

Original Article

Changes in Muscular Strength, Exercise Capacity and Carbohydrate Metabolism in Recreationally Active Men after Self-Supporting Self-Paced Multi-Week High-Volume Adventure Cycling

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Abstract

Objectives: Sustained, high-volume exercise can have both beneficial and deleterious effects on muscular fitness, cardiovascular capacity, and health markers depending on the nature of the activity. This study aimed to investigate the changes in muscular and cardiovascular fitness and carbohydrate metabolism after self-paced recreational adventure cycling over multiple weeks. **Methods:** In this uncontrolled before–after field study, eleven men (age 30.5 ± 10.9 years, BMI 23.4 ± 1.3 kg/m², VO_2max 55.4 ± 4.5 mL/kg/min) were measured for changes in body composition, leg muscle strength, exercise capacity, resting metabolism, whole-body glucose tolerance, blood volume, and hemoglobin mass in response to self-supporting individual cycling on loaded (total weight 21–50 kg) bikes for 50 ± 25 (21–99) days in 6.0 (3.5–7.1) h/day. **Results:** Knee extension and flexion strength, body weight and skinfold-derived body fat, blood volume, and hemoglobin mass remained unchanged. After the tour, VO_2max , reliance on carbohydrates for resting metabolism increased and fasting blood glucose level decreased, while fasting insulin level, glucose tolerance, and insulin response did not change. **Conclusions:** Multi-week high-volume self-paced adventure cycling was associated with increased VO_2max and decreased reliance on fat at rest. No changes in muscle strength, body composition, whole-body glucose tolerance, and insulin sensitivity were observed.

Keywords: Cardiovascular Fitness, Endurance Exercise, Muscle Quality, Self-Sufficient Adventure Cycling, Ultra-Endurance

Introduction

Low levels of habitual exercise are associated with overweight, compromised cardiovascular fitness, metabolic health, and increased risk of chronic non-infectious diseases, leading to impaired quality of life and reduced life expectancy. Cardiovascular and metabolic

health can be improved through regular physical activity, particularly endurance exercise, but the optimal dose remains unclear. In fact, very high-volume daily endurance exercise may leave suboptimal time for recovery and has been reported to result in reduced muscle strength¹, muscle mass², capacity^{3,4} and compromised metabolic health/flexibility, as evidenced by impaired glucose handling^{5,6}.

Most research on multi-day ultra-endurance endeavors so far has focused on changes in dietary energy intake and anthropometrics^{7–9}, while more in-depth studies on changes in metabolism and performance are limited. In addition, previous studies, including cycling tours, have investigated groups of men cycling together with a self-imposed race mindset^{3,10,11}. One such study found that a very high volume of daily cycling over two weeks was associated with greater positive changes in younger

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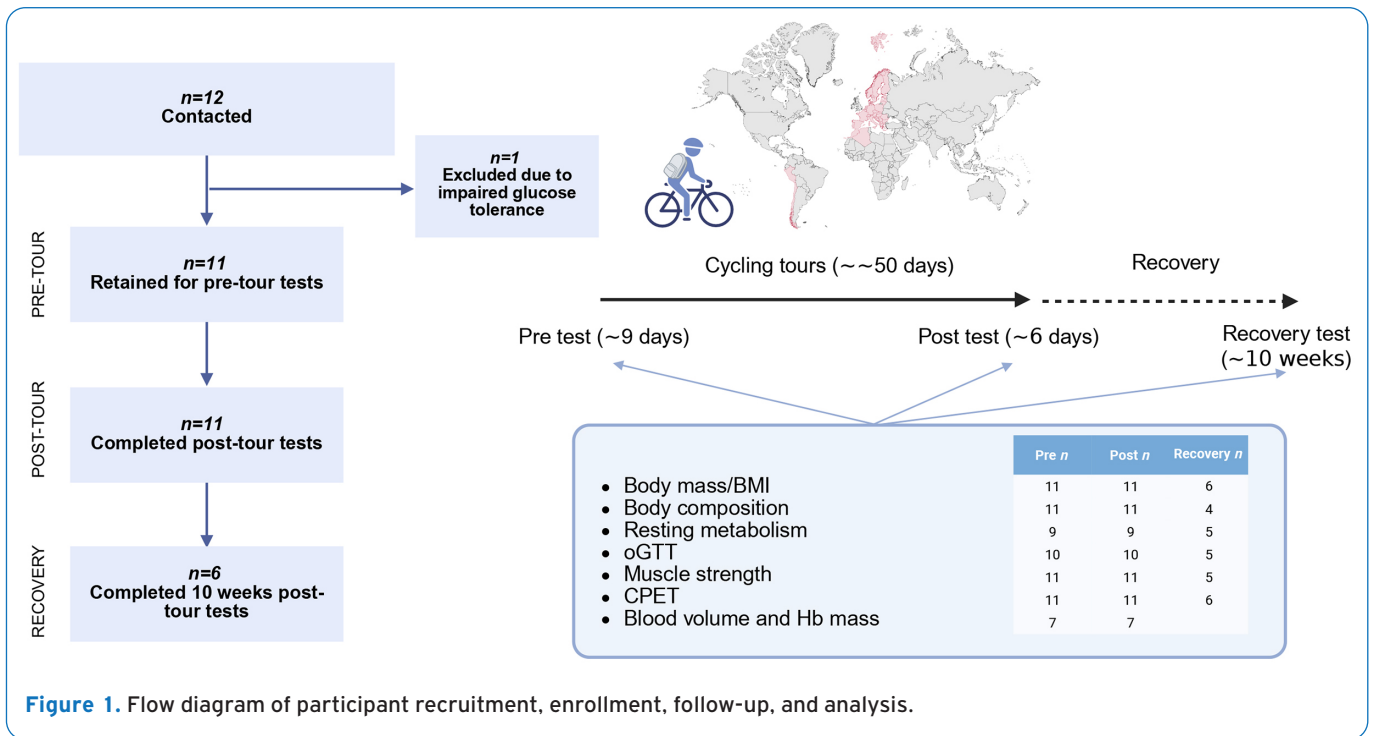


Figure 1. Flow diagram of participant recruitment, enrollment, follow-up, and analysis.

compared with older men¹¹. Secondly, because the cyclists rode in their age group with the adopted competitive attitude, less-fit participants may have struggled to keep up with the group, potentially leading to excessive strain, overtraining, and impaired cardiometabolic health, as evidenced by reduced maximal fat oxidation¹² and impaired glucose tolerance^{5,6}. On the other hand, adequate fitness-adjusted exercise intensity is anticipated to induce beneficial adaptations.

To circumvent the self-imposed competition mindset, the present study examined the changes in fitness and metabolic characteristics in response to self-organized, self-supporting adventure cycling tours among young, recreationally active adults. This design allowed us to explore whether a meaningful increase in cardiovascular and muscular fitness and changes in metabolic health and body composition could occur even in a non-competitive, endurance-context undertaking for personal enjoyment rather than performance augmentation. There is a lack of studies on fitness and metabolic health markers in response to recreational endeavors that are both fun and physically challenging. The participants of the study decided to embark on the tour for the sake of adventure, passion for exploration, and to challenge themselves, but they did not set a primary aim for the highest possible cycling velocity or power, nor did they follow a strict nutrition or supplementation strategy.

The purpose of the present study was to determine

whether several hours of daily non-competitive, self-supporting cycling over multiple weeks provides muscular, cardiovascular and metabolic health benefits in moderately trained healthy young adults. We hypothesized that – by pacing themselves according to internal fatigue rather than targeting the fastest possible speeds or times – participants of the adventure cycling tours would experience an increase in aerobic fitness, glucose tolerance and insulin sensitivity, and would not lose muscle strength.

Methods

Participants

In this uncontrolled prospective observational repeated-measures before–after field study, participants planning multi-day cycling tours were invited to participate after being identified on social media, such as online cycling forums. The inclusion criteria were healthy adults (18–50 years) planning an individual self-supporting cycling tour of at least 20 consecutive days. Exclusion criteria: smoking; fasting blood glucose >5.6 mmol·L⁻¹, blood glucose >10.0 mmol·L⁻¹ at 1 h after and/or 7.8 mmol·L⁻¹ 2 h after ingestion of 1 g·kg⁻¹ glucose during the oral glucose tolerance test; any current or previous injury or condition that would negatively affect any of the laboratory tests or measurements.

Twelve presumably healthy men qualified for the study during the recruitment period. One volunteer was excluded

from the analyses because of the markedly impaired glucose tolerance and evident insulin resistance (Figure 1). The age of the remaining 11 participants was 30.5 ± 10.9 (19–50) years, body height 1.79 ± 0.08 (1.63–1.86) m, body weight 74.9 ± 5.7 (64.5–84.1) kg, and body mass index 23.4 ± 1.3 (21.9–25.7) $\text{kg} \cdot \text{m}^{-2}$. Eight participants were employed full-time pre-tour (5 sedentary or mostly sedentary; 3 physically demanding with ~4 hours per working day of intensive physical activity), and 3 were unemployed (including students). None of the participants had a prior diagnosis of chronic medical conditions, and all were engaged in some habitual physical activity, but levels varied substantially. Eight participants undertook no specific physical preparation for the tour, whereas 3 increased their training (regular cycling to CrossFit) for 3–9 months pre-tour as a means of preparation. The moderate-to-vigorous physical activity of the participants over the last 8 weeks before the tour was 5.6 ± 3.7 (1.0 to 10.5) hours per week. The diet undertaken by the subjects was mostly omnivorous ($n = 10$), while one participant was a vegetarian.

Organization of the study

Testing was conducted on average 9 (range 2–30) days before ($n=11$), 6 (4–10) days after ($n=11$), and 75 (74–84) days (“10 weeks”) after ($n=6$) the tour, with deviations occurring due to logistical constraints. Participants arrived at the laboratory in an overnight fasted state and limited their physical activity to a minimum in the morning. During the last days before the measurements, participants were asked to maintain their typical diet of the preceding weeks. Anthropometrical and resting metabolism measurements were followed by a glucose tolerance test, after which participants took 30–60 min break to snack, visit the toilet, and prepare for the subsequent physical performance tests. Muscle strength was then measured, after which participants performed a cardiopulmonary incremental cycling test until exhaustion. Finally, after subjects drank a glass of water and showered, their blood volume and hemoglobin mass were measured.

The description of the methods used (Anthropometrics, Indirect calorimetry, Glucose tolerance test, Dynamometry, Cardiopulmonary exercise test, Blood volume and hemoglobin mass measurement) is provided in Supplementary Material 1. The primary outcomes of the study were muscle strength, VO_2max , and glucose and insulin responses to glucose challenge.

Intervention

Participants undertook self-organized, self-supporting, self-paced individual cycling tours of 50 ± 25 (21 to 99) days' duration. During the journey, they slept most nights in their tent or hammock; prepared food in the mornings and evenings, most often by themselves; and ate and snacked sporadically but copiously at random shops and/



Figure 2. Representative picture of one study participant during his efforts to reach the southern part of the European continent at Gibraltar. The participant gave written consent for publication of his photograph.

or fast-food buffets. Participants cycled their own touring or hybrid bikes with or without pedal clips. The total bicycle weight, including all the equipment (tent, sleeping bag, food, spare bike gear and parts, spare clothes, etc.), was 36.3 ± 10.3 kg (range: 21–50 kg). Travel was during summer and early autumn, and routes included South America ($n=1$), North Africa ($n=1$), and Europe ($n=9$). Most of the participants measured their heart rate using a chest belt and then shared data for the description of cycling intensity. A picture of one of the participants with the equipment used is presented in Figure 2. The researchers did not influence cycling tours in any way; participants planned, paced, and adjusted their routes and distances on the course individually. Participants were cycling largely alone, even if some were getting occasional company for a short duration of time. Altitude exposure was low in most of the participants: nine spent most of the journey below or at 500 m above sea level with brief elevations above 1000 m; one cycled mostly just above 1000 m, and one predominantly above 2500 m. Ambient temperature fluctuated from +5 to +40°C during the tours.

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test. Although normality was not rejected for most variables, the limited power of normality testing in a small sample led us to apply non-parametric tests throughout

Table 1. Body weight and composition before and after the cycling tour.

Variable	<i>n</i>	Before	After	<i>p</i> value	ES (Hedges' <i>g</i>) [95% CI]
Body weight, kg	11	74.9 (5.7)	74.0 (5.6)	0.451	-0.253 [-0.803, 0.309]
BMI	11	23.4 (1.3)	23.1 (1.1)	0.275	-0.403 [-0.966, 0.179]
Sum of 7 skinfolds, mm	9	66.2 (16.7)	62.0 (9.0)	0.652	-0.278 [-0.875, 0.335]
Fat%	9	8.53 (2.87)	7.84 (1.63)	0.570	-0.308 [-0.907, 0.309]
Fat mass, kg	9	6.27 (2.12)	5.67 (1.01)	0.570	-0.309 [-0.909, 0.309]
Lean body mass, kg	9	67.4 (5.4)	67.4 (6.0)	0.910	-0.053 [-0.642, 0.540]
Peak KE torque, Nm	11	233.3 (38.2)	235.2 (39.5)	0.898	0.104 [-0.446, 0.649]
Peak KF torque, Nm	11	113.4 (17.0)	119.1 (19.2)	0.206	0.360 [-0.215, 0.919]
KF/KE ratio, %	11	48.8 (7.0)	51.3 (6.5)	0.206	0.381 [-0.197, 0.942]

ES, effect size; CI, confidence interval; BMI, body mass index; KE, knee extension; KF, knee flexion.

for consistency. The Wilcoxon signed-rank test was used for comparisons at two time points, and the Friedman test for repeated measures across three time points, followed by Nemenyi's post hoc test for pairwise comparisons. Non-parametric tests were used for hypothesis testing, whereas standardized effect sizes were calculated as Hedges' *g* to quantify the magnitude of paired changes. Ninety-five percent confidence intervals were estimated using the noncentral *t*-distribution method. Given the exploratory nature of the study and the broad range of outcomes assessed, no adjustment for multiple comparisons across outcome variables was applied. Statistical analyses were performed in Python (v3.13.14) using *NumPy*, *pandas*, *SciPy*, *statsmodels*, and *scikit-posthocs*. Data are presented as mean and SD unless stated otherwise. Statistical significance was set at $p < 0.05$.

Results

The tour was on average 50 (range from 21 to 99) days in duration. The cumulative tour distance was 4354 ± 2708 (1600 to 9235) km with a total elevation of 34 ± 15 (6 to 59) km. On average, participants were covering 86 ± 27 (48 to 129) km with 0.75 ± 0.34 km of elevation per day by cycling 6.0 ± 1.0 (3.5 to 7.1) hours per day at 9 to 24 $\text{km} \cdot \text{h}^{-1}$ average speed. Average heart rate was 90 to 130 bpm, which comprised 58 (49 to 66) % of their pre-tour HRmax. Individual values of external load and demands (HR, altitude above sea level) of the cycling journey are provided in Table S5.

Body composition and muscle strength

Body weight and body composition remained stable throughout the study period (Tables 1 and S1). Trunk and leg circumferences remained unchanged, while there was a decrease in upper-arm circumference in both flexed (from 34.1 ± 2.6 to 33.3 ± 2.5 cm; $p = 0.047$;

$n = 10$; $g = -0.788$; CI: -1.443, -0.102) and relaxed (from 31.4 ± 2.2 to 30.3 ± 2.2 cm; $p = 0.027$; $n = 10$; $g = -0.860$; CI: -1.533, -0.156) positions after the tour. No changes were observed in either knee extension or knee flexion strength in response to the cycling tour, and the agonist/antagonist peak torque ratio remained unchanged (Tables 1 and S1).

Glucose tolerance and insulin sensitivity

Fasting glucose was reduced from 5.0 ± 0.3 to 4.7 ± 0.2 $\text{mmol} \cdot \text{L}^{-1}$ ($p = 0.030$; $n = 10$; $g = -0.820$; CI: -1.482, -0.126), while fasting insulin level did not change significantly from 11.6 ± 6.1 before to 15.0 ± 5.1 $\mu\text{U} \cdot \text{mL}^{-1}$ after the tour ($p = 0.084$; $n = 10$; $g = 0.611$; CI: -0.033, 1.229). Glucose and insulin responses (Figure 3) and the response AUC (Figure 4) did not differ significantly before and after the journey. HOMA-IR (from 2.63 ± 1.48 to 3.11 ± 1.05 , $p = 0.230$) and the Matsuda whole-body insulin sensitivity index (from 2.65 ± 1.40 to 3.02 ± 1.04 , $p = 0.720$) remained unchanged in response to the tour.

In a subgroup tested at all three time points, serum insulin concentration at 15 min, 75 min and 120 min after glucose ingestion decreased over time ($p = 0.041$, $p = 0.041$ and $p = 0.022$, respectively; Figure 3d). Compared to before, post-hoc analysis revealed that insulin concentration was decreased at 120 min ($p = 0.031$; $g = -0.73$; CI: -1.36, -0.05) after glucose ingestion after the journey and at 15 min ($p = 0.031$; $g = -1.57$; CI: -2.81, -0.29) and 75 min ($p = 0.031$; $g = -1.31$; CI: -2.41, -0.17) after glucose ingestion 10 weeks after the journey (Figure 3d).

Resting metabolism, exercise capacity, blood volume and hemoglobin mass

Resting metabolic rate did not change significantly, whereas the contribution of CHO oxidation increased from before to after the tour, as evidenced by an increase in RER ($p = 0.004$; Table S2). This translated to an increase in

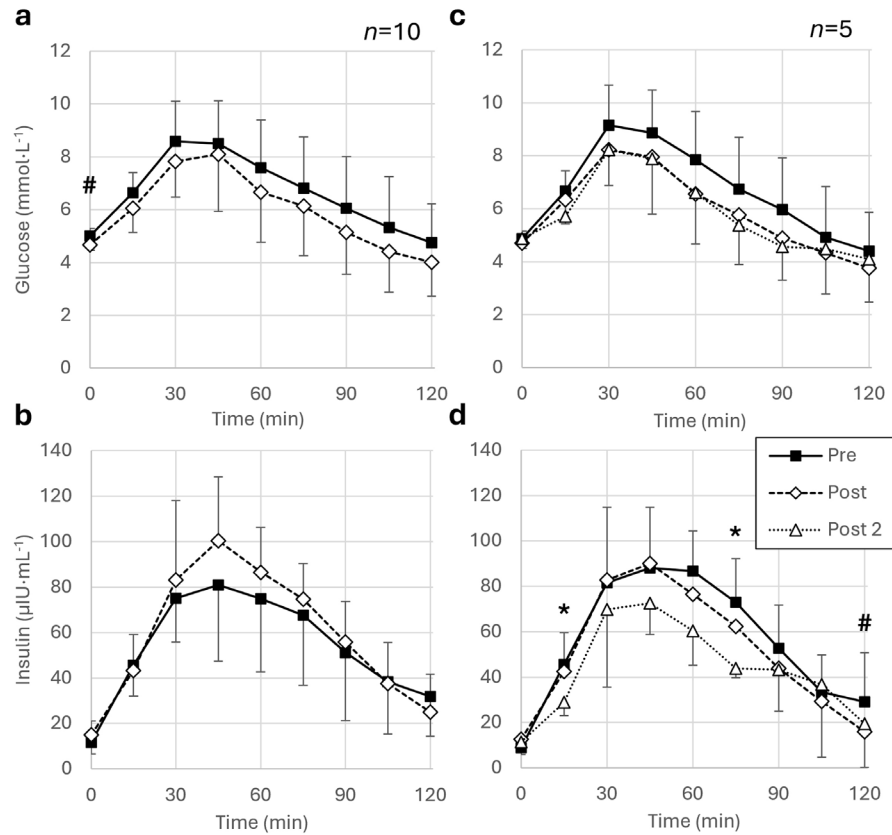


Figure 3. Blood glucose (a, c) and serum insulin (b, d) responses before (Pre, $n=10$), after (Post, $n=10$) and 10 weeks after (Post 2, $n=5$) the cycling tour. Values are means and SD. * $p < 0.05$ Pre vs. Post 2; # $p < 0.05$ Pre vs. Post.

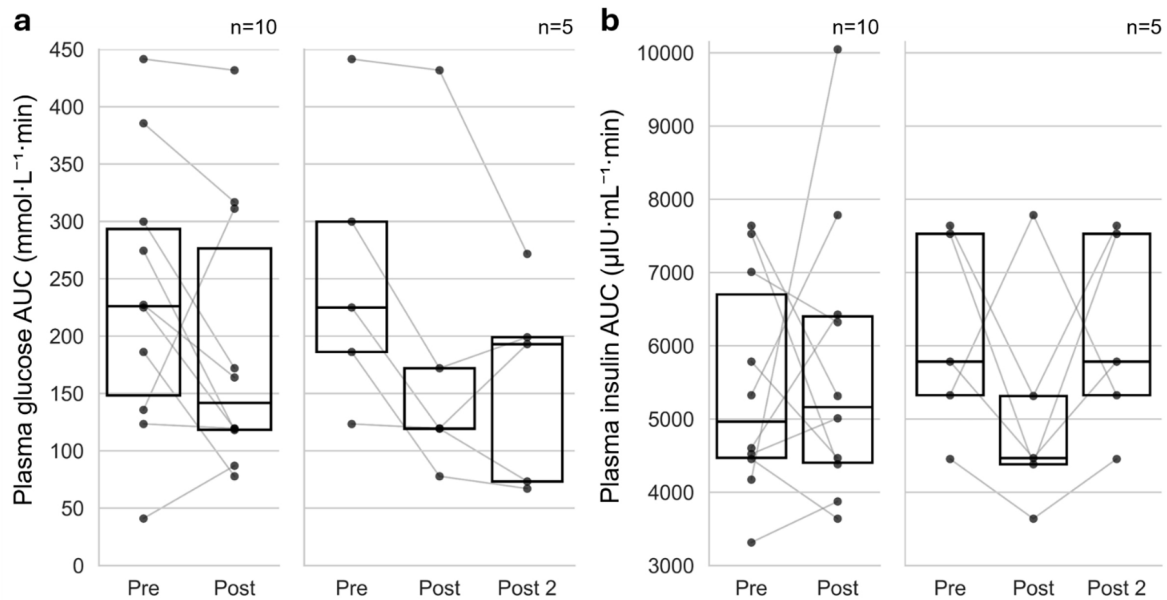


Figure 4. Blood glucose (a, b) and serum insulin (c, d) response incremental area under the curves (AUC) before (Pre, $n=10$), after (Post, $n=10$) and 10 weeks after (Post 2, $n=5$) the cycling tour. Grey lines connect values of the same participant. Box plots show the median and interquartile range.

contribution from CHO oxidation from 26.4 (13.9) to 50.4 (9.8) % and a reciprocal reduction in contribution from fat oxidation from 73.6 to 49.6 %. These changes did not seem to be sustained when measurements were repeated 10 weeks after the tour (Table S3).

An increase in absolute and relative VO_2max , peak power generated during a progressive cardiopulmonary exercise test, and relative VO_2 at cycling power producing an RER of 1.00 was observed following the tour (Table S2). These increases were largely reversed to baseline levels over the 10 weeks following the tour (Table S3). Peak RER, HRmax, and HR and cycling power at cycling power producing RER of 1.00 did not change (Tables S2 and S3).

After the tour, there were no significant changes in plasma volume, hemoglobin concentration, and total Hb mass (Table S4).

Discussion

The main finding of the present study was that participation in non-competitive, self-supporting adventure high-volume cycling over several weeks was associated with increased aerobic capacity and reliance on carbohydrates for resting metabolic needs. Despite the tour's recreational, self-paced nature, participants exhibited a decline in fasting blood glucose levels, while body weight, fasting insulin, glucose tolerance, and the insulin response were similar to those before the tour. Muscle strength, blood volume, and hemoglobin mass were stable throughout the study.

Body composition, muscle strength and cardiovascular fitness

Our participants undertook an adventure tour rather than a race. Its effects might therefore differ from those of some other studies, where athletes were pushing their absolute limits competing over multiple days²⁰, trying to achieve the best possible performance, such as the fastest traverse of the continent⁹, often in association with environmental extremes^{2,21–23}. For example, our study participants did not lose body weight, while in numerous other challenges of similar duration, body weight dropped substantially^{7,9,10,20,22–33}. Indeed, a reduction in body weight is not a universal finding in multi-day adventure races^{34,35} and tours^{36,37}, and in even more demanding challenges, such as ski marching while pulling heavy sledges 9 h·d⁻¹ for 3 months, there is not necessarily a loss of lean body mass or physical performance capacity in young, well-trained men³⁸.

VO_2max of our participants increased in response to the cycling tour and was within the range of VO_2max increases seen in male participants of multi-day adventure journeys from other studies^{11,24,39–42}. These VO_2max values are ~30% lower than those reported in elite male endurance cyclists^{37,43,44} and designate moderate to high aerobic fitness, and since our participants were quite lean in

their body composition, their lower-than-elite VO_2max level is most likely due to inferior cardiovascular fitness. Acute plasma volume expansion in response to cycling simulating long Tour de France stages⁴⁵ or a multi-day, multi-sport race⁴⁰ has been reported, and may result in an increased VO_2max due to augmented cardiac output capacity. Here, however, we did not find any change in plasma or blood volume or total hemoglobin mass, which could be due either to insufficient intensity of the exercise undertaken by the participants and/or moderate temperatures during most of the time for most of the tours, or because of delayed re-test after the last cycling day (i.e., deconditioning). In any case, the increase in VO_2max in our participants is therefore attributable to peripheral (muscular) rather than central adaptations. Indeed, 5.5 h·d⁻¹ of daily skiing in the mountains with a heavy backpack has been shown to increase the oxidative potential of the arm (largely unconditioned) but not leg muscles³⁹. However, a 2-week cycling tour has been shown to acutely reduce VO_2max in older male amateur cyclists⁴, and a decline in VO_2max has also been reported in young males after a 4-week adventure skiing expedition³. The decline in aerobic capacity in these two challenges might be related to their more competitive nature, thus being more demanding compared to the expedition tour and likely more training-like endeavors of our participants. In this line, well-provisioned participation in the ultra-endurance ski trekking for 9 h·d⁻¹ for 3 months not only did not change body composition, but also did not induce any change in VO_2max in young well-trained participants³⁸.

There was no change in muscle strength in our participants, similar to the finding of unchanged functional strength in participants of the ultra-endurance ski trekking for 3 months³⁸. In relation to unchanged body weight and composition, resting metabolic rate remained unchanged in our participants. This suggests that exercise load was well tolerated, as evidenced by no compensation with either an increase in resting energy requirements for augmented needs of tissue repair or suppression of it due to relative energy deficiency⁴⁶.

Shift from fat to carbohydrate (CHO) oxidation

Endurance training is widely associated with increased reliance on fat oxidation during submaximal exercise of various intensities. However, many studies in ultra-endurance sports and multi-day exercise challenges have, in fact, found the opposite. For instance, 2 weeks of daily cycling for > 10 h·d⁻¹ decreased maximal fat oxidation in older cyclists¹². Also, a gradual increase in reliance on CHO as a substrate was observed in a female ultra-endurance athlete during her world record running for the longest distance covered during 7 days on a treadmill³⁵. Further, traversing Antarctica also resulted in a switch to a greater reliance on CHO for daily activities in men and women and during sleep in women^{31,47}. Such an increase in CHO oxidation may result from an increased expression of

GLUT4, as has been observed after 2 weeks of 7–8 h·d⁻¹ of cycling¹¹. Finally, it has been reported that even moderate exercise during prolonged stay in the cold hypobaric conditions of Antarctica resulted in improved VO_2max and increased reliance on CHO during exercise, accompanied by increased lean body mass and decreased fat mass with unchanged body weight⁴⁸.

However, this is not always the scenario that happens. In a study of competitive cycling, RER at easy and moderate workloads decreased substantially after 4–5 ultra-endurance race days during which 1.6 kg of fat reserves had been burned²⁴. In another study, after 3 weeks of ~170 km (close to 5 h) daily cycling in young trained males, exercise RER was also reduced while VO_2max and body weight did not change⁴². Interestingly, after a skiing expedition over 4–6 weeks in moderately trained males RER increased during submaximal leg exercise^{3,10} while decreased¹⁰ or did not change³ during submaximal arm exercise. Finally, in response to simulated Tour de France cycling stages, there was also an increase in reliance on fat for energy both during exercise and recovery, which could be because of the negative energy balance during the cycling days⁴⁹. This switching to increased fat oxidation was reversed when large amounts of CHO were consumed to maintain energy balance during days of large exercise volume⁴⁵.

In the current study, we observed a shift to reliance on CHO (as evidenced by an increase in RER) for resting metabolic needs after the tour in our participants. At the same time, there was no indication of this happening during the progressive cycling test, and glucose tolerance remained normal. Therefore, this switch towards CHO oxidation at rest should not be interpreted as a negative adaptation, but rather a short-term adaptation to copious amounts of dietary energy and CHO intake, as also evidenced by unchanged body weight despite a daily average of 6 h of cycling.

Glucose tolerance and insulin sensitivity

High-intensity cycling days of simulated Tour de France stages, supplemented with sport-type liquid form CHO beverage (“fast” carbohydrates, an analogy to snacks used by our participants) on top of normal food intake, led to larger repeated increases in levels of both blood glucose and insulin. 45 CHO intakes of ~1 kg per day^{12,41,50–55} or even as large as up to 2 kg per day⁵⁶ are consumed during consecutive days of ultra-endurance training and challenges. Our participants admitted in questionnaires and interviews that their major snacks during the tour had been high glycemic index CHO (Tables S6 and S7), their fasting glucose, although remaining in a normal range, was reduced slightly after the tour. In another study, 2 weeks of prolonged, more competitive cycling did not affect baseline glucose levels in older participants⁴. There was no significant change in fasting serum insulin in our participants after the tour, while two weeks of 10.5 h·d⁻¹

of daily cycling increased fasting insulin levels in older participants^{4,12,57}, even if more acutely, namely 3 h after a near-continuous multisport ultra-endurance race for several days, insulin levels were found to be decreased²⁴.

In our participants, insulin and glucose responses to glucose ingestion did not change significantly after the tour, whereas the insulin response was blunted after more intense cycling over 2 weeks¹¹. Glucose tolerance may be acutely reduced after prolonged exercise in endurance-adapted men⁶, and impairment of glucose handling develops with excessively intense endurance training⁵. Daily ultra-endurance ski trekking of 3 months decreased circulating insulin but not glucose response in young, well-trained men 4 days after the expedition³⁸. Therefore, even if the tour was sufficient to increase aerobic capacity and reliance on CHO oxidation at rest in our participants, the average exercise intensity was probably insufficient to induce marked dysregulation in CHO metabolism. Alternatively, the days of rest between the last day of the tour and laboratory tests did not allow for observing more proximate transient effects of the expedition.

It should be appreciated that similar backpacking adventure journeys attract somewhat pre-conditioned, fit individuals. Not being a sport in strict terms, it, however, may be well accessible for a large proportion of the healthy population³⁷, especially when planned and adjusted on an individual basis. Taken together, a large increase in such physical activity over multiple weeks renders improvement in cardiovascular fitness, whilst changes in body composition and carbohydrate metabolism may not be that obvious and likely depend on changes in macronutrient intakes. The diaries and interviews with the participants of the current study revealed a substantial increase in the consumption of fast-food high glycemic index carbohydrate snacks during the tour (Tables S6 and S7). Developing evidence-based dietary recommendations for participants undertaking such tours may be advantageous. Specifically, an individualized nutrient intake strategy, with particular emphasis on carbohydrate consumption and informed by continuous blood glucose monitoring, could help optimize nutritional support throughout the tour.

Limitations

The major limitations of the study were a small sample size and a lack of precise information on dietary intake and exercise intensity distribution. Given the inability to control potentially important confounding factors, including changes in environmental conditions, we decided not to include a control group. The early measurements after the tour were performed only once, and that was 6 days after the tour on average, which served as a compromise between losing detection power of some of the more labile markers of the adaptation by prioritizing a chance of detecting some positive chronic adaptations when the larger portion of accumulated fatigue had disappeared with recovery. Complete blinding of outcome assessors was not

feasible because the research team was directly involved in the organization and monitoring of the tours and was therefore aware of the assessment time points (pre-, post-, and 10 weeks after the tour). No notable adverse events or harm related to the tours were reported, apart from anecdotal reports of transient perineal pressure-related discomfort in some participants. As adverse events were not a predefined outcome of the present study, their occurrence should be systematically evaluated in future investigations.

The study population consisted of young, healthy, recreationally active men; therefore, the findings could not be directly extended to other populations, such as women or inactive people. In addition, participants' individual goals for the journey ranged from leisurely daily cycling for several hours per day, as a form of sightseeing for some, to an extended training modality involving the accumulation of a large amount of cycling for others. Therefore, there was probably also some heterogeneity of the interventional stimulus within the study. Finally, we experienced a substantial attrition of the participants at the last measurement time point (10 weeks after the tour), which was intended to follow the maintenance of the intervention's effects. It should also be acknowledged that this period was not well characterized in terms of the activity pattern and other lifestyle habits, and the remaining participants emerged as those of higher fitness and stronger responders to intervention compared to dropouts.

Conclusions

The current study revealed that a multi-week adventure cycling tour, performed by recreationally active healthy men under self-selected load distribution, was associated with increased maximal oxygen uptake and decreased reliance on fat for resting metabolic needs, but no marked changes in body weight or composition, muscle strength, blood volume, and total hemoglobin mass. It is important to emphasize that, despite the increased reliance on carbohydrates for resting metabolic needs and a modest decrease in fasting circulating glucose level after the tour, insulin sensitivity and glucose tolerance indices did not change. Thus, the study revealed a positive association of a substantially augmented voluntary aerobic exercise volume with increased cardiovascular fitness and suggests neither improvement nor deterioration in whole-body insulin sensitivity, glucose tolerance, body composition, muscle strength, blood volume, and total hemoglobin mass.

Ethics approval

The study was approved by the Regional Biomedical Research Ethics Committee (no. BE-2-90) and complied with the Declaration of Helsinki.

Consent to participate

The participants were informed about the risks of participating in the study and provided written consent before being enrolled.

Authors' contributions

RB conceived the idea, designed the study, collected and analyzed the data, and drafted the manuscript. TV conceived the idea, designed and supervised the study, and wrote the manuscript. ArS, VM, AuS, PM and LC collected and analyzed the data. All authors contributed to the writing and/or editing of the manuscript, revised and approved its final version, and agreed with the order of presentation of the authors.

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Table S1. Body weight and composition and muscle strength before, after, and 10 weeks after the cycling tour.

Variable	n	Before	After	10 wks after	p value	Effect size: Hedges' g [95% confidence interval]		
						Before to After	Before to 10 wks after	After to 10 wks after
Weight, kg	6	74.8 (5.8)	74.1 (6.3)	76.3 (7.3)	0.070	-0.195 [-0.869, 0.499]	0.627 [-0.168, 1.377]	0.714 [-0.110, 1.489]
BMI	6	23.3 (1.4)	22.8 (0.9)	23.6 (1.5)	0.054	-0.447 [-1.154, 0.297]	0.469 [-0.281, 1.180]	0.805 [-0.050, 1.611]
Sum of 7 skinfolds, mm	4	73.5 (20.4)	61.2 (8.1)	67.5 (11.5)	0.368	-0.494 [-1.271, 0.341]	-0.363 [-1.103, 0.426]	0.702 [-0.221, 1.561]
Fat%	4	9.7 (4.0)	7.8 (2.5)	8.8 (2.9)	0.368	-0.555 [-1.353, 0.304]	-0.368 [-1.110, 0.422]	0.684 [-0.231, 1.535]
Fat mass, kg	4	7.2 (3.0)	5.5 (1.5)	6.6 (2.2)	0.472	-0.550 [-1.347, 0.307]	-0.316 [-1.046, 0.458]	0.679 [-0.233, 1.528]
Lean body mass, kg	4	67.3 (7.7)	66.9 (8.0)	68.7 (9.2)	0.105	-0.326 [-1.059, 0.451]	0.637 [-0.257, 1.467]	0.708 [-0.218, 1.569]
Peak KE torque, Nm	5	249.9 (47.9)	249.6 (52.5)	258.5 (57.8)	0.549	-0.010 [-0.711, 0.692]	0.378 [-0.387, 1.104]	0.582 [-0.246, 1.358]
Peak KF torque, Nm	5	117.8 (18.1)	118.9 (20.7)	119.5 (13.9)	0.549	0.049 [-0.655, 0.749]	0.094 [-0.615, 0.793]	0.058 [-0.648, 0.757]
KF/KE ratio, %	5	48.2 (8.3)	48.6 (5.9)	47.4 (7.6)	0.449	0.087 [-0.622, 0.785]	-0.173 [-0.874, 0.548]	-0.210 [-0.913, 0.517]

BMI, body mass index; KE, knee extension; KF, knee flexion.

Table S2. Resting metabolic rate and aerobic capacity before and after the cycling tour.

Variable	n	Before	After	p value	Effect size: Hedges' g [95% CI]
RMR, kcal/24h	9	1816.0 (54.6)	1873.1 (94.4)	0.250	0.451 [-0.190, 1.067]
RER at rest	9	0.779 (0.044)	0.851 (0.031)	0.004	1.279 [0.408, 2.114]
VO ₂ max, L/min	11	4.14 (0.42)	4.42 (0.55)	0.006	1.051 [0.326, 1.746]
VO ₂ max, mL/min/kg	11	55.4 (4.5)	59.7 (5.3)	0.010	1.111 [0.369, 1.823]
HRmax, bpm	10	186.4 (13.2)	185.0 (16.6)	0.787	-0.162 [-0.729, 0.414]
Max power, W	11	321.8 (38.9)	353.9 (37.0)	0.002	0.911 [0.223, 1.569]
Power at RER=1, W	11	258.2 (57.2)	282.5 (43.5)	0.067	0.524 [-0.078, 1.103]
VO ₂ at RER=1, L/min	11	3.42 (0.68)	3.68 (0.63)	0.123	0.412 [-0.171, 0.977]
VO ₂ at RER=1, mL/min/kg	11	45.7 (8.5)	49.5 (6.6)	0.032	0.450 [0.079, 0.802]
HR at RER=1, bpm	10	166.4 (14.5)	164.0 (14.5)	0.492	-0.126 [-0.692, 0.447]
Peak RER	11	1.14 (0.09)	1.18 (0.09)	0.320	0.355 [-0.220, 0.913]

CI, confidence interval; RMR, resting metabolic rate; RER, respiratory exchange ratio; VO₂, oxygen consumption; HR, heart rate. p<0.05 highlighted in bold.

Table S3. Resting metabolic rate and aerobic capacity before, after, and 10 weeks after the cycling tour.

Variable	n	Before	After	10 wks after	p value	Effect size: Hedges' g [95% confidence interval]		
						Before to After	Before to 10 wks after	After to 10 wks after
RMR, kcal/24h	5	1790.6 (59.4)	1897.8 (113.0)	1878.8 (108.0)	0.247	0.697 [-0.173, 1.511]	0.544 [-0.271, 1.309]	-0.155 [-0.854, 0.563]
RER at rest	5	0.773 (0.047)	0.844 (0.031)	0.805 (0.043)	0.165	1.346 [0.183, 2.460]	0.331 [-0.422, 1.049]	-0.607 [-1.391, 0.229]
VO ₂ max, L/min	6	4.21 (0.38)	4.65 (0.48)	4.48 (0.57)	0.042	1.893 [0.555, 3.197]	0.556 [-0.218, 1.288]	-0.391 [-1.089, 0.339]
VO ₂ max, mL/min/kg	6	56.3 (3.3)	62.7 (2.7)	55.1 (9.5)	0.009	2.391 [0.796, 3.963]	-0.117 [-0.789, 0.566]	-0.823 [-1.634, 0.039]
HRmax, bpm	6	191.4 (11.9)	188.9 (15.4)	190.1 (9.0)	0.846	-0.253 [-0.932, 0.450]	-0.154 [-0.827, 0.533]	0.111 [-0.572, 0.783]
Max power, W	6	335.2 (22.9)	367.7 (32.3)	354.0 (26.9)	0.016	1.190 [0.182, 2.148]	0.666 [-0.141, 1.427]	-0.666 [-1.428, 0.141]
Power at RER=1, W	6	259.7 (60.2)	290.0 (37.9)	269.4 (58.8)	0.311	0.441 [-0.301, 1.147]	0.214 [-0.482, 0.890]	-0.424 [-1.127, 0.314]
VO ₂ at RER=1, L/min	6	3.45 (0.77)	3.77 (0.55)	3.56 (0.79)	0.513	0.346 [-0.375, 1.036]	0.169 [-0.520, 0.843]	-0.310 [-0.995, 0.403]
VO ₂ at RER=1 mL/min/kg	6	46.2 (9.8)	50.9 (5.9)	46.9 (10.1)	0.513	0.387 [-0.343, 1.084]	0.084 [-0.596, 0.756]	-0.433 [-1.137, 0.308]
HR at RER=1, bpm	6	167.3 (17.2)	165.0 (16.2)	161.4 (17.2)	0.513	-0.084 [-0.756, 0.596]	-0.303 [-0.987, 0.409]	-0.162 [-0.835, 0.527]
Peak RER	6	1.19 (0.10)	1.19 (0.05)	1.19 (0.06)	0.513	0.050 [-0.627, 0.722]	0.033 [-0.643, 0.705]	-0.015 [-0.688, 0.660]

RMR, resting metabolic rate; RER, respiratory exchange ratio; VO₂, oxygen consumption; HR, heart rate. *p*<0.05 highlighted in bold. Significant differences are highlighted in bold.

Table S4. Blood volume and composition before and after the cycling tour (n=7).

Variable	Before	After	p value	Effect size: Hedges' g [95% CI]
Hb mass, g	945.5 (82.8)	957.8 (86.0)	1.000	0.100 [-0.550, 0.743]
Hb mass, g/kg	12.9 (1.1)	12.7 (0.9)	0.812	-0.152 [-0.796, 0.504]
Red blood cell volume, mL	2898.0 (253.3)	2935.9 (263.8)	1.000	0.101 [-0.550, 0.744]
Red blood cell volume, mL/kg	38.1 (4.6)	38.9 (2.7)	0.938	0.146 [-0.509, 0.789]
Plasma volume, mL	3382.0 (441.2)	3387.2 (499.4)	0.688	0.008 [-0.636, 0.652]
Plasma volume, mL/kg	44.2 (4.6)	44.8 (5.1)	0.812	0.065 [-0.583, 0.707]
Blood volume, mL	6280.4 (553.1)	6323.5 (639.6)	0.688	0.048 [-0.599, 0.691]
Blood volume, mL/kg	82.3 (7.5)	83.8 (5.4)	0.938	0.104 [-0.547, 0.747]
Hematocrit, %	46.3 (3.3)	46.6 (3.6)	0.812	0.096 [-0.554, 0.738]
[Hb], g/L	151.0 (10.8)	152.1 (11.8)	0.734	0.094 [-0.556, 0.736]

CI, confidence interval; Hb, hemoglobin.

Table S5. Individual data of the participants' cycling tours.

Participant	Age	Bike weight (kg)	Total distance cycled (km)	Duration of travel (days)	Total elevation gain (m)	Average daily distance (km)	Average daily elevation gain (m)	Average daily cycling time (h)	Average cycling HR (bpm)	HRmax (bpm)	Average cycling % HRmax	Average cycling speed (km/h)	Average above sea level (m)
P1	27	50.0	9235	99	32000	92.3	320	7.1	90	184	49	20.0	500
P2	22	50.0	5442	59	33978	92.2	576	6.2	111	196	57	14.6	500
P3	20	40.0	1700	21	6000	81.0	286	5.0	120	196	61	16.5	100
P4	31	25.0	7700	62	59000	124.2	952	6.5	112	169	66	19.0	300
P5	33	34.5	7600	68	52250	111.8	768	5.7	103			20.0	300
<u>P6</u>	22	40.0	3712	78	34000	47.6	436	3.5	108	192	56	12.9	300
<u>P7</u>	19	21.0	2700	21	21000	128.6	1000	6.5	110	180	61	24.0	500
<u>P8</u>	42	25.0	3506	40	43262	87.7	1082	5.9	98	172	57	13.6	200
P9	50	45.0	2200	32	43000	68.8	1344	6.5	106	163	65	12.0	1000
<u>P10</u>	24	41.0	2500	41	23600	61.0	576	5.5	130	210	62	19.0	200
<u>P11</u>	44	27.5	1600	30	26401	53.3	880	6.0	102	192	53	9.0	2500
Mean (SD)	30.5 (10.9)	36.3 (10.3)	4354 (2708)	50 (25)	34044 (14992)	86.2 (27.4)	747 (338)	6.0 (1.0)	109 (12)	186 (14)	58 (5)	16.3 (4.6)	582 (681)
Range	19–50	21.0–50.0	1600–9235	21–99	6000–59000	47.6–128.6	286–1344	3.5–7.1	90–130	163–210	49–66	9.0–24.0	100–2500

HR, heart rate. Data collected via semi-structured surveys administered during and after the tours. Participants recorded reported metrics using smartwatches and/or other gadgets. Participants with codes in bold and underlined were measured at three time points.

Table S6. Physical activity and dietary habits of participants before, during, and after the cycling tour.

Participant	Before the tour								During the tour				After the tour		
	Employment	Specific preparation for cycling tour	Typical exercise during leisure time (h/week) (typical activity)	Diet type	Meals per day	Typical snacks	Water per day	Sugar/dessert freq.	Meals/day	Typical snacks	Water per day	Sugar/dessert freq.	Typical exercise during leisure time	Diet habits changed?	Sugar intake changed?
P1	Sedentary office job, 5 d/wk	No	~8 (weight training)	Omnivore	3	Did not snack	1.5	Rare	3	Nuts, chocolate, gummies, sweet buns	3-5	Daily (2-3x)	Unchanged	No	Yes, reduced
<u>P2</u>	Student	No	~1	Omnivore	2-4	Fruits (5x)	2	Rare	3-4	Gummies, nuts, bars	3-4	Constantly (~1000 kcal/d)	Started running (1-2h/wk)	Yes, healthier (more salads)	Desire reduced
P3	Physical construction job, 5 d/wk	No	2-3 (running)	Omnivore	3-5	Pastries, fruits, food bars	2	Daily	5-6	Beef jerky, gummies, chips	3-4	Very often	Unchanged	No	No
P4	Sedentary office job, 5 d/wk	2-3 months cycling (<200km/week) + core exercises	5-6 (cycling)	Omnivore	2	Nuts, dried fruits	2	Rare	3	Gummies/chocolates	4-6	Very often	Unchanged	No	No
P5	Sedentary engineering job, 5 d/wk	7 months dedicated prep	5-16 (running/cycling)	Omnivore	≥4	Fruits, pastries, nuts	1.5	Daily	3	Fruit, chocolate, candy, buns, peanut butter + jam on bread	3-6	Very often	Unchanged	No	No
<u>P6</u>	Lab technician/student, 5 d/wk	No	~1-2 (running)	Vegetarian	3	Healthy snacks, fruits	2	Daily	3	Fruits	2	2-3x/week	Unchanged	No	No
<u>P7</u>	Video editing job (semi-sedentary), 5 d/wk	No	~9 (athletics 6x/wk)	Omnivore	3	Fruit, pastry (1x)	3	Daily	3	Peanut butter, gummies, nuts with dried berries, chocolate	4-5	Daily	Unchanged	No	No

Table S6. (Cont. from previous page).

Participant	Before the tour								During the tour				After the tour		
	Employment	Specific preparation for cycling tour	Typical exercise during leisure time (h/week) (typical activity)	Diet type	Meals per day	Typical snacks	Water per day	Sugar/dessert freq.	Meals/day	Typical snacks	Water per day	Sugar/dessert freq.	Typical exercise during leisure time	Diet habits changed?	Sugar intake changed?
<u>P8</u>	Installer (physical), 5 d/wk	No	7 (swimming/running)	Omnivore	3-4	Does not snack	1	Daily	3-4	Fast food	5	Daily	Increased to 14h/wk	No	No
P9	Unemployed	9 months CrossFit	~10-15 (CrossFit/cycling)	Omnivore	4-5	Nuts, fruits, sweets (2x)	2	Daily	2-3	Sweets	3	Constantly	Unchanged	Yes, fewer meals, no snacking	Increased temporarily then normalized
<u>P10</u>	Unemployed	No	7-11 (cycling/calisthenics)	Omnivore	5	Often; yogurts, fruit, nuts	2-3	Daily	4-6	Yogurt, chocolate, nuts/dried fruit mix, peanut butter with biscuits	4-6	Very often	Unchanged	No	No
<u>P11</u>	Active/physical job, 3-4 d/w	No	6-8 (cycling)	Omnivore	4-5 (frequent small meals)	Nuts, fruits, pastries, sweets (5x)	1.5	Daily	2-3	Fruits, pastries	3-6	Often	Unchanged	No	No
Data collected via semi-structured surveys administered during and after tours. Participants with codes in bold and underlined were measured at three time points.															

Table S7. Dietary intake during the cycling tour.

Participant	Meals/day	Typical meals	Snack frequency & type	Sugar/dessert frequency	Fast food frequency
P1	3	Breakfast: oatmeal; Lunch: salads, yogurt, grilled meat; Dinner: sandwiches, buns, fruit	2-3x at short stops; usually nuts, chocolate, gummies, sweet buns	Daily, 2-3x/day	Rarely, once every 1-2 weeks
<u>P2</u>	3-4	Breakfast: porridge; Lunch: salad, sandwiches or bread + cheese; Dinner: store-bought buns or a proper meal if staying with locals	Every ~30 min; gummies/nuts/bars; less frequent toward end of trip	Very often; ~1 pack of gummies/day (~1000 kcal), various buns	~Half of lunches contained a fast-food element (burgers etc.); notably more than usual
P3	5-6, including snacks	High-calorie meals: kebabs, sandwiches, pizza	Hourly; gummies, chips, beef jerky	Very often	Often
P4	3	Breakfast/dinner: sandwiches, noodles, yogurt, bananas, peanut butter; Lunch: usually burgers/pizza etc.	Gummies/chocolate while riding (frequent); roadside cola etc. at stops	Very often	Usually at least once a day
P5	3	Breakfast: sandwiches, porridge; Lunch: sandwiches, stews, pasta usually with meat/fish/cheese, sometimes canteen/cafe food; Dinner: like lunch	Roughly hourly while riding: fruit, chocolate, candy, buns, peanut butter and/or jam with bread; in heat – lemonade/soft drinks instead of solid snacks	Nearly all snacks were sweets	4x for the whole trip
<u>P6</u>	3	Porridge/pasta cooked on a camp stove, yogurt, fruit	Wild roadside fruit (apples, plums, pears)	2-3x/week	Never
<u>P7</u>	3	Breakfast: 150-250g raw oats with peanut butter, 2 bananas, 100g chocolate; Lunch: 250g pasta with venison or 500g chicken thighs, or 500g beef mince with beans; Dinner: 5-10 eggs	3-5x/day: peanut butter, gummies, nuts with dried berries, chocolate	Daily	Every 3rd-4th day
<u>P8</u>	3-4	Fast food	Constantly	Daily	Daily
P9	2-3	Morning: sandwiches; Evening: usually pasta with tuna	Depending on energy levels, but frequent snacking	Constantly	Rarely, only a few times whole trip
<u>P10</u>	4-6	Breakfast: 4-egg omelet in olive oil + white bread, sometimes bean puree; later fried fish or salad; fried lamb or chicken with potatoes/tomatoes; plus a fair amount of sugar between meals	4-6x: drinkable yogurt, chocolate, almonds/nuts, peanut butter with biscuits, a ground nut/dried-fruit/honey mix	Very often	About once a week (regretted it afterward - acid reflux while riding)
<u>P11</u>	2-3	Meat (chicken/pork/beef), lots of carbs (rice, potatoes, lentils)	Mostly fruits, sometimes baked goods; several times per day	~3-4x/week	Never

Data collected via semi-structured surveys administered during and after tours. Participants with codes in bold and underlined were measured at three time points; others – at two.