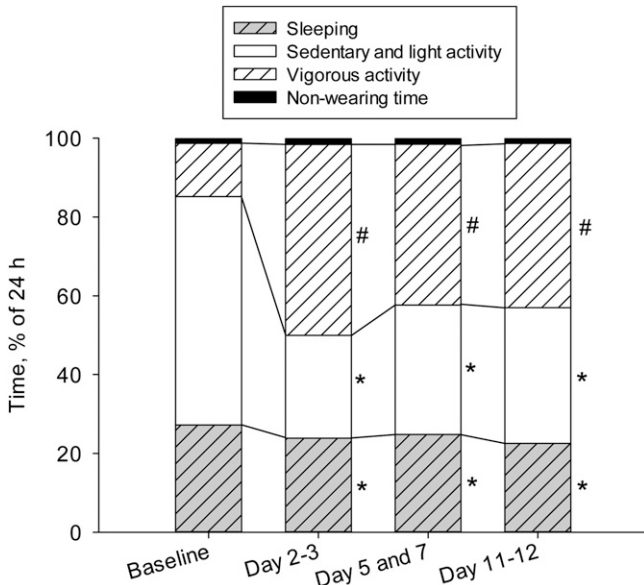


FIGURE 4 Physical activity and sleep during 24 h before (baseline) and on the selected days 2–3, 5 and 7, and 11–12 during a 2706-km bicycle trip from Copenhagen, Denmark to Nordkapp, Norway in 6 older male recreational cyclists. Values are means. *Different from pre, $P < 0.05$; #significant main effect compared with pre, $P < 0.05$. Appetite ratings were analyzed by using a 1-factor repeated-measures ANOVA.

2–5, 6–9, and 10–14; $P = 0.04$ for all) and in which morning hunger ratings tended to increase ($P = 0.07$; **Figure 5**). In the evening, a significant overall effect of cycling with a decrease in ratings of fullness appeared ($P = 0.04$ for all) (Figure 5). No differences in ratings of thirst ($P = 0.16$) or satiety ($P > 0.27$) were detected.

DISCUSSION

The main finding was that the rate of total EE achieved during cycling in older male recreational cyclists, on average, was 30 MJ/d, which was ~4 times the BMR. Hence, the values obtained in older subjects in the present study approach maximally measured EE levels that were reported in professional cyclists competing in the Tour de France (6, 22). The levels reported in the present study are comparable to world-elite cross-country skiers during an intense training period (8) and to values reported

in polar expeditions (23, 24). Another important finding in the present study was the level of negative energy balance (i.e., EI and EE difference) that was registered over the course of cycling, suggesting that EI did not sufficiently match the increase in EE. Although the motivation to eat as expected increased, shown by increased hunger ratings, a conflicting and different response was observed by an increase in fasting hormonal satiety signals (insulin, GLP-1, and PYY_{3–36}) after cycling.

To put the EE of the older subjects in our study into perspective, the rate of EE determined by DLW during a case study in the Ironman World Championship was 37.8 MJ (25). This is a 1-d event that requires extreme physiologic effort. The upper values of expenditure reported in the literature are, in most cases, obtained in younger subjects who are trained for demanding situations. Thus, older, recreationally very-well-trained male subjects were able to achieve and sustain near-maximal levels of EE during 14 d of strenuous and prolonged cycling. To our knowledge, no studies have presented such high rates of EE in a group of subjects with an age similar to that in the present study.

Ainslie et al. (9) reported an average EE of 21.4 ± 3.2 MJ/d measured by using DLW during a 10-d strenuous walking expedition in the Scottish highlands in a comparable group of older male subjects (56 ± 3 y). In that study, younger (24 ± 3 y) and older subjects were compared. Body fat decreased in both groups, but more so in the older group because food intake was reported to be lower in that group. As an indication of fatigue, $\dot{V}O_{2max}$ declined in the present study, suggesting that these rates of EE are beyond what can be tolerated in this age group under these conditions.

Although somewhat counterintuitive, the decline in both absolute (O_2/min) and relative (to body weight) maximal oxygen uptake is not surprising. Indeed, all of the subjects experienced the experimental criteria for obtaining $\dot{V}O_{2max}$ (e.g., leveling off in oxygen uptake); as such, the values obtained are valid and represent maximal obtainable values at the time of testing. We cannot refute that the decline may be transient, and the present study is not a usual exercise intervention in which increases in $\dot{V}O_{2max}$ are expected. Rather, subjects are well trained (high $\dot{V}O_{2max}$) when they start the cycling trip (10, 11). It should be understood that the present study was a study of performance, rather than training, and the decrease in $\dot{V}O_{2max}$ could be

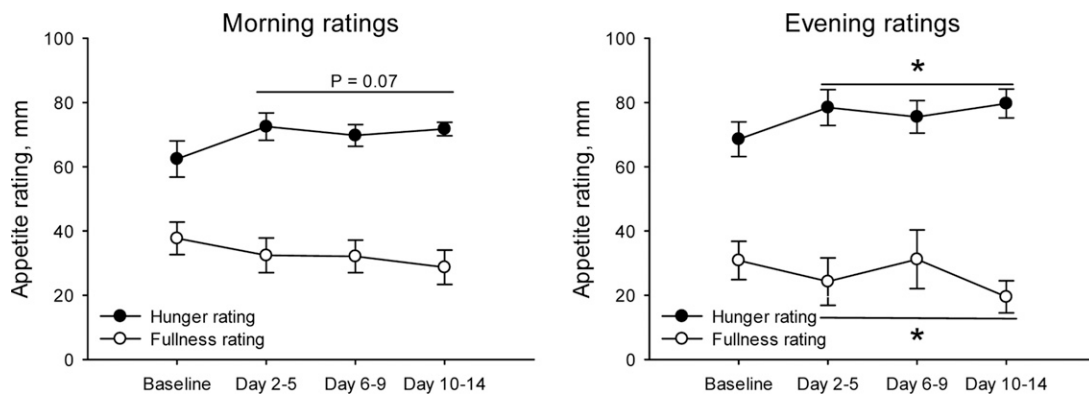


FIGURE 5 Average of 4 d of subjective ratings of hunger and fullness immediately before breakfast (morning ratings; left panel) and immediately before supper (evening ratings; right panel) before (baseline) and during days 2–5, 6–9, and 10–14 during a 2706-km bicycle trip from Copenhagen, Denmark to Nordkapp, Norway in 5 older male recreational cyclists. Values are means \pm SEs. *Significant main effect of cycling, $P < 0.05$.

caused by several factors that were not addressed in the present study.

Under normal physiologic conditions, factors limiting $\dot{V}O_2$ max are normally considered as systemic oxygen delivery and especially oxygen delivery to the locomotor muscles. Mitochondrial work efficiency is widely considered to exceed maximal oxygen delivering capacity, and we suspect the decline in $\dot{V}O_2$ max was caused by fatigue, most prominently central fatigue (26). In some of our other work, 96–112 h of ultra-endurance exercise in New Zealand (27) and studies in persons crossing the Greenland ice cap (exercising 5.5–6.5 h/d) (28, 29), we also observed either similar or even decreased maximal oxygen uptake. Under the latter conditions, this was concurrent with weight loss. In younger (24 ± 2 y) trained male cyclists, body weight and fat mass as well as $\dot{V}O_2$ max were maintained after 21 d of intense cycling training (3211 km in total, 170 km/d) (30), implying that younger, well-trained athletes can maintain energy balance under comparable conditions. In addition, Tour de France cyclists only experienced minimal declines in body fat (-0.4 kg) during the first 2 wk of the race, despite maximally measured EE rates (6). Some of the conditions, however, separate the findings of the present study from those for younger trained athletes. We did not manipulate the diet, although food and supplements were readily available throughout the day over the course of cycling. An upper limit of EI (~ 20 MJ) has been hypothesized when consuming a normal carbohydrate-rich diet (without supplements) under prolonged, repeated heavy exercise performed in the laboratory (31). Interestingly, EI without supplements was 20.7 MJ/d in the present study, suggesting that a threshold might have been reached in the present study. Although energy consumed from supplements accounted for approximately one-fifth of energy consumed, EI was insufficient to compensate for the increase in total EE. Altogether, this could suggest that aging could be associated with a blunted physiologic drive to eat in response to very high rates of EE.

Within the context of the present study (age, training status, EE, etc.) our findings confirm the original hypothesis proposed by Jean Mayer, that beyond a certain threshold level of EE increases in intake are not directly coupled with increases in expenditure (1, 2), resulting in a negative energy balance. However, it has been hypothesized that the matching of EI to increases in EE are time-dependent, such that increases in EI are delayed after increases in EE (32). Although we cannot rule out such a delay in EI in the present study, the ranges of EE in previous studies that showed a delay in EI to increased EE (33, 34) are only $\sim 50\%$ of those in the present study. Nevertheless, the exact mechanisms for a delay in EI to EE are not well elucidated.

A phenomenon of exercise-induced anorexia has been described (35), such that both subjective control over and physiologic regulation of appetite are transiently suppressed, leading to decreased EI in relation to an acute bout of exercise (36). Obviously, anorexia would be detrimental in the setting of high rates of EE. Because of the increase in the motivation to eat (increase in hunger and decrease in fullness) and the amount of EI, we speculate that this “anorexia” did not occur. On the other hand, a negative energy balance was observed over the course of cycling, and we speculate that this could be the result of a reversed or inadequate physiologic stimulus under the present dietary conditions. Energy homeostasis is governed by a complex

system integrating signals from the gut, pancreatic islets of Langerhans, and adipose tissue within central neural endocrine pathways. Adiposity signals, such as leptin and insulin, exert a long-term control over energy balance and food intake, whereas signals from the gastrointestinal tract fluctuate with meal patterns to mediate more episodic control of energy intake and satiety (37). In addition to its role in regulating glucose homeostasis, insulin has been implicated as a central nervous mediator of long-term positive energy balance limiting food intake (38); and in the present study, fasting concentrations of insulin increased after cycling. Signals from the gastrointestinal tract implicated in satiety regulation, namely GLP-1 (39) and PYY_{3–36} (40), were increased in the fasted state, whereas the only gastrointestinal hormone known to stimulate hunger, ghrelin (41), was lower in 5 of 6 subjects (not significantly different). Although the acute effects of these gastrointestinal hormones in relation to meals are well described, it has been hypothesized that fasting concentrations of these hormones may also contribute to the regulation of the chronic stimulus to eat (37). In the present study, citrulline, a marker of enterocyte capacity and mass (18), tended to increase over the 14 d, possibly induced by the rather dramatic increase in food consumption and thus energy intake. This may suggest that the increase in gastrointestinal hormones and thus the somewhat divergent appetite hormone response may, at least in part, be due to an increased enterocyte mass and thus larger secretory capacity.

The data from the present study were obtained under extraordinary conditions and reflect extreme work durations. A major limitation of the present study is that the selection of subjects was not from a random pool, which causes a potential risk of bias. Because the subjects were highly (self) motivated, this challenges the external validity of our findings. On the other hand, recruitment of subjects for this kind of study requires motivated subjects in all cases, and the fact that the study was carried out in the field most certainly speaks to the applicability of the results. Furthermore, we performed state-of-the-art measurements of many components of energy balance. Another limitation is that changes in body composition with the use of the dilution methodology occurred after 8 d and not 14 d. For logistical reasons, the measurements of body composition by DXA could not be performed after 14 d of cycling and the determination of body water (and body composition) by isotope dilution was only performed after day 8. Although body weight was stable over the course of cycling, it is possible that fat mass was even further decreased and FFM additionally increased. As such, we may have underestimated the amount of energy imbalance. In conclusion, older male subjects who completed a 14-d cycling expedition achieved rates of 30 MJ/d, approaching near-maximal rates of sustained EE. Despite increases in their motivation to eat, energy balance was not maintained, possibly due to a counteracting physiologic stimulus that inhibited the upregulation of EI.

We thank Anna Vitek West for providing the Senswear armbands (Maribo Medico A/S, Denmark). We also thank Thomas Beck and Jeppe Bach for technical assistance.

The authors' responsibilities were as follows—MR, KW, AS, and JWH: designed the research; MR, TM, PRA, KK, HR, and JWH: performed the research; MR, JJH, FD, KW, AS, and JWH: analyzed the data; MR and JWH: performed the statistical analysis; MR: wrote the manuscript; JWH

had primary responsibility for the final content; and all authors: substantially contributed to the manuscript and had access to the final version. The authors declared no conflicts of interest. The supporters had no role in the design, implementation, analysis, or interpretation of the results of the present study.

REFERENCES

- Mayer J, Roy P, Mitra KP. Relation between caloric intake, body weight, and physical work: studies in an industrial male population in West Bengal. *Am J Clin Nutr* 1956;4:169–75.
- Mayer J, Marshall NB, Vitale JJ, Christensen JH, Mashayekhi MB, Stare FJ. Exercise, food intake and body weight in normal rats and genetically obese adult mice. *Am J Physiol* 1954;177:544–8.
- Hill JO, Wyatt HR, Peters JC. Energy balance and obesity. *Circulation* 2012;126:126–32.
- Church TS, Thomas DM, Tudor-Locke C, Katzmarzyk PT, Earnest CP, Rodarte RQ, Martin CK, Blair SN, Bouchard C. Trends over 5 decades in U.S. occupation-related physical activity and their associations with obesity. *PLoS One* 2011;6:e19657.
- Hoyt RW, Friedl KE. Field studies of exercise and food deprivation. *Curr Opin Clin Nutr Metab Care* 2006;9:685–90.
- 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* (1985) 1986;61(6):2162–7.
- Hammond KA, Diamond J. Maximal sustained energy budgets in humans and animals. *Nature* 1997;386:457–62.
- Sjödín AM, Andersson AB, Hogberg JM, Westerterp KR. Energy balance in cross-country skiers: a study using doubly labeled water. *Med Sci Sports Exerc* 1994;26:720–4.
- Ainslie PN, Campbell IT, Frayn KN, Humphreys SM, MacLaren DP, Reilly T, Westerterp KR. Energy balance, metabolism, hydration, and performance during strenuous hill walking: the effect of age. *J Appl Physiol* 2002;93:714–23.
- Rogers MA, Hagberg JM, Martin WH III, Ehsani AA, Holloszy JO. Decline in VO₂max with aging in master athletes and sedentary men. *J Appl Physiol* (1985) 1990;68(5):2195–9.
- Tanaka H, Seals DR. Endurance exercise performance in Masters athletes: age-associated changes and underlying physiological mechanisms. *J Physiol* 2008;586:55–63.
- 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(Suppl 1):49–57.
- Westerterp KR. Body composition, water turnover and energy turnover assessment with labelled water. *Proc Nutr Soc* 1999;58:945–51.
- Black AE, Prentice AM, Coward WA. Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Hum Nutr Clin Nutr* 1986;40:381–91.
- Pace N, Rathbun PE. Studies on body composition III: Total body water and chemically combined nitrogen content in relation to fat content. *J Biol Chem* 1945;158:685–91.
- Elia M, Livesey G. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. *World Rev Nutr Diet* 1992;70:68–131.
- Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord* 2000;24:38–48.
- Demacker PN, Beijers AM, van Daal H, Donnelly JP, Blijlevens NM, van den Ouweland JM. Plasma citrulline measurement using UPLC tandem mass-spectrometry to determine small intestinal enterocyte pathology. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009;877:387–92.
- Hall KD. What is the required energy deficit per unit weight loss? *Int J Obes (Lond)* 2008;32:573–6.
- Racette SB, Das SK, Bhapkar M, Hadley EC, Roberts SB, Ravussin E, Pieper C, Delany JP, Kraus WE, Rochon J, et al. Approaches for quantifying energy intake and %calorie restriction during calorie restriction interventions in humans: the multicenter CALERIE study. *Am J Physiol Endocrinol Metab* 2012;302:E441–8.
- Westerterp KR, Donkers JH, Fredrix EW, Boekhoudt P. Energy intake, physical activity and body weight: a simulation model. *Br J Nutr* 1995;73:337–47.
- 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(Suppl 1):S26–31.
- Stroud MA, Ritz P, Coward WA, Sawyer MB, Constantin-Teodosiu D, Greenhaff PL, Macdonald IA. Energy expenditure using isotope-labelled water (²H₂¹⁸O), exercise performance, skeletal muscle enzyme activities and plasma biochemical parameters in humans during 95 days of endurance exercise with inadequate energy intake. *Eur J Appl Physiol Occup Physiol* 1997;76:243–52.
- Frykman PN, Harman EA, Opstad PK, Hoyt RW, Delany JP, Friedl KE. Effects of a 3-month endurance event on physical performance and body composition: the G2 trans-Greenland expedition. *Wilderness Environ Med* 2003;14:240–8.
- Cuddy JS, Slivka DR, Hailes WS, Dumke CL, Ruby BC. Metabolic profile of the Ironman World Championships: a case study. *Int J Sports Physiol Perform* 2010;5:570–6.
- Noakes TD. Physiological models to understand exercise fatigue and the adaptations that predict or enhance athletic performance. *Scand J Med Sci Sports* 2000;10:123–45.
- Helge JW, Rehrer NJ, Pilegaard H, Manning P, Lucas SJ, Gerrard DF, Cotter JD. Increased fat oxidation and regulation of metabolic genes with ultraendurance exercise. *Acta Physiol (Oxf)* 2007;191:77–86.
- Helge JW, Lundby C, Christensen DL, Langfort J, Messonnier L, Zacho M, Andersen JL, Saltin B. Skiing across the Greenland icecap: divergent effects on limb muscle adaptations and substrate oxidation. *J Exp Biol* 2003;206:1075–83.
- Helge JW, Damsgaard R, Overgaard K, Andersen JL, Donsmark M, Dyrskog SE, Hermansen K, Saltin B, Dagaard JR. Low-intensity training dissociates metabolic from aerobic fitness. *Scand J Med Sci Sports* 2008;18:86–94.
- Slivka DR, Dumke CL, Hailes WS, Cuddy JS, Ruby BC. Substrate use and biochemical response to a 3,211-km bicycle tour in trained cyclists. *Eur J Appl Physiol* 2012;112:1621–30.
- Brouns F, Saris WH, Stroecken J, Beckers E, Thijssen R, Rehrer NJ, ten Hoor F. Eating, drinking, and cycling: a controlled Tour de France simulation study, part I. *Int J Sports Med* 1989;10(Suppl 1):S32–40.
- Blundell JE, King NA. Effects of exercise on appetite control: loose coupling between energy expenditure and energy intake. *Int J Obes Relat Metab Disord* 1998;22(Suppl 2):S22–9.
- Stubbs RJ, Hughes DA, Johnstone AM, Whybrow S, Horgan GW, King N, Blundell J. Rate and extent of compensatory changes in energy intake and expenditure in response to altered exercise and diet composition in humans. *Am J Physiol Regul Integr Comp Physiol* 2004;286:R350–8.
- Whybrow S, Hughes DA, Ritz P, Johnstone AM, Horgan GW, King N, Blundell JE, Stubbs RJ. The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding ad libitum. *Br J Nutr* 2008;100:1109–15.
- King NA, Burley VJ, Blundell JE. Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *Eur J Clin Nutr* 1994;48:715–24.
- King JA, Wasse LK, Ewens J, Crystallin K, Emmanuel J, Batterham RL, Stensel DJ. Differential acylated ghrelin, peptide YY3-36, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *J Clin Endocrinol Metab* 2011;96:1114–21.
- Cummings DE, Overduin J. Gastrointestinal regulation of food intake. *J Clin Invest* 2007;117:13–23.
- Schwartz MW, Woods SC, Porte D Jr., Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000;404:661–71.
- Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev* 2007;87:1409–39.
- Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR. Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med* 2003;349:941–8.
- Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001;50:1714–9.