Original article

The impact of physical inactivity on glucose homeostasis when diet is adjusted to maintain energy balance in healthy, young males

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SUMMARY

Background & aims: It is unclear if dietary adjustments to maintain energy balance during reduced physical activity can offset inactivity-induced reductions in insulin sensitivity and glucose disposal to produce normal daily glucose concentrations and meal responses. Therefore, the aim of the present study was to examine the impact of long-term physical inactivity (60 days of bed rest) on daily glycemia when in energy balance.
Methods: Interstitial glucose concentrations were measured using Continuous Glucose Monitoring Systems (CGMS) for 5 days before and towards the end of bed rest in 20 healthy, young males (Age: 34 ± 8 years; BMI: 23.5 ± 1.8 kg/m^2). Energy intake was reduced during bed rest to match energy expenditure, but the types of foods and timing of meals was maintained. Fasting venous glucose and insulin concentrations were determined, as well as the change in whole-body glucose disposal using a hyperinsulinemic-euglycemic clamp (HIEC).
Results: Following long-term bed rest, fasting plasma insulin concentration increased 40% ($p = 0.004$) and glucose disposal during the HIEC decreased 24% ($p < 0.001$). Interstitial daily glucose total area under the curve (tAUC) from pre-to post-bed rest increased on average by 6% ($p = 0.041$), despite a 20 and 25% reduction in total caloric and carbohydrate intake, respectively. The nocturnal period (00:00—06:00) showed the greatest change to glycemia with glucose tAUC for this period increasing by 9% ($p = 0.005$). CGMS measures of daily glycemic variability (SD, J-Index, M-value and MAG) were not changed during bed rest.
Conclusions: Reduced physical activity (bed rest) increases glycemia even when daily energy intake is reduced to maintain energy balance. However, the disturbance to daily glucose homeostasis was much more modest than the reduced capacity to dispose of glucose, and glycemic variability was not negatively affected by bed rest, likely due to positive mitigating effects from the contemporaneous reduction in dietary energy and carbohydrate intake.
Clinical trials record: NCT03594799 (registered July 20, 2018) (https://clinicaltrials.gov/ct2/show/NCT03594799).

1. Introduction

Bed rest studies employed by space agencies to examine deconditioning (as a model for microgravity) also represent a unique model to investigate the physiological effects of physical inactivity isolated from potential confounders such as energy imbalance [1,2]. During most bed rest studies, habitual dietary energy intake is reduced to match energy requirements to maintain energy balance, and feeding regimens and sleep/wake cycles are tightly controlled [3]. Sustained bed rest-induced physical inactivity causes whole body metabolic dysfunction characterized by altered substrate utilization and impaired glucose control/insulin sensitivity, substantial bone and muscle deterioration, and redistribution of adipose tissue [4–6].

A primary causal factor in impaired whole-body glucose control and insulin sensitivity with bed rest-induced physical inactivity is the reduction of skeletal muscle insulin sensitivity and glucose uptake [7–10]. Prior observations have typically characterized the changes in glucose control induced by bed rest following a specific stimulus both before and after bed rest (e.g., 75 g of glucose in an oral glucose tolerance test) [11]. Such studies provide strong evidence for changes in functional capacity and/or physiological responses to an absolute and fixed stimulus. However, these comparisons do not provide information about daily metabolic regulation *during* the period of physical inactivity. It is unclear whether impaired insulin sensitivity with reduced physical activity translates into poorer daily glucose control when energy (and carbohydrate) intake is reduced to match energy requirements—or, whether the dietary adjustments that are introduced to maintain energy balance partially or wholly offset the effect of reductions in insulin sensitivity and produce normal daily glucose concentrations and meal responses.

High-resolution continuous glucose monitoring enables the measurement of glucose responses across extended periods of time (days/weeks) [12–14]. In the present study, we used continuous glucose monitoring to examine glucose control during long-term bed rest (60 days) when energy intake was reduced to match requirements; and compared the magnitude and nature of changes in daily glycemia with bed rest-induced changes in glucose control, including a hyperinsulinemic-euglycemic clamp (HIEC).

2. Materials and method

2.1. Experimental design

Twenty healthy, young (20–45 years old) males undertook 60 days of head-down (−6° tilt) bed rest, preceded by a 14-day ambulatory control period, and a 14-day recovery period, as previously described [15]. The study was conducted in accordance with European Space Agency (ESA) Standardization of Bed Rest Study Conditions guidelines [3] at the Médecine et de Physiologie Spatiales (MEDES) clinical institute in Toulouse, France. The study was sponsored by the ESA and French National Space Agency (CNES). The protocol was reviewed and approved by the local ethics committee (CPP Sud-Ouest et Outre-Mer I, France, RCB: 2016-A00401-50) and was registered on *ClinicalTrials.gov* (NCT03313869). All procedures conformed to the Declaration of Helsinki. A flow diagram of participant recruitment, adapted from Austermann et al. [16], is presented in Fig. 1. Screening/selection processes and volunteer selection processes are presented elsewhere [15]. Briefly, participants were recruited by online advertisements and press announcements, followed by tiered medical and psychological screening to select the final 20.

One of the original aims of this bed rest study was to assess the efficacy of an anti-oxidant/anti-inflammatory nutritional countermeasure on the deleterious effects of bed rest [15]. As described in previous published reports from this bed rest investigation, the nutritional countermeasure (cocktail; *ingredients detailed in* Supplementary Table 1) did not affect measures of muscle deconditioning, bone turnover, oxidative stress, mitochondrial biogenesis, whole-body insulin sensitivity, glucose disposal, adipose tissue inflammation, lipid metabolism, carbohydrate metabolism, B cell function, or a range of cardiovascular indices [15–21]. There was also no effect from the cocktail on the outcomes in the present report (*subgroup results provided in* Supplementary Table 2). Given the small sample size, which is typical of intensive and demanding long-term bed rest studies, data from both groups was merged to enable a more robust and rigorous investigation of the impact of bed rest *per se*.

Continuous interstitial glucose monitoring (CGMS), HIEC, body composition assessment, venous blood, and urine sample collections were carried out before and within the final eight days of bed rest, as outlined in Fig. 2.

2.2. Physical activity baseline standardization

During the 14-day pre-bed rest period in the MEDES clinical facility, participants were required to undertake approximately 8000 steps/d measured with a wrist-mounted accelerometer (Polar Loop; Polar; France) accounting for ~70% of active energy
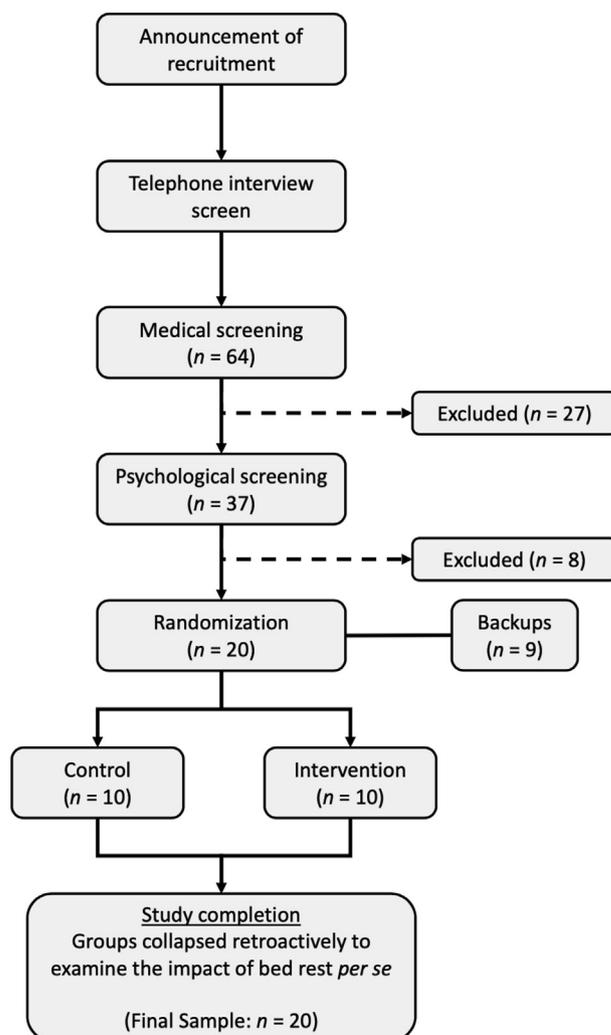

Fig. 1. Flow chart of participant recruitment, eligibility, and group allocation, adapted from Austermann et al. (2021).
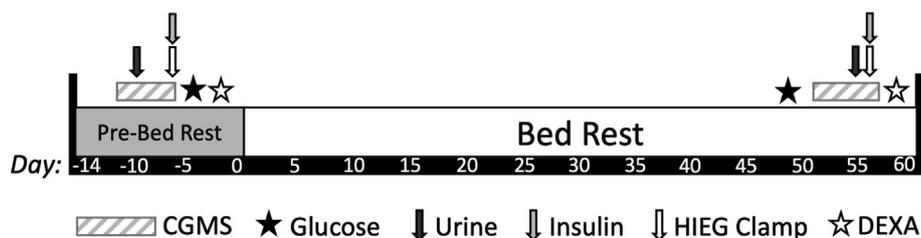
Fig. 2. Summary of the study protocol and points of sample collections. All bloods were collected in the fasted state upon waking. Abbreviations: CGMS, continuous glucose monitoring system; HIEC, hyperinsulinemic-euglycemic clamp.

expenditure. Participants also undertook bouts of supervised structured exercise during this standardization period on a treadmill and cycling ergometer, accounting for ~30% of active energy expenditure. Specifically, during the CGMS pre-bed rest monitoring period, participants undertook a structured exercise session and a VO_{2max} test on days 7 and 8 pre-bed rest, respectively. An overview of all structured physical activity during the pre-bed rest period is detailed in Trim et al. [15]. No exercise was undertaken on the HIEC measurement days.

2.3. Dietary control

Diet was controlled throughout the study period according to guidelines detailed in Heer et al. [3]. Energy intake was based on basal metabolic rate (BMR) measured by indirect calorimetry [3]. During the pre-bed rest period dietary energy intake equivalent to 140% of BMR was provided, which was reduced to 110% BMR during the bed rest period in order to maintain fat mass stable [3]. An additional 1000 IU of vitamin D3 was supplemented daily by oral administration [3]. Macro- and micronutrient intake for the pre-bed rest and bed rest period are presented in Trim et al. [15].

Throughout the study (both pre- and during bed rest), all participants consumed the same food stuffs but with energy reduced to match requirements during bed rest, with a 7-day menu that cycled each week. The MEDES dieticians adjusted participants' diets to maintain the types of foods consumed prior to bed rest, and to maintain protein intake, but to reduce carbohydrate and fat intake and thus reduce overall energy intake. All participants ate within the same time windows on each day, split into three main meals (breakfast, lunch, evening meal), with an additional mid-afternoon snack. Sleep-wake cycles were maintained for all participants throughout the entire protocol with lights on at 07:00 and lights off at 23:00.

2.4. Continuous glucose monitoring

Interstitial glucose was measured continuously using Freestyle Libre continuous glucose monitoring systems (CGMS) (Abbott Diabetes Care Ltd.; Oxon, UK). Sensor probes were inserted into the back of the upper left arm, according to manufacturer instructions. Data was recorded in 15-min intervals throughout the period of wearing (Fig. 2).

During the CGMS measurements (Fig. 2), we excluded days to avoid potentially confounding measures being made by other research teams (e.g., lipid tolerance tests). After these exclusions, there were two periods (pre- and during bed rest) where foods were matched for food-type, but at different quantities, across two days (Supplementary Table 3). These periods were analyzed in isolation, with the mean of both days for each respective outcome

used for the final analysis. Consequently, from the six days of collection pre-bed rest, and five days during bed rest, two days were selected for the final analysis (days 8 and 7 pre-bed rest, and days 53 and 55 during bed rest). Fig.3 displays the 24-h CGMS trace for all participants combined ($n = 20$) across one day pre- and one day during bed rest. We examined glucose responses across 24-h, as well as further splitting into 6-h periods throughout the day to capture feeding times (morning, afternoon, and evening), and nocturnal fasting glucose responses to bed rest.

2.5. Hyperinsulinemic-euglycemic clamp analysis

A hyperinsulinemic-euglycemic clamp (HIEC) was performed on the morning of day 6 pre-bed rest, and on day 56 of bed rest [18]. Briefly, an anterograde cannula was inserted into an antecubital fossa vein for simultaneous infusion of insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) and 20% glucose (Baxter Healthcare, Norfolk, UK). A second cannula was inserted retrograde into a superficial dorsal vein on the non-dominant hand. The cannulated hand was warmed to arterialize venous drainage of the hand using a hand-warming unit (circulating air temperature: 50—55 °C). Throughout the 3-h HIEC period, insulin (0.5 IU/mL) was infused at 60 mU/m^2/min as described [18]. Arterialized-venous whole blood glucose concentration was measured at 5-min intervals during the HIEC (YSI2300; Yellow Springs Inc, Ohio, USA and maintained at 4.5 mmol/L by adjusting the 20% glucose infusion rate. Samples
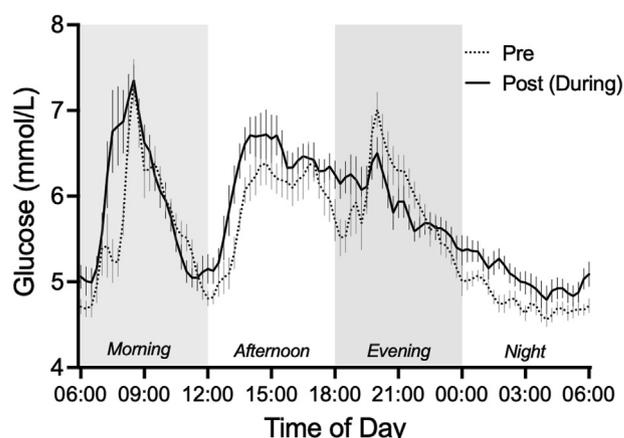

Fig. 3. 24 h continuous glucose data in all 20 participants recorded by CGMS at 15 min intervals pre- and post-bed rest. Note; all measures denoted at 'Post' refer to at the end of the bed rest protocol, but during its final week. Six-hourly intervals represent segmentation of the 24-h period presented in Fig. 5. Data are presented as group mean ± SEM.

were collected at baseline and every 5 min during the final hour of the clamp for measurement of glucose concentrations). Whole-body glucose disposal was calculated thereafter according to DeFronzo et al. [22]), and normalized to DEXA-determined lean body mass. Steady-state glucose disposal was determined between minutes 135–165 of the clamp [22].

2.6. Blood sampling and biochemical analysis

Fasted venous blood samples were collected from an ante-cubital vein at 07:00 into EDTA-containing tubes (BD Vacutainer; BD Biosciences, USA) as shown in Fig. 2. Glucose was measured in venous blood using fresh whole-blood samples at the clinical facility using an automated analyzer (Architect C8000; Abbott, CA).

Blood samples were immediately centrifuged upon collection and plasma stored at −80 °C until analysis. Plasma insulin was measured by enzyme-linked immunosorbent assay (Mercodia, Mercodia AB; Sweden).

2.7. Urine analysis

Urine was collected over a 24-h period from the second void on the day of collection to the first void on the following day (i.e., days 10 into 9 pre-bed rest and days 55 into 56 of bed rest; Fig. 2). Urine (2 mL) was collected from each void and stored at −20 °C for 2–4 h until being transferred to −80 °C. Urinary glucose was measured with an automated spectrophotometric analyzer by hexokinase reduction (Cobas Substrates®; Mannheim, Germany).
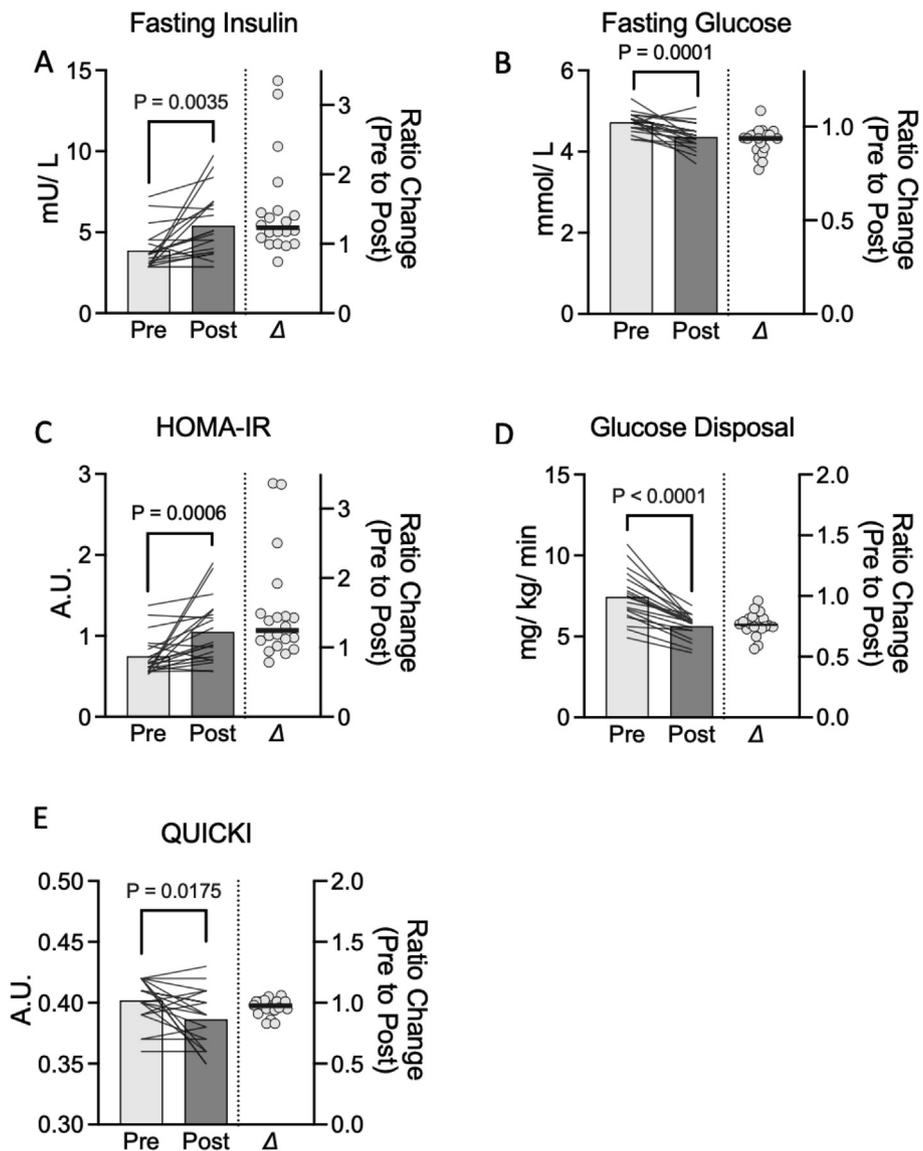

Fig. 4. Effects of bed rest on glucose homeostasis and insulin sensitivity. A, fasting plasma insulin before and after bed rest. B, fasting blood glucose before and after bed rest. C, homeostatic model of insulin resistance responses before and after bed rest from fasting blood glucose and plasma insulin collected on days 4 and 6 prior to bed rest, respectively. D, glucose disposal during between minutes 120 and 180 of the hyperinsulinemic-euglycemic clamp before and after bed rest. E, quantitative insulin sensitivity check index responses before and after bed rest from fasting blood glucose and plasma insulin collected on days 4 and 6 prior to bed rest, respectively. Note; all measures denoted at 'Post' refer to at the end of the bed rest protocol, but during its final week. All data are presented as group means with individual responses overlaid, and individual ratio change values for changes from pre-to post-bed rest (denoted as Δ on the x axis), with the median change of all participants signified by an overlaid line. Data were analysed by paired t-tests or non-parametric Mann-Whitney U equivalents where non-normally distributed. P values are reported above each pair of bars. Abbreviations: A.U, arbitrary units.

2.8. Body composition

Body mass (kg) was measured using a weighing gurney, in pre-weighed underwear accurate to 0.1 kg on day 2 pre-bed rest, and day 58 of bed rest. Body composition was determined using dual-energy X-ray absorptiometry (DEXA; Discovery, Hologic; Bedford, UK).

2.9. Statistical analysis

Serial measurements of interstitial glucose (by CGMS) were converted into summary statistics to illustrate the net response of each parameter. The trapezoid method for total area under the curve (*t*AUC) was performed [23]. Homeostatic model of insulin resistance (HOMA-IR) was estimated using the equation by [24]: fasting glucose (mmol/L) x fasting insulin (mU/L)/22.5. Insulin sensitivity was estimated using the quantitative insulin sensitivity check index (QUICKI) [25]. Summary statistics (mean, standard deviation, J-index, MAGE, CGMS-derived M-value, and MAG) were performed using the easy glucose variability (EasyGV v.9.0.) [26]. Missing data points (<1%) were interpolated using a straight line estimation method, for missing data durations <2 h. For CGMS-derived M-value calculations, the reference glucose concentration around which glycemic variability was calculated was set at 6.67 mmoL/l (120 mg/dL) (suggested default set by Hill et al. [26]). For MAGE calculations, a fuzzy logic algorithm was applied to eliminate potential short-term fluctuations due to sensor inaccuracy [26]. Descriptive data are presented as mean ± standard deviation (SD), unless otherwise stated. Pre-to during-bed rest comparisons were assessed by paired *t* tests. Wilcoxon signed rank tests were performed if Shapiro-Wilks distribution tests on the paired data were violated ($p < 0.05$). Standardized effect sizes for comparisons pre-to during-bed rest were performed using Cohen's *d* calculations [27]. Effect sizes were defined as follows <0.2 = no effect, 0.2—<0.5 = small effect, 0.5—<0.8, moderate effect, and ≥0.8 = large effect. Statistical analysis was performed using GraphPad Prism v.9.2.0 for Mac (GraphPad Software; California, US). Significance was set at $p \leq 0.05$.

3. Results

3.1. Insulin resistance, insulin sensitivity, and glucose disposal

Fasting plasma insulin concentration significantly increased by 40% following bed rest from 3.87 ± 1.27 mU/L pre-, to 5.41 ± 2.04 mU/L during bed rest ($p = 0.0035$, $d = 0.95$; Fig. 4A). Fasting venous whole blood glucose concentrations declined from 4.73 ± 0.24 mmol/L pre-, to 4.36 ± 0.32 mmol/L during bed rest ($p = 0.0001$, $d = 1.28$; Fig. 4B). There was a corresponding 1.24 ± 0.74 median fold increase in HOMA-IR from 0.75 ± 0.24 pre-, to 1.05 ± 0.39 during bed rest ($p = 0.0006$, $d = 0.97$; Fig. 4C) and a 24% decrease in the rate of glucose disposal during the HIEC clamp from 7.45 ± 1.50 pre-, to 5.63 ± 0.73 mg/kg LBM/min during bed rest ($p < 0.0001$, $d = 1.53$; Fig. 4D). There was a significant reduction in fasting insulin sensitivity estimated by QUICKI from 0.402 ± 0.019 pre-, to 0.387 ± 0.024 during bed rest ($p = 0.017$, $d = 0.70$; Fig. 4E).

3.2. Daily glucose concentrations (CGMS AUC)

Interstitial glucose *t*AUC across the 24-h period (from 6am to 6am) increased significantly with bed rest ($p = 0.0413$, $d = 0.63$; Fig. 5A). The increase in *t*AUC over 24 h was approximately 6% on average, despite a 20% reduction in total daily energy intake and a 25% reduction in carbohydrate intake during bed rest (Fig. 5B). Fat intake was reduced 21% whereas protein intake was not adjusted during bed rest and remained constant throughout (Fig. 5B).
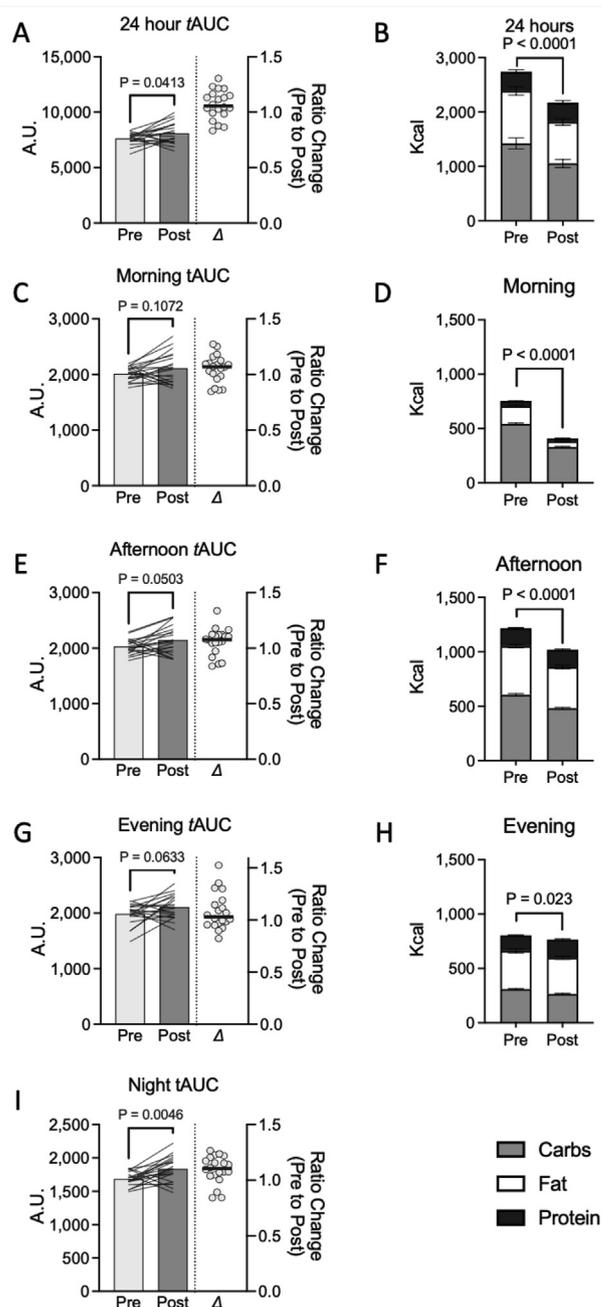

Fig. 5. Glycemic variability throughout days matched for food types consumed, split by time-of-day, before and at the end of bed rest. Data in Panels A, C, E, G and I represent glycemic responses, as measured by *t*AUC analysis of all interstitial glucose concentrations assessed by CGMS at 15-min intervals, for the whole 24-h period (Panel A), the 6 am—12 pm Morning period (Panel C), the 12 pm—6 pm Afternoon period (Panel E), the 6 pm—12 am Evening period (Panel G), and 12 am—6 am Night period (Panel I), across two sets of days matched for food types consumed before and at the end of bed rest. Panels B, D, F, and H represent a summary breakdown of the total caloric intake split by macronutrient across these two sets of days matched for food types consumed before and at the end of bed rest, corresponding to the whole day (Panel B), 6 am—12 pm Morning period (Panel D), the 12 pm—6 pm Afternoon period (Panel F), and the 6 pm—12 am Evening period (Panel H). Data in Panels A, C, E, G and I are presented as group means with individual responses overlaid, and individual ratio change values for changes from pre-to post-bed rest (denoted as Δ on the x axis), with the median change of all participants signified by an overlaid line. Data in Panels B, D, F, and H are presented as group means with SEM for each macronutrient. Note: all measures denoted at 'Post' refer to at the end of the bed rest protocol, but during its final week. Data were analysed by paired t-tests or non-parametric Mann-Whitney U equivalents where non-normally distributed. P values are reported above each pair of bars.

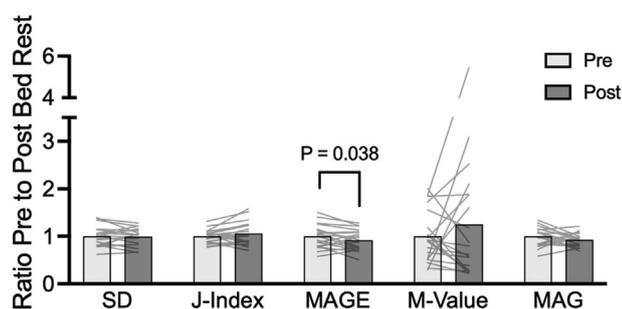Abbreviations: A.U, arbitrary units; tAUC, total area under the curve.

Fig. 6. Summary statistics for interstitial glucose concentrations during days paired for foods consumed before and after bed rest. Data are presented as individual responses from pre-to post-bed rest, normalized to pre-bed rest values. Note; all measures denoted at 'Post' refer to at the end of the bed rest protocol, but during its final week. Data were analysed by paired t-tests or non-parametric Mann-Whitney U equivalents where non-normally distributed. P values are reported above each pair of bars.
Abbreviations: MAG, mean absolute glucose; MAGE, mean amplitude of glycemic excursions.

Interstitial glucose *t*AUC for specific 6-h periods when meals were consumed (morning, afternoon, evening) increased ~5−7% on average (*p* = 0.107, 0.050, and 0.063 *d* = 0.45, 0.57, and 0.61 for Fig. 5C, E and G, respectively). As shown in Fig. 5I, the increase in nocturnal interstitial glucose concentrations was even more substantial (~9%), at a time when no food was consumed (12am−6am) (*p* = 0.0046, *d* = 0.98). During bed rest, there was a considerably greater reduction in energy intake at breakfast (−46% energy intake; Fig. 5D) and lunch (−16% energy intake; Fig. 5F), compared to the evening meal (−5% energy intake; Fig. 5H).

3.3. Daily glucose variability indices (CGMS)

Indices of daily glycemic variability are presented in Fig. 6. There was no change in the SD, J-Index or MAG, but MAGE was significantly lower during bed rest (−6.3 ± 17.9%; *p* = 0.038, *d* = 0.38; Fig. 6). There was considerable heterogeneity in the CGMS-derived M-Value response to bed rest (Fig. 6).

3.4. Urinary glucose output and concentration

There was no evidence for increased glucosuria in response to bed rest. Total urinary output increased by ~300 mL (Table 1), whereas average glucose concentrations, standard deviation, and maximal concentrations were significantly reduced in response to bed rest (Table 1). After correcting for total urinary output, assuming the molecular weight of glucose at 180.156 g/mol, total glucose excretion across 24 h was modestly reduced ~10% (~9 mg/d; *p* = 0.074) in response to bed rest (Table 1).

3.5. Body composition

Total body mass decreased by −1.7 ± 1.2 kg, combined fat-free mass and bone mineral content decreased by−3.0 ± 1.0 kg, and total fat mass increased modestly by 1.2 ± 0.7 kg (all *p* < 0.001) (Table 2).

4. Discussion

This is the first study to examine how a long-term reduction in physical activity (60 days of bed rest) impacts continually measured interstitial glucose homeostasis. Bed rest increased daily glycemia by ~6% (*t*AUC), even with dietary adjustments to maintain energy balance. However, the change to daily glycemia was much more modest than the change in capacity for glucose disposal (which

Table 1
Twenty-four hour urinary glucose excretory patterns before and at the end of 60 days of bed rest (n = 20).

	Pre-bed rest	Post-bed rest	*p*	*d*
Daily average glucose conc. (mmol/L)	0.28 ± 0.10	0.21 ± 0.07	0.014[a]	0.75
Daily glucose SD (mmol/L)	0.15 ± 0.07	0.11 ± 0.04	0.053[a]	0.67
Daily glucose CV (%)	53.8 ± 14.8	53.4 ± 15.8	0.925[a]	0.03
First daily void conc. (mmol/L)	0.33 ± 0.20	0.35 ± 0.13	0.674[a]	0.14
Daily minimum glucose conc. (mmol/L)	0.11 ± 0.03	0.08 ± 0.04	0.108[a]	0.52
Daily maximum glucose conc. (mmol/L)	0.50 ± 0.21	0.38 ± 0.13	0.031[a]	0.73
Voids per day	6 ± 2	6 ± 2	0.815[a]	0.06
Total urinary output (mL)[c]	1865 ± 519	2196 ± 466	0.021[a]	0.67
Total glucose excretion (mg)[c]	87.7 ± 22.0	78.5 ± 13.6	0.074[b]	0.50

Note; all measures denoted at 'Post' refer to at the end of the bed rest protocol, but during its final week. Data represent mean ± SD.
Abbreviations: Conc., concentration; CV, coefficient of variation; SD, standard deviation.
[a] Paired-samples t-tests were performed.
[b] Non-parametric Wilcoxon signed-rank tests were performed. Effect size calculations were performed using Cohen's d effect sizes.
[c] These results represent *n* = 18.

declined 24%); and there were no negative changes to measures of daily glycemic variability, most likely due to the mitigating effects of a reduction in energy and carbohydrate intake.

Bed rest has been consistently shown to impair glucose control and insulin sensitivity [9,28−32], largely as a consequence of reduced muscle mass and/or muscle-specific insulin sensitivity [7−10,29−31]. These earlier investigations examined the physiological response to the same metabolic challenge pre- and during bed rest. In line with these observations, the present study reports a 24% decrease in glucose disposal during a HIEC clamp and a 40% increase in HOMA-IR. In contrast, the change in daily glucose concentrations was more modest, and 24 h *t*AUC glucose increased by ~6%. Thus, chronic physical inactivity increases daily glycemia (*t*AUC), even when dietary intake is adjusted to maintain energy balance, but the magnitude of the change in glycemia is much more modest than the change in the capacity for glucose disposal and HOMA-IR. Furthermore, most indices of glucose variability (SD, J-Index, CGMS-derived M-value and MAG) did not change during bed rest. Thus, whilst long-term bed rest reduces insulin sensitivity and glucose tolerance in response to the same challenge (i.e., a set bolus of glucose or HIEC), adjustments to dietary intake prevent this from translating into a deterioration in within-individual daily glycemic variability.

The increase in glycemia due to bed rest was similar at all times of the day when meals were consumed (~5−6%, Cohen's *d* = 0.5−0.6), despite a variable degree of dietary adjustment during

Table 2
Participant characteristics and body composition (DEXA) pre-bed rest compared to post-bed rest (n = 20).

	Pre-Bed Rest	Post-Bed Rest	*p*
Age (years)	34 ± 8	—	—
Height (m)	1.76 ± 0.05	—	—
Body mass (kg) (DEXA)	72.89 ± 7.09	71.17 ± 6.81	<0.0001[a]
Body mass index (kg/m^2) (DEXA)	23.5 ± 1.8	22.9 ± 1.8	<0.0001[b]
Fat-free mass + BMC (kg) (DEXA)	53.69 ± 4.95	50.73 ± 4.45	<0.0001[a]
Fat mass (kg) (DEXA)	19.20 ± 3.83	20.44 ± 4.08	<0.0001[a]

Note; all measures denoted at 'Post' refer to at the end of the bed rest protocol, but during its final week. Data represent mean ± SD.
Abbreviations: BMC, bone mineral content; DEXA, dual X-ray absorptiometry.
[a] Paired-samples *t* tests were performed.
[b] Non-parametric Wilcoxon signed-rank tests were performed.

these periods. Energy intake in the morning was reduced by almost 50% compared to pre-bed rest, whereas energy intake in the evening was only reduced by around 5%. The increase in nocturnal glycemia, when food had not been consumed for many hours, was even more pronounced (~9%, Cohen's d = 1.0). The remarkably similar effect on glycemia over 24 h, during periods with variable dietary adjustments, may indicate a readjustment of the homeostatic set point for glucose [33] and/or a decrease in insulin-stimulated carbohydrate oxidation [18]. Whether this degree of increased glycemia has long term health consequences cannot be ascertained from the present study, but increased glycemia is a risk factor for cardiovascular disease in people with [34] and without diabetes [35]. Thus, dietary changes implemented to maintain energy balance do not completely offset the impact of reduced physical activity on daily glycemia.

The increase in nocturnal glucose concentrations in the present study is intriguing. Sleep is normally associated with a reduction in both glucose disposal and hepatic glucose output, with relatively stable glucose concentrations [36]. We previously reported that morning fasting leptin increased >60% with bed rest [15]. Leptin concentration is highest at night [37], and leptin is known to be influenced by glycemia and glucose availability [38—40]. Thus, the increase in glycemia (especially nocturnal glycemia) may influence average leptin concentrations and/or the biological rhythm for leptin (e.g., amplitude), and contribute to the high morning leptin after bed rest. Further studies are needed to examine the interplay between diurnal variation in glycemia and leptin during bed rest or other models of low physical activity.

One exception to the other measures of glycemic variability was MAGE which, counterintuitively given the metabolic disturbance caused by bed rest, *decreased* during bed rest. However, it is important to note that, prior to bed rest, participants performed substantial physical activity and consumed larger meals. Given the impact of acute physical activity on glucose uptake and oxidation [41], and the counter effect on circulating glucose from the larger meals, it is not so surprising that some measures of daily within-individual variability in glycemia were greater before, rather than during, bed rest (i.e., during bed rest, both positive and negative effectors of glycemia were reduced). Thus, this change in MAGE is context-specific and unlikely to carry the same physiological significance as changes to MAGE in the context of type 1 or 2 diabetes or cardiovascular disease.

This present study has many strengths. To our knowledge, it is the first report of the impact of bed rest as a model of physical inactivity on daily glycemia. These novel findings characterize the extent of changes to glycemia experienced during bed rest when diet is adjusted to match energy requirements, rather than changes in functional capacity and/or responses to a fixed dietary challenge. The clinical facility study setting is another major strength, with carefully controlled feeding/waking cycles, homogeneous dietary composition, pre-intervention standardization, plus rigorous control of physical activity during the intervention period. The current design cannot establish what would have happened if dietary intake was *not* adjusted during reduced physical activity. This would be an interesting but challenging experiment, given that ideally there would be a similar level of control of physical activity (bed rest), but with the freedom to choose and consume an *ad libitum* diet. We also do not know what would have happened if all meals had been adjusted equally, especially given the unknown potential interaction with established meal-timing interaction effects [42—44]. The greater nocturnal glycemia could be partly related to the fact that dietary adjustments to the evening meal were more modest than the adjustments to other meals. However, adjusting all meals equally whilst maintaining the type and nature of foodstuffs consumed (and overall daily protein intake) would be

very challenging. We used a single continuous glucose monitor placed in the subcutaneous adipose tissue of the upper arm. The placement of continuous glucose monitors in a different anatomical location has been reported to provide different postprandial and nocturnal glycemic responses [45,46], although other studies report only minor variation due to the type of device and/or location [47].

5. Conclusions

In conclusion, long-term (60 days) physical inactivity in the form of bed rest causes increased glycemia, even when dietary energy intake is adjusted to match reduced energy requirements. Although reductions to energy and carbohydrate intake do not completely offset the detrimental impact of reduced physical activity on daily glycemia, the effects on glycemia were more modest than the effects on the capacity to dispose of glucose, and measures of daily glucose variability did not deteriorate during bed rest, probably due to the mitigating effects of the simultaneous reductions in energy and carbohydrate intake.

Author contributions

Conceptualization: WVT, FK, NFS, EJS, IAM, PLG, DT.
Data curation: WVT, J—PW, FK, JET, NFS, EJS, DT.
Formal analysis: WVT, J—PW, FK, JET, DT.
Writing — Original draft: WVT, J—PW, FK, JET, NFS, EJS, IAM, PLG, DT.
Writing — Review and editing: WVT, J—PW, FK, JET, NFS, EJS, IAM, PLG, DT.
Funding acquisition: DT, IAM, PLG.
Project administration: WVT, J—PW, FK, JET, NFS, EJS, IAM, PLG, DT.
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Supervision: DT, FK, JPW, JET, IAM, EJS, PLG.
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Had primary responsibility for final content: DT.
All authors have read and approved the final manuscript.

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Data availability

Data described in the manuscript, code book, and analytic code will be made publicly and freely available without restriction at the University of Bath Data Repository (https://doi.org/10.15125/BATH-01052).

Conflicts of interest

I.A.M. was a member of the Mars Scientific Advisory Council, member of the Mars Europe Nutrition Advisory Board, and Scientific Adviser to the Waltham Centre for Pet Nutrition, and was also a member of the Nestle Research Scientific Advisory Board, and of the Novozymes Scientific Advisory Board. He withdrew from all of these roles in 2020 and on 1 August 2020 became Professor

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2023.02.006.

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