In Amateur Athletes With Type 1 Diabetes, a 9-Day Period of Cycling at Moderate-to-Vigorous Intensity Unexpectedly Increased the Time Spent in a State of Hyperglycemia, Which Was Associated With Impairment in Heart Rate Variability.

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Title

In amateur athletes with type 1 diabetes, a 9-day period of cycling at moderate-to-vigorous intensity unexpectedly increased the time spent in hyperglycemia, which was associated with impairment in heart rate variability.

Short running title: Exercise and diabetic autonomic dysfunction

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Abstract: 250 words

Main text: 4000 words

1 figure and 3 tables

1 table and 2 figures as supplementary data

ABSTRACT

Objective

In type 1 diabetes, autonomic dysfunction may occur early as a decrease in heart rate variability (HRV). In nondiabetic populations, the positive effects of exercise training on HRV are well documented. However, exercise in individuals with type 1 diabetes, particularly if strenuous and prolonged, can lead to sharp glycemic variations, which can negatively impact HRV. This study explores the impact of a 9-day cycling tour on HRV in this population, with a focus on exercise-induced glycemic excursions.

Research Design and Methods

Twenty amateur athletes with uncomplicated type 1 diabetes cycled 1500km. HRV and glycemic variability were measured by heart rate and continuous glucose monitoring. Linear mixed models were used to test the effects of exercise on HRV, considering concomitant glycemic excursions and subject characteristics as covariates.

Results

Nighttime HRV tended to decrease with the daily distance traveled. The more time the subjects spent in hyperglycemia, the lower the parasympathetic tone was. This result is striking given that hyperglycemic excursions progressively increased throughout the 9 days of the tour, and to a greater degree on the days a longer distance was traveled, while time spent in hypoglycemia surprisingly decreased. This phenomenon occurred despite no changes in insulin administration and a decrease in carbohydrate intake from snacks.

Conclusions

In sports enthusiasts with type 1 diabetes, multiday prolonged exercise at moderate-to-vigorous intensity worsened hyperglycemia with the latter being negatively associated with parasympathetic cardiac tone. Considering the putative deleterious consequences on cardiac

risks, future work should focus on understanding and managing exercise-induced hyperglycemia.

MAIN TEXT

In type 1 diabetes, cardiac autonomic neuropathy results from dysfunction of sympathetic and/or parasympathetic nervous system activity, and is associated with an increased risk of ventricular arrhythmia and cardiovascular morbidity and mortality (1). Long before the appearance of autonomic neuropathy clinical signs, subtle cardiac autonomic dysfunction can manifest as a decrease in heart rate variability (HRV) and its components (2). A large body of literature describes an altered parasympathetic tone in individuals with uncomplicated type 1 diabetes compared to healthy controls, resulting in relative sympathetic overactivity (2).

In an 11-year follow-up study of 83 subjects with type 1 diabetes, Mäkimattila et *al.* (2000) showed that chronic hyperglycemia (high HbA_{1c}) was a strong predictor of a lower HRV (2). Chronic hyperglycemia might be attenuated by interventions such as exercise training (3), which has indeed been suggested as a way to improve HRV in type 1 diabetes (4; 5).

However, aerobic exercise, particularly when prolonged, intense and/or unusual, may also trigger glycemic variability (6). Hypoglycemic episodes are common due to the increased muscle glucose disposal associated with high peripheral insulin concentrations, while non-decreased insulin levels in the portal vein prevent glucose release from the liver. Transient hyperglycemic episodes may also occur, for example during early recovery from intense exercise performed in post-absorptive state. Notably, it is not only sedentary or inactive patients who are prone to these exercise-induced glycemic fluctuations but also the increasing number of sports enthusiasts with type 1 diabetes engaging in outdoor ultraendurance events.

Interestingly, outside the context of exercise, it has been suggested that acute glycemic excursions impair cardiac autonomic activity. Thus, Nguyen et *al.* (2013) provided pilot data in 6 subjects with type 1 diabetes, showing that periods of naturally occurring hyperglycemia (measured over one night) were associated with an impaired global HRV and parasympathetic tone, compared to the non-hyperglycemic periods (7). Besides, Koivikko et *al.* (2005) showed

a reduction in HRV and parasympathetic tone in response to hyperinsulinemic-hypoglycemic clamp as compared to euglycemic clamp in subjects with type 1 diabetes (8). Additionally, a greater glycemic variability towards low blood glucose values, registered over a regular 5-day period, was associated with impaired HRV in adults with type 1 diabetes (9). However, the literature offers no data about cardiac autonomic activity changes accompanying exercise-induced glycemic fluctuations, even though exercise-induced hypoglycemic episodes may appear long (24h) after the exercise session.

The aim of this observational study was to explore, in riders with uncomplicated type 1 diabetes, the impact of a 9-day cycling tour on HRV, taking into consideration concomitant exercise-induced glycemic excursions and their influencing factors (*i.e.,* diet and insulin).

Research Design and Methods

Subjects

Twenty-three riders agreed to participate in this investigation, traveling the 1456 km that separates Brussels and Geneva over 10 days (mHealth Grand Tour, 3-12 September 2015), including a recovery day (day 4) (Supplemental table S1). The inclusion criteria were: aged 18 years or older, a history of type 1 diabetes for more than one year, an HbA_{1c} (dating back no more than 3 months) below 9% [75mmol.mol^{-1}], and to have already experienced a one-day ride over 160km as well as rides of 100km on consecutive days. All participants were free from overt micro- and macrovascular complications, except one who suffered from arteriopathy; thus, the latter was excluded from the analyses. Written informed consent was obtained, and data collection was granted approval by CNIL (MMS/TDG/ALU/AE151191). Usual physical activity was assessed using the short version of the International Physical Activity Questionnaire. Additionally, 10 riders had undergone an incremental maximal exercise test (VO_{2max}) as part of independent medical monitoring of athletes. Whether participants suffered

from hypoglycemia unawareness was also reported. According to VO_{2max} and/or training status, the participants were recreationally-trained or trained cyclists (10; 11).

The cycling tour

Throughout the 10 days of the tour, subjects wore a CGM (Dexcom G4 Platinum® ≥3 calibrations/day by capillary fingerstick measurement, Tapcheck glucometer) to evaluate glycemic excursions and variability. They concomitantly wore a heart rate monitor (Polar H7®) to assess HRV at night plus time spent at different exercise intensities during cycling (12).

Helped by the onsite dietician, riders reported their self-estimation of carbohydrates consumed at every meal (breakfast, lunch and dinner). The day prior to the start of the tour, riders were interviewed by the dietician to assess their ability to accurately count carbohydrates. Those who were less accustomed to this practice benefited from a closer follow-up by the dietician throughout the tour. Riders also reported the exact times and types of snacks consumed. For better standardization, they were encouraged to use the gels, bars and recovery drinks provided by the staff. Among the 13 individuals treated with continuous subcutaneous insulin infusion (CSII), 8 made their insulin pump data available for the study analyses. Every morning of the tour, just before breakfast, blood pressure and body composition (bioelectric impedance) were noted. Data from the heart rate monitor, CGMs, carbohydrate intake, capillary blood glucose, and symptomatic (awareness) episodes of hypoglycemia were gathered via Bluetooth on smartphones and thereafter downloaded with specific software for further analyses.

HRV analysis

The HRV analysis was performed with Kubios HRV software® in accordance with the Task Force of the European Society of Pacing and Electrophysiology (13). HRV was analyzed during a standardized calm (sleeping) period between midnight and 4:00 AM throughout the 9 days of

cycling. We analyzed time domain parameters [SDNN (standard deviation of normal to normal R-R intervals), pNN50 (percentage of differences >50ms between successive NN intervals), and RMSSD (the root mean squared of differences of successive NN intervals)] as well as frequency domains of HRV by the Fast-Fourier Transform (FFT) [high-frequency (HF: 0.15-0.40 Hz) and low-frequency (LF: 0.04-0.15 Hz)].

Glycemic variability analysis

Glycemic excursions and variability were calculated from CGM recordings over several specific periods: *(i)* from midnight to 4:00 AM, concomitant with the period of HRV analysis, *(ii)* the day before, from the beginning of breakfast to 2h post-dinner, *(iii)* over periods of 24h, *(iv)* during the cycling periods excluding the lunch break, *(v)* during early and late recovery (2 and 6h following the cycling periods). Glycemic excursions considered were the percentage of time spent in range [between 70 and 180 $mg.dL^{-1}$ (3.9 and 10 $mmol.L^{-1}$)], below range [level 1 hypoglycemia, <70 $mg.dL^{-1}$ (<3.9 $mmol.L^{-1}$); level 2 hypoglycemia, <54 $mg.dL^{-1}$ (<3.0 $mmol.L^{-1}$)] and above range [level 1 hyperglycemia, >180; level 2 hyperglycemia, >250; and hyperglycemia >300 $mg.dL^{-1}$ (14) (10, 13.9 and 16.7$mmol.L^{-1}$)] levels (15). Glycemic variability was assessed through coefficient of variation (%CV) (15), SD, mean amplitude of glycemic excursions (MAGE), continuous overlapping net glycemic action 1&2 (CONGA 1&2), average daily risk ratio (ADRR) indexes (16).

Statistics

All statistical analyses were performed with SPSS software (Inc., IBM company©, Version 19). The quantitative data are described as the mean ± SD. Normality was checked with the Shapiro-Wilk test. A logarithm transformation was applied to data with a non-Gaussian distribution. In

all of the following models, covariates were added as fixed effects and subjects as random effects to consider between - and within - participant variability.

In the *first set of models*, HRV parameters were studied as dependent variables in linear mixed models with time (*i.e.,* days 1 to 10, except for day 4, which was a recovery resting day) as covariates. In a *second set of models*, we then successively analyzed the effects of subjects and exercise characteristics during the tour, with time kept as a covariate. In a *third set of models*, the effects of time as well as glycemic variability and excursions were tested as covariates, in addition to exercise characteristics if significant in the second set of models.

In a *fourth set of models,* glycemic variability and excursions were studied as dependent variables in mixed models or multinomial & binary logistic regressions with time (*i.e.,* days 1 to 10, except for day 4) and circadian (*i.e.,* night *vs.* day periods, only for analyses over the periods of 24h) effects as covariates. Multinomial or binary logistic regressions were specifically used to assess the percentage of time spent in hypo- and hyperglycemia (see details in the legend of Table 3). In *the fifth and sixth sets of models*, with glycemic variability and excursions as dependent variables, we tested the effects of exercise characteristics and subject characteristics, respectively, with time and, for the 24h periods, circadian effects as covariates. Subsequently, we tested the effects of carbohydrates (grams) ingested (*seventh set of models*) and the effects of carbohydrates ingested and insulin administered (n=8 subjects on CSII, *eighth set of models*) on glycemic variability and excursions, considering the time effect and, when significant in the *fifth set of models*, the exercise characteristics.

A *P*-value <0.05 was considered statistically significant. All results are expressed as the mean estimation “e”. A particular focus was also given to the difference magnitude in addition to inferential statistical tests expressed using *p*-values.

Results

Technical problems were encountered in the collection of nighttime beat-to-beat heart rate and/or interstitial glucose (*e.g.,* disconnection of sensor, synchronization problems) for 3 subjects. Thus, 20 subjects were included in the final analyses. Their characteristics are displayed in table 1.

Carbohydrate data were processed for 19 of the 20 riders because one of them incorrectly completed the food questionnaire. Among these 19 riders, fifteen were considered as having mastered advanced carbohydrate counting including appropriate carbohydrate gram estimation. The other four participants benefited from closer assistance from the dietician with counting carbohydrates at every meal throughout the tour. Exercise intensities and exact duration of the cycling periods (supplemental table S1) were obtained only for 46 (*i.e.*, 25.6%) full days over the 180 (*i.e.,* 9 days for 20 riders) days of cycling analyzed because of problems with transient disconnection between the belt and heart rate watch monitor. Riders spent a large part of the cycling period at moderate (*i.e.,* 160.0 ± 38.3 min per day) and vigorous (*i.e.,* 155.1 ± 27.0 min per day) intensities. Morning body mass, percent of fat mass, muscular mass and hydration did not change over the 9-day period. While systolic blood pressure remained unchanged throughout the tour, morning diastolic blood pressure and mean blood pressure decreased significantly (main time effect e:−0.43 and e:−0.39, respectively, $P<0.05$). Anthropometric, demographic, and diabetes-related variables were not significantly related to blood pressure throughout the tour.

HRV during the Tour

The results of HRV are presented in 16 individuals because 4 riders did not wear their heart rate monitor correctly at night. The results of mixed models for the association of time, subjects' exercise characteristics, and glycemic excursions, with temporal and frequency HRV domains displaying significant main effects are presented in table 2.

Parasympathetic tone parameters (*i.e.,* HF 910.4 ± 1440.2 ms^2, pNN50 17.2 ± 20.2%, RMSSD 43.5 ± 32.5ms, mean over the 9 days) and sympathetic-vagal balance (*i.e.,* LF:HF 4.1 ± 2.2, mean over the 9 days) did not significantly change with time throughout the tour and were not altered by the characteristics of the exercise performed each day. However, global HRV (as reflected by SDNN, 101.5 ± 39.0 ms over the 9 days) tended to decrease with the number of kilometers traveled the day before, without the influence of exercise intensity or time.

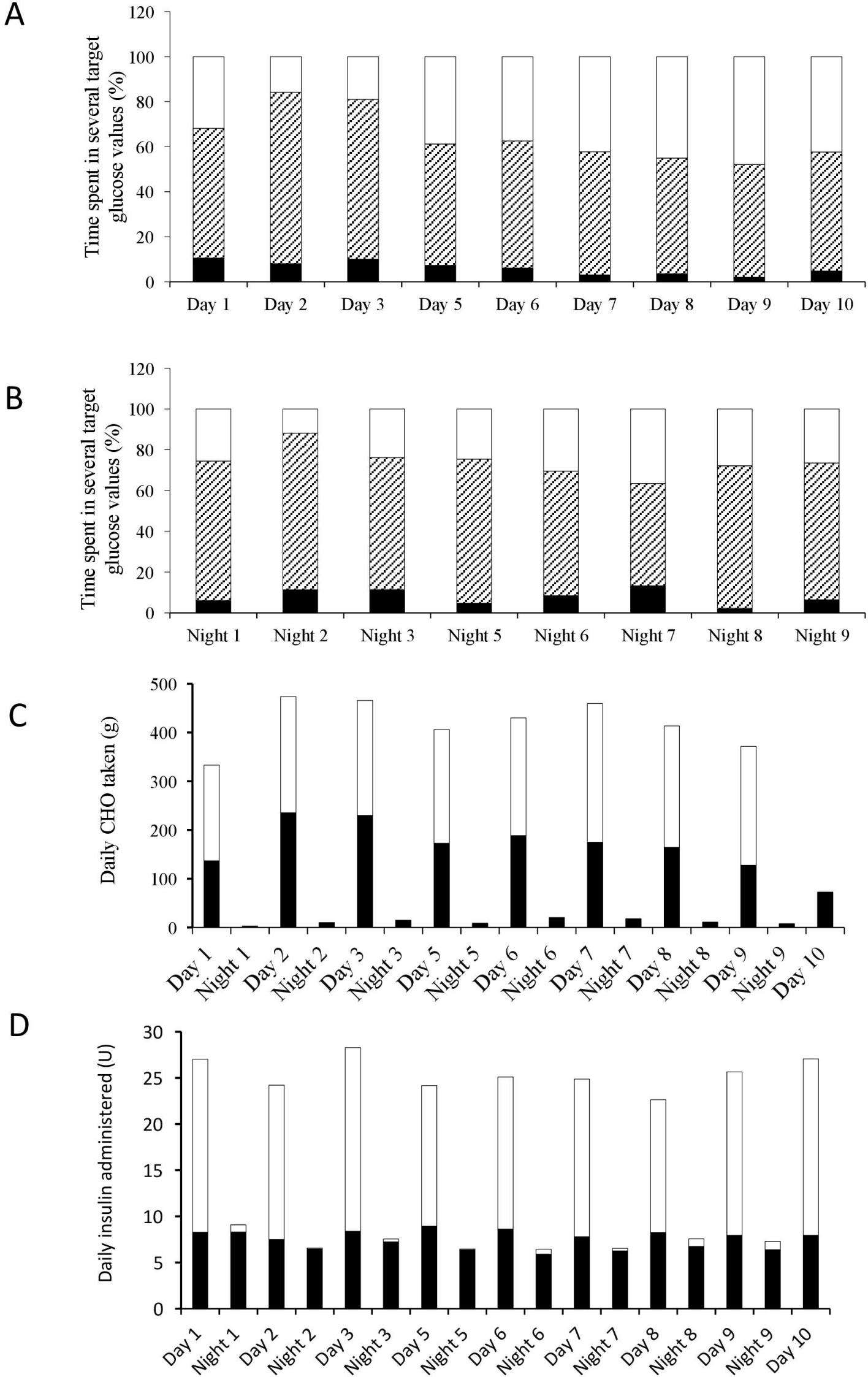
The 12 men had a higher sympathetic-vagal balance than the 4 women. Age decreased parasympathetic tone. Aerobic fitness (VO_{2max}) was positively associated with global HRV and parasympathetic tone.

Global HRV was not linked with glycemic excursions. A decrease in sympathetic-vagal balance and an increase in parasympathetic tone during the night were associated with longer time spent in alert low glucose values during the previous day and 6-h post-exercise recovery. A decrease in parasympathetic tone during the night was associated with a longer time spent in level 2 hyperglycemia during the concomitant night, as well as in hyperglycemia >300 $mg.dL^{-1}$ during the previous day.

Glycemic excursions and variability

Change in time spent in hypo-, normo-, and hyperglycemia throughout the tour is presented in figure 1 (for the 24h periods) and supplemental figure S1 (for cycling, early and late recovery periods). Glycemic variability is reported in supplemental figure S2. Factors influencing glycemic outcomes are presented in table 3.

Hypoglycemia unawareness (subjectively reported) did not influence hypoglycemic excursions. A higher HbA_{1c} level was associated with a longer time spent above range but lower glycemic variability. Neither mode of insulin therapy nor the habit of using CGMs influenced glycemic outcomes.


A
Time spent in several target glucose values (%)
120
100
80
60
40
20
0
Day 1
Day 2
Day 3
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
B
Time spent in several target glucose values (%)
120
100
80
60
40
20
0
Night 1
Night 2
Night 3
Night 5
Night 6
Night 7
Night 8
Night 9
C
Daily CHO taken (g)
500
400
300
200
100
0
Day 1
Night 1
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Notably, while the number of aware hypoglycemic episodes did not change during the tour (0 to 1 episode/day among the riders over the 9 days), riders decreased (time effect) the percentage of time spent below range (hypoglycemia levels 1 and 2), as measured over all the periods studied. However, this decrease was at the expense of glycemic variability and hyperglycemic excursions (as measured over all the periods studied), which worsened throughout the tour (time effect) and were more frequent the days a longer distance was ridden (for hyperglycemia >300 $mg.dL^{-1}$, MAGE, SD), without a significant effect of exercise intensity. This was accompanied by a decrease in time in range. Throughout the tour, subjects experienced less time in range and more time above range during the daytime compared to the nighttime.

Throughout the tour, the riders progressively decreased the daily carbohydrate content ingested via daytime snacks (e:−10.29, $P<0.001$) but did not change the carbohydrate content of the nighttime snacks or the 3 meals (Figure 1C). In this context of sustained repeated exercise, neither the carbohydrates from meals nor the carbohydrates from nighttime or daytime snacks (which decreased with time) were significantly associated with glycemic excursions throughout the tour (*seventh set of models*). In the sample of subjects providing insulin pump data, bolus and basal rates were not significantly changed throughout the tour (*eighth set of models*) (Figure 1D). Notably, when comparing insulin doses used during normal daily life (data obtained from 6 of the 8 subjects during a usual week before the tour) with those administered during the tour, no significant difference appeared (paired t-test) (insulin basal rate: 14.9 ± 7.5 *vs.* 20.9 ± 10.0 $U.day^{-1}$; bolus: 18.8 ± 10.7 *vs.* 23.9 ± 13.0 $U.day^{-1}$ throughout the 9 days of the tour *vs.* during one week of daily life, respectively). Nevertheless, during the tour, the larger the daily amount of insulin bolus (either in U or in $U.kg^{-1}$) was, the greater the extent of time spent below range (level 2 hypoglycemia, e:+8.87 or e:+0.11, respectively, $P<0.05$) experienced by riders from breakfast to 2h post dinner. Glycemic outcomes during the night (between midnight and 4:00

AM) were not significantly associated with the concomitant insulin basal rate throughout the tour.

Conclusions

Our study of 20 riders with type 1 diabetes highlighted, for the first time, that the repetition of long-duration exercise bouts at moderate-to-vigorous intensity over 9 days may trigger hyperglycemic excursions, which were negatively associated with parasympathetic tone, while time spent in hypoglycemia decreased. The type of statistical model used was chosen to ensure that the observed relationships (*e.g.,* between hyperglycemia – a covariate, and parasympathetic tone – the dependent endpoint) were not due to simultaneous changes in time or in exercise characteristics, but appeared for any given value of these 2 outcomes, which were added as covariates in the model.

While demographic characteristics such as age (17) and sex (2) are well-known predictors of sympathetic-vagal balance, as confirmed in our study, the impact of exercise training on cardiac autonomic function in type 1 diabetes is documented less frequently. Interestingly, in agreement with a recent study (18), we found that VO_{2max}, as a reflection of regular aerobic training level, was positively associated with cardiac autonomic function (parasympathetic tone in our study). However, nighttime beat-to-beat heart rate recordings throughout the tour revealed that unusual multiday sustained moderate-to-vigorous exercise, without appropriate recovery, may conversely trigger impairment of HRV in type 1 diabetes. The longer the distance ridden during the day, the lower the global HRV tended to be during the subsequent night. This phenomenon could reflect a state of overreaching as already observed in nondiabetic recreationally trained runners (19). The latter displayed a significant reduction in indexes of HRV (including SDNN) up to 24h after an ultramarathon (64km distance, 1572m accumulative altitude change). It should however be noted that studies on the effect of overreaching on heart rate variability in

healthy athletes remain few and far between, with sometimes the finding of no significant change in HRV (20; 21)

In our study, it is worth noting that some cardiac autonomic function parameters were actually linked with glycemic excursions.

Thus, a longer time spent at low glucose levels (corresponding both to level 1 or 2 hypoglycemia) (15) during late recovery (*i.e.,* the 6h period post-exercise) was associated with increased parasympathetic activity during the subsequent night. To the best of our knowledge, the only studies having explored the link between hypoglycemia and cardiac autonomic balance specifically in the context of physical exercise (only one session) have considered either the changes occurring during the 60min before the hypoglycemic episode (22) or the changes occurring concomitant with the hypoglycemic period (*e.g.,(23; 24)*), and showed either a decrease, or an increase, in parasympathetic tone, respectively. Further studies are needed to confirm the increase in nocturnal parasympathetic tone associated with post-exercise hypoglycemic periods and to understand its clinical implications.

While time spent in hypoglycemia decreased throughout the tour, we observed a surprisingly significant decrease in time spent in euglycemia due to a considerable increase in time spent in hyperglycemia. While the deleterious impact of chronic hyperglycemia (HbA_{1c}) on HRV and parasympathetic tone is already well documented in youth with type 1 diabetes (25), this study is the first to show a link between cardiac autonomic imbalance and acute hyperglycemic periods in the context of physical exercise. To our knowledge, only two studies, with quite controversial results, have attempted to explore the possible acute impact of hyperglycemia on HRV in individuals with type 1 diabetes, but without involving concomitant physical exercise and based only on a limited number of glycemic values (*i.e.,* only one measure taken in a fasting state and the other 30min after a regular meal (26); or one measure every 30min during one night in only 6 subjects (7)).

As impaired cardiac vagal control is associated with higher cardiac mortality (27), it appears crucial to elucidate the factors involved in the worsening of hyperglycemia observed during the tour. Although reducing insulin administration and/or increasing carbohydrate intake are commonly recommended for avoiding hypoglycemic episodes around physical exercise in type 1 diabetes (6), these measures may not be needed in athletes for whom insulin doses and diet are already well adjusted to their usual intensive exercise training. Accordingly, in our work, the cyclists did not increase carbohydrate intake throughout the tour and presumably did not change their usual insulin dose (as verified among the individuals using an insulin pump). Thus, while insulin and diet might not be the direct cause of exercise-induced hyperglycemia worsening, the characteristics of the multiday exercise, *i.e.,* prolonged and including a significant portion of vigorous intensity, may play a fundamental role. Intense exercise (>85% VO_{2max}) to exhaustion is known to induce an increase in glycemia during the early recovery period because plasma catecholamines and glucagon take time (~30min and 30-50min, respectively) to return to resting concentrations. In nondiabetic subjects, the increase in glycemia during early recovery following intense exercise is counteracted by a 2-fold increase in plasma insulin, whereas individuals with type 1 diabetes are prone to transient hyperglycemia unless a bolus of insulin is delivered (28). Consistent with this theory, the only cyclist in our study who did not experience significant post-exercise hyperglycemia received a frequent insulin correction bolus in the hours following exercise.

Additionally, when exercise becomes extremely prolonged, glucose metabolism might be disrupted long after the end of exercise, as revealed in a study in nondiabetic subjects (29). Equally of interest, in line with this result, we noticed a positive association between the number of kilometers traveled during the day with time spent in hyperglycemia as well as with glycemic variability during the surrounding 24h. To the best of our knowledge, blood metabolite and hormonal response to multiday long-distance sports events has only been the topic of one single

case report in an athlete with type 1 diabetes, showing progressive increase in markers of inflammation (CRP) and muscle damage (creatine kinase), which are two factors of insulin resistance (30; 31), while changes in cortisol, an activator of hepatic gluconeogenesis, did not exactly follow trends of hyperglycemic excursions (32). Future studies on larger sample sizes, will be needed to ascertain the cause of the observed persistent hyperglycemia. Data from a study using non-repeated sustained exercise, *i.e.,* a single marathon, suggest that persistence of free fatty acid oxidation long after exercise may suppress carbohydrate oxidation (33). Additionally, in non-diabetic subjects, the exercise-induced increase in lactatemia has been shown to be enhanced throughout the week following a marathon (29), and lactate can then serve as an alternative muscle substrate for sparing blood glucose (34).

Finally, it is noteworthy that in addition to the negative link between glycemic excursions and cardiac autonomic activity during the 9-day repeated moderate-to-vigorous exercise, such activity was associated with a progressive decrease in morning diastolic blood pressure. This phenomenon has already been observed in response to long and strenuous events in healthy athletes and was suggested to be due to metabolic vasodilatation and/or ineffective transduction of sympathetic outflow from arterial smooth muscle (35). Therefore, better attention should be paid to vulnerability to orthostatic challenges after multiday prolonged exercise in athletes with type 1 diabetes.

While our results are particularly relevant given the growing popularity of long distance or multiday running or cycling events, including for persons with type 1 diabetes, the findings should be interpreted in the context of the study limitations. The non-standardization of the resting day and the limited data obtained during riders' daily-life prevent drawing comparisons between control resting periods and the multiday cycling challenge. We obtained insulin data only from a small proportion of the participants, making generalization difficult. In addition, the accuracy of CGMs may be reduced during exercise bouts due to multiple factors, such as

subcutaneous dehydration, temperature variations, rapid decreases in glycemia, etc. To address these limitations, the CGMs were calibrated at least three times daily by capillary finger prick testing, and body hydration was controlled every morning.

Given the observational study design, we cannot draw conclusions about causality. However, the current cross-sectional study was a necessary first step towards future implementation of interventional randomized control trials. Based on the current results, these future trials could contribute to reducing hyperglycemia induced by outdoor endurance sports, instead of focusing only on hypoglycemia, and provide observations of the subsequent impact on heart rate variability. This will make it possible to partition off the possible impact of hyperglycemia from that of overreaching, the latter having already been reported in non-diabetic athletes. As far as we are aware, our study is the first to confirm empirical data (*i.e.,* based on patients' narrative and on a single case report (36)) on the increased risk of hyperglycemia triggered by multiday ultra-endurance events, in amateur athletes with type 1 diabetes, with concomitant careful consideration of exercise intensity and carbohydrate intake. Notably, two other recent reports are available (37; 38), but on professional cyclists, whose adaptations to elite stage races are certainly different from those of amateur athletes. In addition to offering widely generalizable data, the unique nature of our study lies above all in the novel way the putative links between sustained exercise-induced glycemic excursions and cardiac autonomic activity are examined. Further studies are needed to understand the mechanisms involved in the hyperglycemic effect of such multiday moderate-to-vigorous prolonged exercise events and to gain more insight into the health consequences of accompanying cardiac autonomic imbalance.

In conclusion, this study on amateur athletes with type 1 diabetes demonstrates that multiday prolonged exercise at moderate-to-vigorous intensity increased the time spent in hyperglycemia, and this was negatively associated with parasympathetic cardiac tone. These results are important considering the putative consequences on future cardiac mortality risk. In

this context, beyond research on hypoglycemia prevention strategies, future work on understanding and managing exercise-induced hyperglycemia should be promising.

Acknowledgments

The authors thank all members of the Orange HealthCare team, particularly, J. Braive, V-D. Dam, O. Graille, M. Montaner-Gomez, and P. Chopineau for their help in providing all the material needed for linking, via a connected phone, glycemic data with other data (carbohydrate intake, HRV, body composition, arterial pressure, etc.). Data extraction was facilitated by O. Beltoise (Glooko Diasend) and by B. Klinkenbijl and S. Guerra from ClinInfo teams. We also thank the crucial logistical support of A. Denton from Hydon Consulting Society, as well as C. Hugues (diabetes dietician) for collecting diet data throughout the tour, M. Gonnet (Another Brain Society), S. Tagougui (Lille University), P. Morel (University of the Littoral Opale Coast) for data analysis assistance, J-F Gautier (Department of Diabetes and Endocrinology, Lariboisiere Hospital, Paris, France) for his contribution to the study design, L. Canipel (Société Française de Télémédecine) for her help with ethics approval, P. Fontaine (Endocrinology unit, Lille University Hospital) and M. Garcia Vigueras for their scientific advice, A. Bocock (Melun), A. Denton (Hydon Consulting Society), and B. Heyman (University of Rennes) for revising the English, and A. Bertrand (Statistical Methodology and Computing Service (SMCS), UCLouvain) for checking the statistical analyses. All the above mentioned support was supplied at no charge.

Funding. This study was supported by a donation from Linde Homecare France, which allowed to recruit E.L. as a postdoctoral researcher for 1 year.

Duality of interest. There are no potential conflicts of interest relevant to this article to report.

Authors contributions. E.H. and M.D. conceived the experiments. E.L. and E.H. performed the experiments, collected and analyzed data and wrote the manuscript. O.B. analyzed insulin data. J.H. created algorithms for analyses of glycemic variability. B.P. contributed to statistical analyses. FX.G. and B.S. contributed to the HRV analyses. O.B., J.H., B.P., B.S., FX.G., and M.D. contributed to the performance of the experiments and reviewed the manuscript. E.H. is the guarantor of this work and as such, had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Some preliminary data related to this study have been previously presented at the congress of 'Société de Physiologie et Biologie Intégrative' in 2018 (abstract in Acta Physiologica, 2018, Vol.224, Issue S715: A5).

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Tables

Table 1. Anthropometric, demographic and physical activity characteristics of the riders

Sex, male/female	16/4
Age (years)	37.9 ± 10.5 (19.0-54.0)
BMI ($kg.m^{-2}$)	23.8 ± 2.6 (18.3-30.8)
Fat mass (%)	17.6 ± 5.7 (7.4-36.9)
Waist/hip circumference	0.9 ± 0.1 (0.7-1.0)
Diabetes duration (years)	19.6 ± 7.7 (5.0-35.0)
HbA_{1c} ($mmol.mol^{-1}$)	54.1 ± 9.1 (42.1-74.9)
HbA_{1c} (%)	7.1 ± 0.8 (6.0-9.0) (n=19)
Habit to wear a CGM/no habit	12/8
Brands of CGM used	*N=4 from Medtronic®, N=8 from Dexcom®*
CSII/MDI	13/7
ICR (grams/unit of insulin)	11.2 ± 4.9 (n = 15)
Other drugs	n=1 calcium antagonists n=2 thyroid drug
VO_{2max} ($mL.min^{-1}.kg^{-1}$)	53.1 ± 7.9 (38.6-67.4) (n=10)
IPAQ score ($MET\text{-}min.wk^{-1}$)	7559.0 ± 5104.4 (2187.0-25194.0) (n=19)
Mean number of kilometers/year traveled (cycling) in daily life	6339 ± 3518 (500-15000)

Data are expressed as the mean ± SD (min-max). The number of subjects is indicated for outcomes with some lacking data. CSII, continuous subcutaneous insulin infusion; MDI, multiple daily insulin injections; ICR, insulin-to-carbohydrate ratio: IPAQ, International Physical Activity Questionnaire

Table 2. Results of mixed models for influence of subject's and exercise characteristics, glycemic variability (during day or night) and excursions on HRV

Dependent variables (midnight – 4:00 AM HRV)	**Global HRV** **SDNN (ms)**	**Parasympathetic tone** **pNN50 (%)**	**RMSSD† (ms)**	**HF† (ms^2)**	**Sympathetic-vagal balance** **LF:HF**
Second set of models					
Effect of subject's characteristics [no effect of glycated hemoglobin, diabetes duration, mode of insulin therapy, BMI, percent fat mass or, riders' regular physical activity (subjectively assessed by the IPAQ)]					
Sex	NS	NS	NS	NS	e: −2.68; P=0.08
Age	NS	NS	e: −0.01; P=0.08	e: −0.03; P<0.05	NS
Aerobic fitness (VO_{2max})	e: +3.63; P<0.01	e: +1.76; P<0.05	e: +0.03; P<0.01	e: +0.05; P<0.01	NS
Effect of exercise characteristics (no effect of altitude changes, duration of cycling, or exercise intensity)					
Kilometers traveled	e: −0.32; P=0.08	NS	NS	NS	NS
Third set of models					
Effects of glycemic excursions and variability measured in the following periods:					
During the concomitant night (midnight – 4:00AM)					
Time (%) spent >250 $mg.dL^{-1}$†	NS	e: −4.76; *P=0.06*	e: −0.09; *P*<0.05	e: −0.21; *P*<0.05	e: +0.84; *P<0.05*
Throughout the day before (from beginning of breakfast to 2 h post dinner)					
Time (%) spent <70 $mg.dL^{-1}$†	NS	NS	NS	NS	e: −1.14; *P*<0.05
Time (%) spent >300 mg/dL†	NS	NS	e: −0.13; *P*<0.05	e: −0.28; *P*<0.05	NS
During the cycling period, the day before					

SD	NS	NS	e: +0.007; $P<0.05$	NS	NS
During the 6-hour post-exercise period, the day before					
Time (%) spent <54 mg.dL^{-1}†	NS	e: +4.78; $P<0.05$	NS	NS	NS
Time (%) spent <70 mg.dL^{-1}†	NS	e: +3.58; $P<0.05$	e: +0.09; $P<0.06$	e: +0.24; $P<0.05$	NS

N=16; † data values are log-transformed; SD, Standard Deviation of glycemia; Glycemic and heart rate variability data from the last (i.e., the 10th) night were not used because several riders decided to remove their collection devices before that night.

In the first set of models, no significant effect of time was detected. In the second set of models, the subject characteristics analyzed were *(i)* anthropometric (% fat mass) and demographic (age, sex) characteristics, *(ii)* disease & treatment (HbA_{1c}, diabetes duration, mode of insulin therapy, habit of wearing a CGM), and *(iii)* physical activity and fitness (IPAQ score, VO_{2max}) characteristics. In the second set of models, the exercise characteristics analyzed were kilometers daily traveled, cumulative altitude change, and cycling duration, combined in a first model. Then, the effect of percentage of time spent in moderate- and vigorous-intensity activity was tested with the exercise characteristic(s) kept as (a) covariate(s) if significant in the preceding model.

In the third set of models, glycemic variability and excursions during the cycling period and during the 6-hour recovery period were obtained for 42 (*i.e.,* 26.2%) and 85 (*i.e.,* 53.1%) days, respectively, over the 160 days of analysis, since these periods were determined based on heart rate data from morning and afternoon, or afternoon only, respectively (*cf.* problems of transient disconnection between the belt and heart rate watch monitor). No significant effects were detected for time spent in range (70-180 mg.dL^{-1}), above range level 1 (>180 mg.dL^{-1}) or most of the glycemic variability indexes (%CV, ADRR, MAGE, CONGA 1&2). The km traveled was added as a covariate for all models with SDNN as the dependent variable.

	Hypoglycemic excursions		Euglycemia	Hyperglycemic excursions			Glycemic variability				
	Time (%) spent <54 mg.dL^{-1}*	**Time (%) spent <70 mg.dL^{-1}***	**Time (%) spent between 70-180 mg.dL^{-1}**	**Time (%) spent >180 mg.dL^{-1}***	**Time (%) spent >250 mg.dL^{-1}***	**Time (%) spent >300 mg.dL^{-1}***	**MAGE**	**ADRR**	**CONGA1**	**CONGA2**	**SD**
Effect of time throughout the tour ***(fourth set of models)***											
Effect of time	e:−0.21; *P*<0.001	e:−0.10; *P=0.06*	e:−1.67; *P*<0.001	e:+0.33; *P*<0.001	e:+0.16; *P*<0.01	e:+0.18; *P*<0.001	e:+2.54; *P*<0.01	e:+0.57; *P*<0.05	e:+1.82; *P*<0.001	e:+1.97; *P*<0.001	e:+1.07; *P*<0.01
Circadian effects throughout the tour ***(fourth set of models)***											
Circadian effects	NS	NS	e:−7.50; *P*<0.01	e:+1.21; *P*<0.01	e:+1.59; *P*<0.001	e:+1.26; *P*<0.001	NS	NS	NS	NS	e:+11.24; *P*<0.001
Effects of exercise characteristics ***(fifth set of models)*** (no effect of altitude change, cycling duration or exercise intensity)											
Effects of kilometers	NS	NS	NS	NS		e:+0.03; *P*<0.05	e:+0.62; *P=0.06*	NS	NS	NS	e:+0.26; *P*=0.06
Effects of subject characteristics ***(sixth set of models)***											
Anthropometric characteristics (no effect of age or sex)											
Fat mass (%)	NS	NS	NS	NS	NS	e:+0.07; *P=0.09*	NS	NS	NS	NS	NS
Disease and treatment											

characteristics (no effect of mode of treatment nor habit to use a CGM)											
HbA_{1c}	NS	e:−0.03; *P=0.08*	NS	e:+0.04; *P*<0.05	NS	e:+0.04; *P*<0.05	NS	NS	e:−0.71; *P*<0.01	e:−0.51; *P=0.06*	NS
Diabetes duration	NS	e:+0.06; *P=0.08*	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 3. Results of mixed models and logistic regression for parameters influencing glycemic excursions and variability over periods of 24 hours (8:00 AM-8:00 PM and 8:00 PM-8:00 AM)

*For binary and multinomial logistic regressions, a positive or a negative 'e' represents an increase or a decrease, respectively, of the probability to be in the following categories <54, <70, >180, >250, >300 $mg.dL^{-1}$. The multinomial or binary logistic regressions were specifically used to assess for the percentage of time spent in hypo- and hyperglycemia. Ordinal categories 1, 2 and 3 (*i.e.,* no time spent in the target glucose range, below the median and above the median time spent in the target glucose range, respectively) were derived from time spent <70, >180 or >250 $mg.dL^{-1}$ [3.9, 9.9, 13.9 $mmol.L^{-1}$]. Additionally, categories 0 and 1 (*i.e.,* no time spent in the target glucose range or some time spent in the target glucose range, respectively) were designed to reflect time spent <54 or >300 $mg.dL^{-1}$ [3.0 or 16.7 $mmol.L^{-1}$].

MAGE, Mean Amplitude of Glycemic Excursions; CONGA 1 & 2, Continuous Overlapping Net Glycemic Action 1&2; ADRR, Average Daily Risk Ratio; For the circadian effect, night was chosen as the reference. For the mode of treatment effect, CSII was chosen as the reference. For habit with CGM use, the fact that the rider was not familiar with the wear of a CGM was chosen as a reference.

Glycemic data from the last (i.e., the 10^{th}) night were not used because several riders decided to remove their CGM before that night.

In the fifth set of models, the exercise characteristics analyzed were kilometers daily traveled, cumulative altitude change, and cycling duration, combined in a first model. Then, the effect of percentage of time spent in moderate- and vigorous-intensity activity was tested with the exercise characteristic(s) kept as (a) covariate(s) if significant in the preceding model. In the sixth set of models, the subject characteristics analyzed were *(i)* anthropometric (% fat mass) and demographic (age, sex) characteristics, *(ii)* disease & treatment (HbA_{1c}, diabetes duration, mode of insulin therapy, habit of wearing a CGM), and *(iii)* physical activity and fitness (IPAQ score, VO_{2max}) characteristics. Neither daily physical activity (IPAQ) nor $VO_{2\,max}$ significantly influenced glycemic variability and excursions.

When we focused our analysis on glycemic outcomes measured in the periods between breakfast and 2 h post dinner, the effects of time during the tour (days) were comparable to those presented here, *i.e.,* for the 24 h periods. The negative effect of kilometers traveled on glycemic variability was also found when we focused specifically on subsequent early (2 h) and late (6h) post-exercise recovery periods (early recovery: MAGE, e: +2.32, $P < 0.01$; late recovery: SD, e: +0.41, $P = 0.09$; MAGE, e: +2.03, $P < 0.01$). In addition, cumulative altitude change increased glycemic variability during late post-exercise recovery periods (%CV, $P < 0.05$, e +0.01; SD, e: +0.02, $P < 0.05$; MAGE, e: +0.07, $P < 0.01$). There was no significant result for %CV in other models except for the circadian effect in the 4^{th} set of models ($P < 0.05$, e +3.25). The percentage time in vigorous intensity tended to decrease the time spent < 70 $mg.dL^{-1}$ during subsequent late recovery period (e: − 0.04, P = 0.08) and to increase the

time in range during cycling as well as subsequent early and late recovery periods (e: +0.44, $P = 0.09$; e: +0.97, $P < 0.05$; e: + 0.67, $P < 0.05$, respectively). The percentage time in moderate intensity also decreased the time spent < 70 $mg.dL^{-1}$ during subsequent late recovery periods (e: − 0.08, $P < 0.05$).

FIGURE

Figure 1. Percentage of time spent in hypo-, normo- and hyperglycemia in relation to insulin administration and carbohydrate intake

Legend

1A. Between 8 AM and 8 PM

1B. Between 8 PM and 8 AM the next day; N=20; black bars, percentage of time spent below 70 $mg.dL^{-1}$; clear bold hatch bars, percentage of time spent between 70 and 180 $mg.dL^{-1}$; white bars, percentage of time spent above 180 $mg.dL^{-1}$. The effects of time on these glycemic outcomes are displayed in table 3. SD values varied between 3.02 and 16.12%, 9.18 and 28.07%, 11.78 and 30.17% for daytime data, and between 3.81 and 20.36%, 16.44 and 31.30%, 12.52 and 29.26% for nighttime data, for time spent in hypoglycemia, euglycemia, and hyperglycemia, respectively. Glycemic data from the last (*i.e.,* the 10^{th}) night were not used in analyses because several riders decided to remove their CGM before that night.

1C. N=19; Day, from breakfast to 2 h post-dinner; Night, from 2h post-dinner to breakfast the next day; black bars, snacks during the tour; white bars, 3 meals of the day. Carbohydrates from meals of day 10 were not taken into account because most of the riders did not correctly report their intake of the dinner following the end of the tour. The effects of time on carbohydrate ingestion are indicated in the results section. SD values varied between 15.0 and 28.4, 0.6 and 4.6, 10.5 and 21.5 g for carbohydrates (CHO) from the daytime snacks, the nighttime snacks, and from the 3 meals, respectively.

1D. N=8 who were treated with an insulin pump; Day, from breakfast to 2 h post-dinner; Night, from 2 h post-dinner to breakfast the next day; White bars, insulin bolus; Black bars, basal rates. The effects of time on insulin administration are indicated in the results section. SD values varied between 7.7 and 19.3, 0.3 and 2.5, 3.2 and 4.7, 3.6 and 4.2 U for daytime and nighttime insulin bolus, and for daytime and nighttime basal rates, respectively.

Data from day 4 are not displayed because they represent a resting day.

SUPPLEMENTAL MATERIAL

Supplemental Table S1. Details of the cycling tour

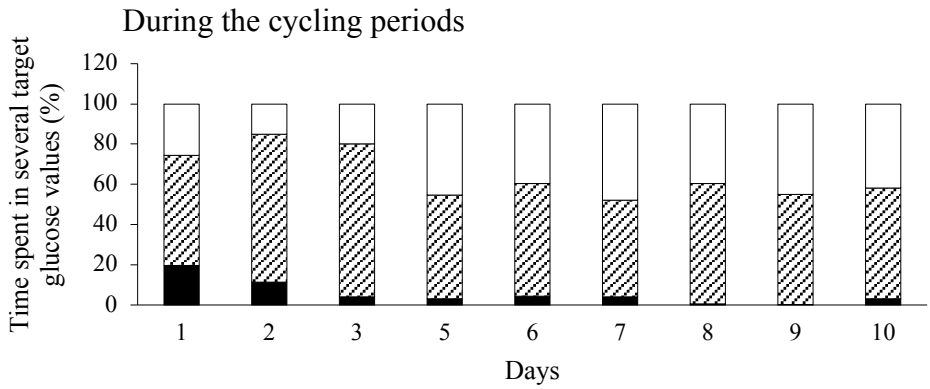
Days	City of departure	City of arrival	Kilometers	Altitude change (m)	Cycling duration (hours)	Lunch break (min)	Time spent in very light activity (%)	Time spent in light activity (%)	Time spent in moderate activity (%)	Time spent in vigorous activity (%) [among which, very vigorous and maximal intensities]	Percentage of time CGM was active
Day 1	Brussels	Cambrai	157	1000	5.5 ± 1.1	55.9 ± 23.3	0.32 ± 0.59	18.6 ± 11.4	39.5 ± 15.6	41.6 ± 15.9 [0.81 ± 2.07 and 0.03 ± 0.08]	71.1 ± 36.7
Day 2	Cambrai	Reims	163	1724	6.6 ± 1.5	47.5 ± 8.8	0.02 ± 0.03	10.0 ± 8.2	36.4 ± 13.9	53.6 ± 20.9 [0.80 ± 2.25 and 0.03 ± 0.07]	82.4 ± 30.4
Day 3	Reims	Paris	167	1767	7.2 ± 1.6	41.9 ± 9.0	0.09 ± 0.23	13.1 ± 15.7	46.9 ± 15.0	39.9 ± 24.8 [0.19 ± 0.50 and 0.00 ± 0.00]	73.3 ± 30.6
Day 4	Paris	Paris	0	0	0	/	/	/	/	/	/
Day 5	Paris	Troyes	187	1678	6.3 ± 1.4	42.9 ± 13.1	0.17 ± 0.49	15.1 ± 15.8	49.4 ± 10.8	35.4 ± 23.4 [0.00 ± 0.00 and 0.00 ± 0.00]	83.2 ± 21.8
Day 6	Troyes	Langres	161	1876	6.0 ± 1.3	39.1 ± 15.4	0.25 ± 0.59	11.6 ± 12.9	46.8 ± 14.5	41.3 ± 23.9 [0.14 ± 0.42 and 0.00 ± 0.01]	87.8 ± 24.0
Day 7	Langres	Belfort	188	2239	6.5 ± 1.5	50.1 ± 10.7	0.21 ± 0.28	19.7 ± 18.7	45.7 ± 12.7	34.4 ± 23.2 [0.02 ± 0.06 and 0.02 ± 0.05]	84.5 ± 18.6

Day 8	Belfort	Yverdon-les-Bains	167	3395	7.1 ± 1.5	52.4 ± 9.2	0.64 ± 1.91	12.1 ± 9.8	45.5 ± 15.4	41.3 ± 23.9 [0.09 ± 0.20 and 0.00 ± 0.00]	80.9 ± 18.9
Day 9	Yverdon-les-Bains	Morzine	141	3455	5.9 ± 1.2	64.1 ± 18.8	0.49 ± 0.80	15.9 ± 11.4	37.9 ± 12.9	45.7 ± 14.5 [0.39 ± 1.16 and 0.00 ± 0.01]	88.5 ± 23.2
Day 10	Morzine	Geneva	125	3582	4.3 ± 0.8	50.8 ± 15.1	1.83 ± 3.92	16.9 ± 12.2	35.4 ± 12.5	45.7 ± 12.2 [0.00 ± 0.00 and 0.03 ± 0.06]	92.2 ± 10.0
Total (for km and altitude) or mean ± SD, over the 9 days	**Brussels**	**Geneva**	**1456**	**20 716**	**6.2 ± 1.3**	**39.4 ± 13.7**	**0.44 ± 0.98**	**14.8 ± 12.9**	**42.6 ± 13.7**	**42.2 ± 18.9 [0.27 ± 0.74 and 0.01 ± 0.03]**	**/**

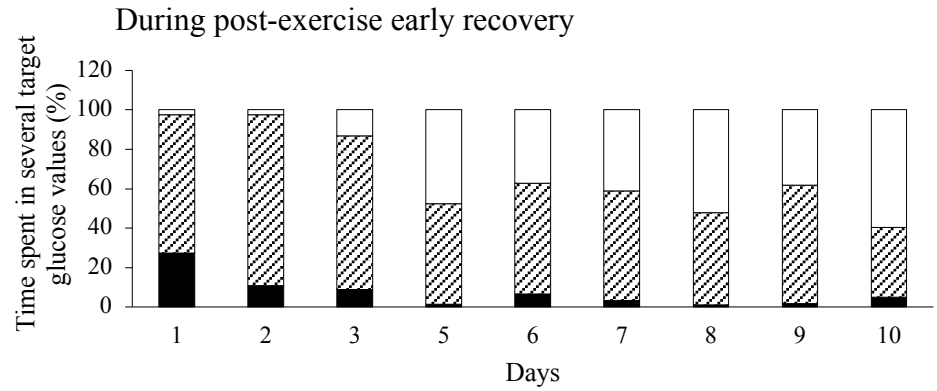
Mean ± SD; Relative intensity as % of maximal heart rate for the different target intensity ranges: very light activity, <35 %; light activity, 35-54 %; moderate activity, 55-69 %; vigorous activity >70 % [including very vigorous activity, 90-100%, and maximal activity, >100 %]; Riders spent a major part of the cycling period at moderate (i.e., 160.0 ± 38.3 min, mean over the 9 days) and vigorous (i.e., 155.1 ± 27.0 min, over the 9 days) intensities, while time spent in very light, light, very vigorous or maximal intensities was shorter (i.e., 1.43 ± 2.13, 52.40 ± 10.40, 1.00 ± 1.17, and 0.03 ± 0.03 min, respectively).

Supplemental Figure S1: Percentage of time spent in hypo-, normo- and hyperglycemia during cycling and post-exercise recovery periods throughout the tour

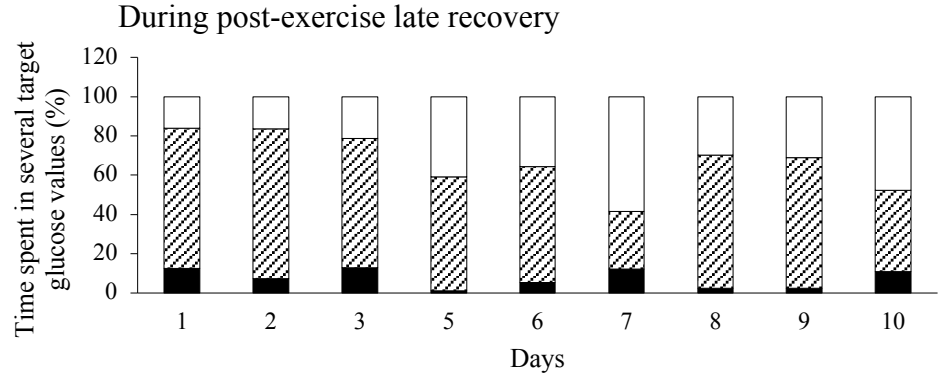
A



B



C



Legend.

Black bars, percentage of time spent below 70 mg.dL^{-1}; Clear bold hatch bars, percentage of time spent between 70 and 180 mg.dL^{-1}; White bars, percentage of time spent above 180 mg.dL^{-1}.

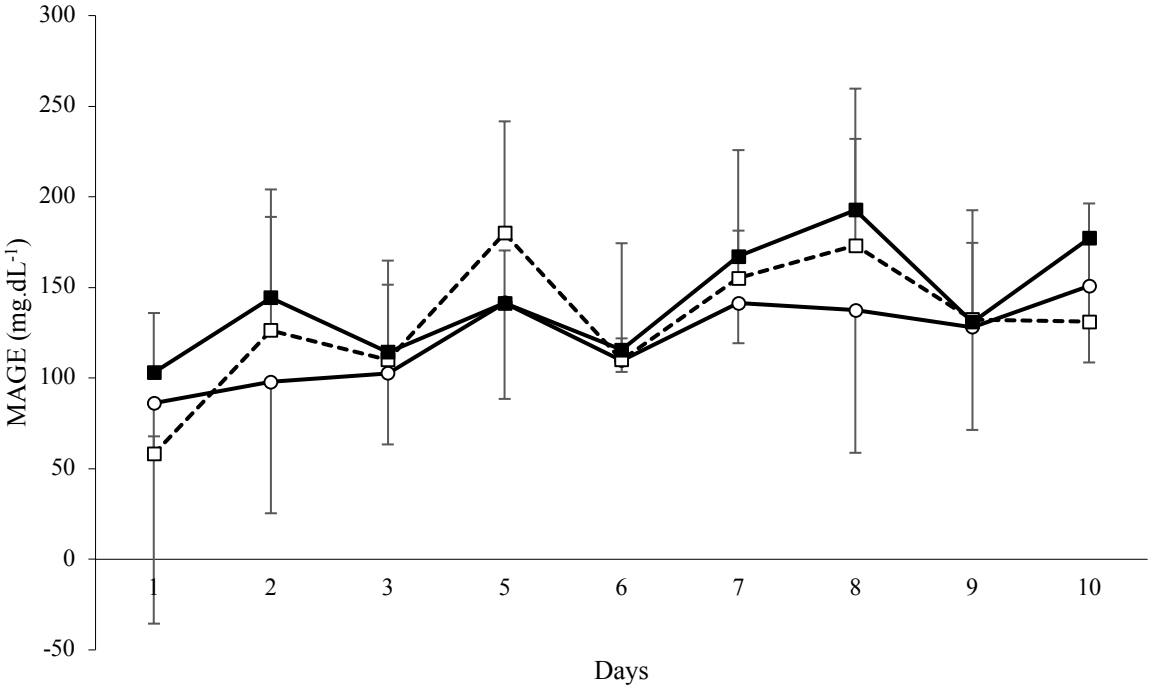
1A. N=9; during the cycling period, effects of time (*i.e.,* days 1 to 10, except for day 4) for percentage of time spent: < 70 mg.dL^{-1}, e: − 0.28, $P < 0.05$; > 180 mg.dL^{-1}, e: + 0.49, $P = 0.06$; in addition, there was also a decrease in time (%) spent < 54 mg.dL^{-1}, e: − 0.28, $P < 0.05$ and an increase in time (%) spent > 250 mg.dL^{-1}, e: + 0.31, $P < 0.05$ and > 300 mg.dL^{-1}, e: + 0.35, $P < 0.05$; SD values varied between 2.69 and 22.85%, 18.95 and 44.64%, 4.29 and 47.37% for time spent below range, in range, and above range, respectively.

1B. N=19; during the 2 hours of post-exercise recovery, effects of time for percentage of time spent: < 70 mg.dL^{-1}, e: − 0.23, $P < 0.05$; 70-180 mg.dL^{-1}, e: − 3.95, $P < 0.01$; > 180 mg.dL^{-1}, e: + 0.26, $P < 0.001$; in addition, there was also a marginal increase in time (%) spent > 300 mg.dL^{-1}, e: + 0.34, $P = 0.07$; SD values varied between 2.38 and 29.49%, 16.41 and 36.18%, 12.00 and 40.32% for time spent below range, in range, and above range, respectively.

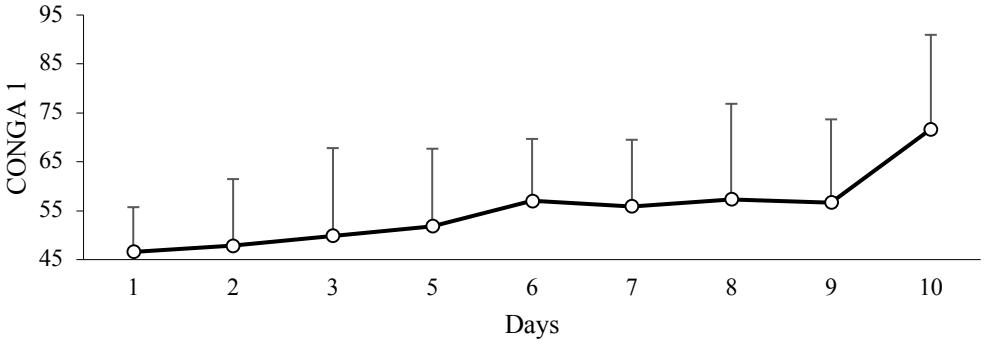
1C. N=19; during the 6 hours of post-exercise recovery, effects of time for percentage of time spent: < 70 mg.dL^{-1}, e: − 0.15, $P < 0.05$; 70-180 mg.dL^{-1}, e: − 1.57, $P = 0.09$; > 180 mg.dL^{-1}, e: + 0.17, $P < 0.05$; in addition, there was also a significant decrease in time (%) spent < 54 mg.dL^{-1}, e: − 0.20, $P < 0.05$; SD values varied between 3.64 and 23.76%, 26.50 and 39.97%, 21.55 and 40.88% for time spent below range, in range, and above range, respectively.

Supplemental Figure S2: Glycemic variability throughout the cycling tour

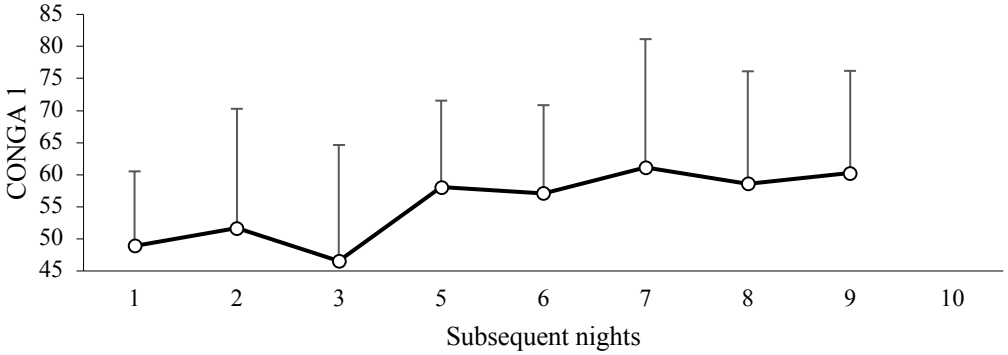
A



B



C



Legend.

2A. Mean Amplitude of Glycemic Excursions (MAGE) throughout the tour

Solid line with white circles -○- during the cycling periods (n=9), effect of time, e: + 5.79, $P < 0.05$; Dotted line with white squares --□-- during the 2 hours of post-exercise recovery (n=19), effect of time, e: + 4.59, $P = 0.07$; Solid line with black squares -■- during the 6 hours of post-exercise recovery (n=19), effect of time, e: + 4.26, $P < 0.05$.

2B. Daytime (8 AM to 8 PM) Continuous Overall Net Glycemic Action (CONGA) 1 throughout the tour (n=20); effect of time, e +2.16, $P < 0.001$; in addition, there was also a significant increase in daytime CONGA 2 and SD throughout the tour, e: + 2.02, $P < 0.001$ and e: + 1.28, $P < 0.01$.

2C. Nighttime (midnight to 4 AM) Continuous Overall Net Glycemic Action (CONGA) 1 throughout the tour (n=20); CONGA 1 from the last (i.e., the 10^{th}) night is not indicated because several riders decided to remove their CGM before this night; effect of time, e: + 1.67, $P < 0.001$; In addition, there was also a significant increase in nighttime CONGA 2 throughout the tour, e: + 2.17, $P < 0.001$.

In addition, there was a significant increase in Standard Deviation of glycemia (SD), CONGA 1, and CONGA 2 during the cycling periods (effect of time, e: + 1.54, $P < 0.05$; e: + 1.85, $P < 0.01$; and e: + 2.02, $P < 0.01$, respectively).