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Fuel Homeostasis during Physical Inactivity Induced by Bed Rest*

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ABSTRACT

The consequences of physical inactivity on fuel homeostasis were evaluated during 7 days of head-down bed rest (HDBR), a model mimicking weightlessness. Eight men (32.4 ± 1.9 yr; body mass index, 23.9 ± 0.7 kg/m^2) and eight women (27.9 ± 0.9 yr; body mass index, 20.9 ± 0.6 kg/m^2) underwent an oral glucose tolerance test (OGTT; 1 g/kg) before and after HDBR. The glucose load was labeled with ^{13}C and associated with D-[6,6-$^2H_2$]glucose infusion, indirect calorimetry, breath tests, and plasma measurements to determine the glucose turnover and biodisponibility, substrate oxidation, and endocrine responses. Body composition was assessed using $H_2{}^{18}O$ dilution. In addition, hormones were measured in daily blood and 24-h urine samples. No change in body composition was noted. Daily fasting insulin increased during HDBR (men, 34%; women, 26%), as did the insulin to glucose ratio (men, 30%; women, 25%). The normetanephrine level dropped (men, 30%; women, 16%), but metanephrine was unchanged. During OGTTs, the insulin response was increased after HDBR (men, 47%; women, 67%), whereas plasma glucose levels were similar. Nonesterified fatty acids and β-hydroxybutyrate levels were lower. Endogenous glucose production dropped (28%), and exogenous glucose oxidation increased (28%) only in men. Resting energy expenditure was unchanged, but nonproteic respiratory quotient increased (men, 10%; women, 14%). Basal levels of lipid oxidation dropped in both sexes (~90%), but those of carbohydrate oxidation increased in men (40%); as did lipogenesis in women (570%). In response to OGTTs, lipid oxidation was 80% reduced in both sexes after HDBR, but carbohydrate oxidation increased (25%) in men. Lipogenesis occurred in men (304%) and women (74%), but the latter had higher absolute levels. Therefore, 7 days of HDBR resulted in 1) reduced sympathetic activity, 2) insulin resistance suggested at the muscle level in men and at both the muscle and liver levels in women, 3) no changes in glucose biodisponibility, suggesting no alterations in the gastrointestinal function, and 4) a shift toward carbohydrate oxidation in men and a net lipogenesis in women. Such results suggest gender differences in response to sedentary life style and warrant further analysis. (*J Clin Endocrinol Metab* **85:** 2223–2233, 2000)

SEDENTARY lifestyle-triggered physical inactivity has been implicated in the onset of some pathologies such as impaired glucose tolerance, insulin resistance, type 2 diabetes, orthostatic hypotension and obesity (see Refs. 1–3 for review). The deleterious consequences of sedentary behaviors have emerged from the ability of exercise training to prevent or reduce some clinical manifestations of these pathologies. Overall, the basic adaptive mechanisms of inactivity have been poorly studied (1).

A reliable model of physical inactivity is commonly used to mimic human exposure to weightlessness (4). Volunteers are confined to bed in a head-down position of −6° for variable durations. These head-down bed rest (HDBR) experiments reproduce the headward shift of fluids, the hypokinesia, and the hypodynamia observed in space. Among the physiological adaptations to HDBR, fluid loss, cardiovascular dysfunction, muscle atrophy, and bone demineralization are the most important (5). The combined losses of fluid, muscle, and bone affect body composition and mass. Studies by our group (4) and others (6–8) have previously demonstrated that HDBR resulted in a decrease in lean body mass (LBM). Conversely, fat mass (FM) increased (8, 9) or did not increase (4). These changes were observed in a context of a mild metabolic stress but in a state of energy balance, and the enforced physical inactivity achieved during HDBR was mainly responsible for the observed adaptation (4).

Basically, the steady state of weight maintenance requires that the daily oxidation of a fuel mix matches the amount and composition of the nutrients of the diet (10). As mentioned above, energy balance is achieved during bed-rest, and thus, one may expect that body composition changes are linked to alterations in the substrate oxidation balance. This paradigm has been scanty investigated, and only one short-term (11) and one long-term (6) bed rest experiment are available. Fat oxidation has been shown to either increase (11) or decrease (6), and carbohydrate oxidation has been shown to either increase (6) or not change (11). Both studies demonstrated a higher nonproteic respiratory quotient (NPRQ). Even if clear discrepancies exist, these results suggested that the substrate oxidation balance is altered during HDBR. However, the actual mechanisms of adaptation are still under debate. Moreover, the extent to which this alteration is dependent of the endocrine status or other factors, such as gastrointestinal dysfunction, remains to be defined.

Therefore, this study was carried out to investigate the consequences of 7 days of enforced physical inactivity and simulated weightlessness (HDBR) on the relation between

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substrate oxidation and biodisponibility, and body composition. The implication of endocrine status and the gender differences have been studied. Because over the short term the body's carbohydrate economy is a more important determinant of fat oxidation than fat intake and thus of the substrate oxidation balance (10), we choose the paradigm to appraise the metabolic fate of an oral glucose load using stable isotope tracers (D-[6,6-$^2H_2$]glucose and naturally labeled [^{13}C]glucose) to assess the study's aims. The objectives were a better understanding of the mechanisms involved in 1) the body composition changes occurring during simulated weightlessness and 2) the deleterious adaptations to physical inactivity.

Subjects and Methods

Subjects

A group of 16 individuals (8 men and 8 women), whose physical characteristics are presented in Table 1, served as subjects in this study. All were in a good state of general health on the basis of medical history, physical and psychological examination, and routine urine and blood biochemical screening. None had a family history of diabetes mellitus or gastrointestinal disease or was taking any medication for 3 months before the study. Within the same period, women were required to stop oral contraception. The subjects were fully informed of the purpose and potential risks of the experimental protocol. They gave their written consent according to the Huriert law before entering the study.

Volunteers were fed with conventional foods calculated to ensure 146 kJ/kg·day of initial body mass. Dietary records were kept for calculation of nutrient intakes using the Regal software (GENI, Micro 6, Nancy, France) and the French Food Tables (12). A senior dietitian ensured the accuracy of the diet records. Dietary sodium was limited to 3 g/day and water intake to 2.5 L/day. No medication, smoking, alcohol, or caffeinated drinks were allowed during the study, and no extra food was allowed between the three daily meals taken at control times.

Materials

$H_2{}^{18}O$ (2% and 10%) was obtained from Isotec (St. Quentin en Yvelines, France). The labeled water was sterilized before the subjects drank it. Naturally enriched [^{13}C]glucose (pure maize glucose) was obtained from Aguettant (Lyon, France). D-[6,6-$^2H_2$]Glucose (99% mol excess) was obtained from Commissariat à l'Energie Atomique (Gif-sur-Yvette, France). Chemical and isotopic purity was confirmed by gas chromatography-selected ion monitoring mass spectrometry analysis. It was dissolved in sterile isotonic saline (9 g/L) and passed through a 0.22-μm pore size filter (Millipore Corp., Bedford, MA) before infusion. The preparation was pyrogen free. Its actual concentration was determined at the end of each test.

Experimental protocol

General scheme. The experimental schedule, resumed in Fig. 1, was broken down into three periods. A control period of 4 days was followed by 7 days of HDBR at −6° and then 4 days of a recovery period. During the first and last periods, the subjects remained in the bed rest facility at MEDES, Clinique de l'Espace (CHU Rangueuil, Toulouse, France).

Body composition was determined by $H_2{}^{18}O$ dilution on control day −4 and HDBR day 6. On the same days, an oral glucose tolerance test (OGTT) was performed as described below. Hormone levels were also determined in daily blood samples during the fasting state in men, whereas in women, blood samples were realized every 2 days. The daily 24-h urine was collected for assessment of hormones and metabolites. The subjects were weighed daily in the supine position with a special weighing machine.

Daily endocrine and metabolite measurements. Insulin and glucose were assayed daily in men and every 2 days in women in the fasting state at 0700 h. The 24-h urine samples were pooled each day for determination of normetanephrine (NMN), metanephrine (MN), and cortisol. Additionally, the days of OGTT, urea, and creatinine levels were assessed. Plasma and urine samples were stored at −80 C for further analysis.

Body composition. The subjects were awakened at 0500 h, allowed to void, and then were weighed. After collection of baseline saliva samples, subjects drank 0.9 g/kg $H_2{}^{18}O$ 2% on control day −4 and 0.7 g/kg $H_2{}^{18}O$ 10% on HDBR day 6. Two different enrichments were used to overcome differences in the background enrichments due to the first probe. Then, salivary samples were collected 3, 4, 5, and 6 h after the ingestion of labeled water to optimize the equilibration plateau determination of isotope with body fluids. After a 10-min centrifugation at 6000 rpm, samples were stored at −80 C in cryogenically stable tubes until analysis.

OGTT and substrate oxidation. All OGTTs were performed in the postabsorptive state 12 h after the last evening meal. Intravenous catheters were inserted into forearm veins in each arm for D-[6,6-$^2H_2$]glucose infusion on one side and blood sampling on the other side. A continuous infusion of D-[6,6-$^2H_2$]glucose (0.06 mg/kg·min) was used for the determination of glucose turnover rates before and after glucose absorption. A priming bolus was realized at 80 times the infusion rate over 1 min.

After 150 min of D-[6,6-$^2H_2$]glucose equilibration, the subjects ingested 1 g/kg [^{13}C]glucose. As shown in Fig. 1, blood samples were sequentially collected each 15 or 30 min during 5 h after the ingestion to determine metabolites [nonesterified fatty acids (NEFA), glucose, and β-hydroxybutyrate], hormones [insulin, epinephrine (E), norepinephrine (NE), and GH], and isotopic enrichments (D-[6,6 $^2H_2$]glucose and [^{13}C]glucose). Blood was centrifuged immediately at 3500 rpm for 10 min after being drawn, and plasma was then separated and frozen at −80 C until analysis.

An open circuit indirect calorimeter (Deltatrac Metabolic Monitor, Datex Corp., Helsinki, Finland) measured the respiratory gas exchanges for 6 h (one in the basal state and five after the oral glucose load). It was hourly stopped to realize breath samples for measurements of ^{13}C enrichments in the expired CO_2. Expired gas samples were collected in a

TABLE 1. Anthropometric data and body composition

	Units	Men (n = 8)		Women (n = 8)	
		Control d-4	HDBR d+6	Control d-4	HDBR d+6
Age	yr	32.4 ± 9		27.9 ± 0.9	
Ht	m	1.77 ± 0.99		1.64 ± 0.01	
BMI	kg/m^2	23.9 ± 0.7	23.9 ± 0.9	20.9 ± 0.6	20.8 ± 0.6
Body mass	kg	74.8 ± 2.8	75.1 ± 2.7	56.3 ± 2.0	56.0 ± 2.0
Total water	L	44.9 ± 1.4	44.5 ± 1.3	30.1 ± 1.1	29.2 ± 1.0
	%	60.2 ± 0.9	59.5 ± 1.0	53.7 ± 2.1	52.2 ± 1.1
Lean mass	kg	61.4 ± 2.0	60.8 ± 1.8	41.1 ± 1.5	39.9 ± 1.4
	%	82.3 ± 1.2	81.2 ± 1.4	73.4 ± 2.8	71.3 ± 1.6
Fat mass	kg	13.4 ± 1.2	14.3 ± 1.4	15.2 ± 2.0	16.2 ± 1.2
	%	17.7 ± 1.2	18.7 ± 1.4	26.6 ± 2.8	28.7 ± 1.6

BMI, Body mass index. %, Mean percentage of individual body masses. No significant differences were triggered by RM-ANOVA.

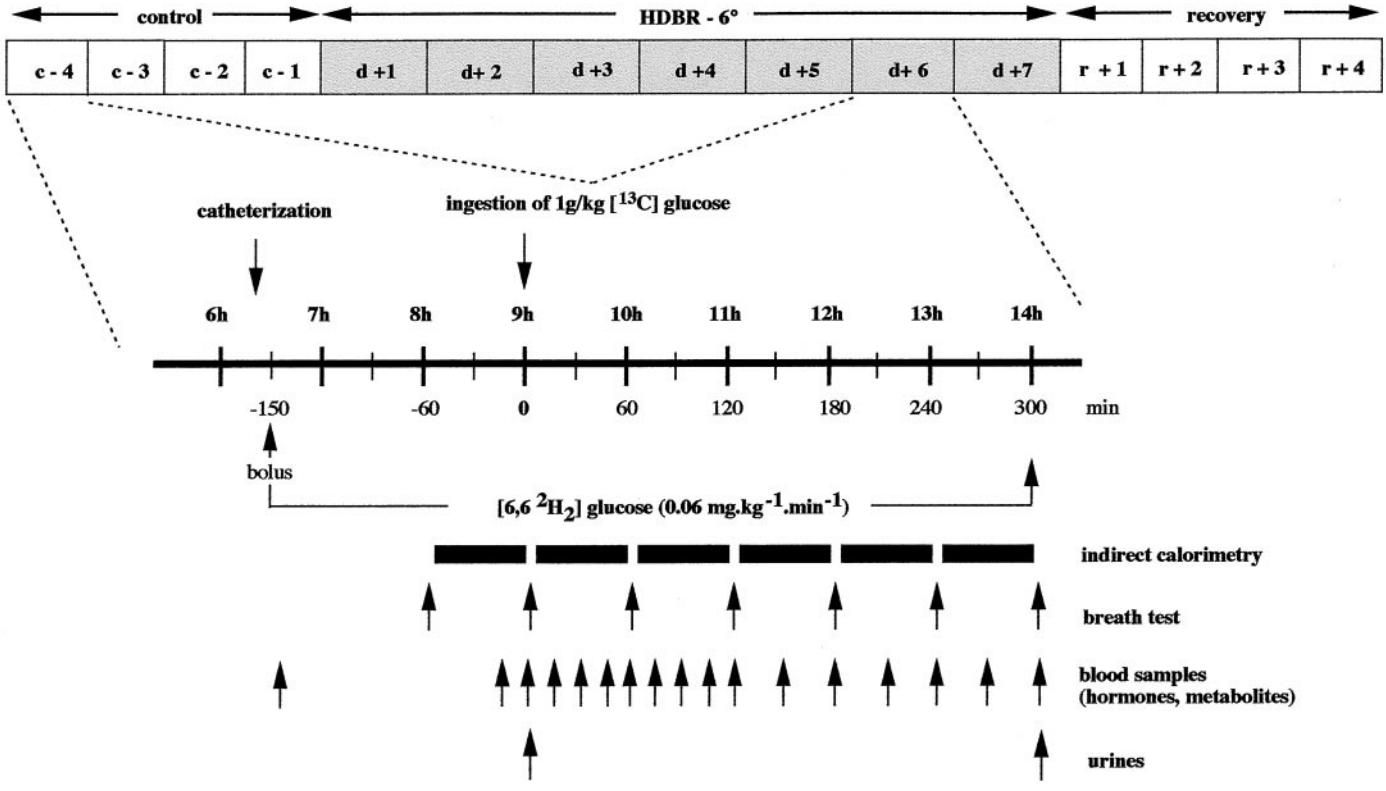

FIG. 1. Experimental schedule of the OGTTs.

5-L Douglas bag, and two aliquots were immediately transferred into 10-mL Vacutainers (Becton Dickinson and Co., Rutherford, NJ). Urine samples were collected before and during the test to determine nitrogen excretion. During the measurements, subjects were allowed to watch television, read, and listen to the radio while under constant supervision of one of the investigators; at no time were they allowed to sleep.

Analytical procedures

Isotope analysis. $H_2{}^{18}O$ enrichments were measured using isotope ratio mass spectrometry (OPTIMA, Fisons, UK), as previously described (4). Plasma glucose isotope enrichments were determined on neutral fractions of deproteinized plasma samples partially purified over sequential anion cation exchange resins, as described previously (13, 14). Plasma D-[6,6-$^2H_2$]glucose was measured by gas chromatography-organic mass spectrometry (model 5890, Hewlett-Packard Co., Evry, France) using an electron impact mode and a selective monitoring of m/z 297 and 299 after derivation to acetyl-bis-butane-boronyl glucose (15). Plasma [^{13}C]glucose enrichment was assessed by gas chromatography-combustion-isotope ratio mass spectrometry (SIRA 10 VG Isogas, Middlewich, UK) after derivation to pentaacetyl glucose as previously described (13, 14). Plasma glucose isotopic enrichments were calculated *vs.* plasma glucose determined before the infusion of deutered glucose and the ingestion of ^{13}C-labeled glucose. The ^{13}C enrichment of ingested glucose was determined using an isotope ratio mass spectrometer (SIRA 10, VG Isogas) on-line with an elemental nitrogen and carbon analyzer (NA 1500, Carlo Erba, Massy, France) as previously described (14).

Breath sample analysis. The ^{13}C enrichment of expired CO_2 was determined on an isotope ratio mass spectrometer (SIRA 10, VG Isogas) as previously described (16). Breath CO_2 isotopic enrichments were calculated *vs.* breath CO_2 obtained before labeled glucose ingestion.

Hormones, metabolites, and urinary nitrogen. Classical and widely described methods were used. Insulin was determined by RIA (17); E, NE, MN, and NMN were determined by HPLC with electrochemical detection (18); glucose and NEFA were determined by enzymatic methods (19); urinary nitrogen was determined by chemiluminescence (Antek 703C, Sopares, Paris, France); urinary urea was determined by UREA-KIT S (sensitivity, 0.15 g/L; Laboratoires BioMerieux, Lyon, France); and urinary creatinine was determined by the colorimetric method of Jaffe. The GH assay was performed using a GH immunoradiometric assay kit (Immunotech, Marseilles, France) with a sensitivity below 0.05 μg/L and intra- and interassay coefficients of variation of 1.15% and 1.35%, respectively. Urinary cortisol measurements were made by high performance liquid chromatography with UV detection using a classical method (20) modified in the laboratory (in these conditions, the intra- and interassay coefficients of variation were 1.97% and 6.98%, respectively, and the sensitivity was <1 ng/mL).

Calculations

Body composition. Total body water (TBW) was determined from the dilution space of oxygen-18 after adjusting it by a factor of 1.01 to account for isotope exchange as previously described (4). It has been reported that the hydration of LBM is unchanged (73.2%) during the head-down tilt position (4). Therefore, LBM and FM were deduced from TBW and body mass.

Glucose turnover. The rates of glucose appearance (Ra) and disappearance (Rd) were calculated from plasma D-[6,6-$^2H_2$]glucose enrichment (RaT and RdT; T for total glucose) and from plasma [^{13}C]glucose enrichment (RaE and RdE; E for exogenous glucose), using Steele's equation for nonsteady state (21, 22), as previously described (13). The glucose distribution space was taken as 0.2 L/kg, and the pool fraction as 0.75. Endogenous glucose production was calculated as RaT − RaE.

Concerning the determination of ^{13}C enrichment in the expired CO_2, special considerations were assumed. Effectively, in the steady state, $^{13}CO_2$ production is calculated as: $V^{13}CO_2$ (L/min) = $VCO_2 \times$ APE $^{13}CO_2$, where VCO_2 (liters per min) is the CO_2 production determined by indirect calorimetry, and APE $^{13}CO_2$ is ^{13}C enrichment in expired gas, expressed as the atom percent excess compared to baseline. In fact, the actual production of $^{13}CO_2$ must be calculated in nonsteady state conditions by taking into account the changes in the [^{13}C]bicarbonate/CO_2 pool with time and the incomplete $^{13}CO_2$ recovery in expired gas (23).

Taken together these conditions yield: $V^{13}CO_2 = \{[VCO_2 \times (APE_1\ ^{13}CO_2 + APE_2\ ^{13}CO_2)/2] + [0.318 \times BW \times (APE_2\ ^{13}CO_2 - APE_1\ ^{13}CO_2)/(t_2 - t_1)]\} \times 1/R$, where APE_1 and APE_2 are the ^{13}C enrichment in expired gas at times t_1 and t_2, 0.318 is the estimated mass of CO_2 in the organism (liters per kg), BW is the body weight (kilograms), and R is the $^{13}CO_2$ recovery in expired gas taken as 0.733 (23). VCO_2 (liters per min) was averaged every 60 min.

Then, the $[^{13}C]$ glucose oxidation was calculated as: $[^{13}C]glucose_{oxidized}$ (g/min) = $[(V^{13}CO_2(t) \times 180)/(22.4 \times 6)] \times (1/APE([^{13}C]glucose_{ingested})$, where $V^{13}CO_2$ is the actual $^{13}CO_2$ production (liters per min) at time t, 180 is the molecular weight of glucose, 22.4 is used for the conversion of liters into moles of CO_2, 6 is the number of carbon atoms per molecule of glucose, and APE ($[^{13}C]glucose_{ingested}$) is the ^{13}C enrichment of ingested glucose.

Substrate oxidation. The resting metabolic rate was calculated as the mean of the hourly indirect calorimetry measurements. The thermic effect was calculated by dividing the suprabasal energy expenditure by the energy content of the ingested glucose load, expressed as a percentage. The urinary nitrogen values (determined before and during the OGTT) were used to calculate the NPRQs during the test. Carbohydrate, fat, and protein oxidation rates were calculated from the oxygen consumed and the carbon dioxide produced after correction for changes in the urea nitrogen pool. Calculations were performed according to the method of Acheson *et al.* (24). When lipogenesis occurred (total lipid oxidation <0), other equations were used to determine the total carbohydrate oxidation (25).

Statistical analysis

Each subject acted as his own control, with the pre-HDBR ambulatory period taken as the control period. A two-way repeated measure ANOVA (RM-ANOVA) was used to detect any differences between time and gender. Fisher's protected least significant differences test was used for *post-hoc* comparisons. All analyses were performed with StatView (1992, Abacus Concepts, Inc., Berkeley, CA), and reported values are the mean ± SEM, with $P < 0.05$ considered statistically significant.

Results

To clarify this section, the F and P values of the RM-ANOVA tests are mentioned only when $P < 0.05$ and when relevant for the purpose of the study. In the same way, the classical and well described basal gender differences in hormones and metabolites, mainly attributed to FM, are not detailed.

Energy intake, body weight, and composition

The body mass, TBW, LBM, and FM, expressed either in kilograms or as a percentage of body weight, were unaltered during HDBR (Table 1). We did not observed any change in either energy intake or the macronutrient composition of the diet (Table 2).

Daily hormone and metabolite measurements

The daily fasted plasma levels of insulin and glucose and the insulin to glucose ratio are presented in Table 3. Bed rest resulted in an increased insulin levels in both men (34%; F = 7.362; $P = 0.0065$) and women (26%; F = 4.465; $P = 0.0355$). No normalization was noted during the recovery period. Glucose levels were not modified in men, and a slight, but significant, hypoglycemia was noted in women (7%; F = 21.424; $P = 0.0002$). Consequently, the insulin to glucose ratio followed the insulin pattern, with a higher ratio during both HDBR and the recovery period (30% in men: F = 5.895; $P = 0.0139$; 25% in women: F = 3.852; $P = 0.0500$).

The urinary excretion rates of NMN, MN, and cortisol are shown in Table 3. NMN was decreased in both men (30%; F = 3.773; $P = 0.0489$) and women (16%; F = 11.298; $P = 0.0012$) during HDBR, and the level returned to control values during the recovery period. MN did not change throughout the experiment in either men or women. Excretion of cortisol was unaltered by HDBR in men, but a significant effect was observed in women (F = 4.609; $P = 0.0290$). However, in these later, the PLSD Fisher's test raised evidence that cortisol excretion decreased between the control and recovery periods. Thus, we can conclude that HDBR did not induce significant changes in urinary excretion of cortisol.

OGTT results

Hormones and metabolites. The results presented below are compared with the area under curve (AUC) integrated over the 300 min after the oral glucose load.

Insulin and glucose: The time courses of insulin and glucose are presented in Fig. 2. The AUCs showed that insulin secretion was higher in response to the glucose load on HDBR day 6 compared to control day −4 in both men (48%; F = 7.215; $P = 0.0313$) and women (63%; F = 5.915; $P = 0.0453$). The glucose response was unchanged, but the insulin to glucose ratio was significantly higher after 6 days of HDBR in both men [from 5,594 ± 1,037 (control day −4) to 7,841 ± 865 (HDBR day 6); F = 7.875; $P = 0.0263$] and women [from

TABLE 2. Dietary intake

Nutrients	Men (n = 8)			Women (n =8)		
	Control	HDBR	Recovery	Control	HDBR	Recovery
Energy intake						
MJ/day	10.5 ± 0.2	10.3 ± 0.3	10.3 ± 0.3	7.9 ± 0.3	7.9 ± 0.3	8.1 ± 0.4
Water						
g/day	3098 ± 91	2861 ± 66	2908 ± 94	1604 ± 246	1248 ± 158	1625 ± 195
Carbohydrate						
g/day	321 ± 7	312 ± 5	316 ± 6	243 ± 10	232 ± 13	243 ± 12
%	51 ± 1	51 ± 1	51 ± 0	52 ± 1	49 ± 1	51 ± 1
Fat						
g/day	84 ± 2	86 ± 2	86 ± 2	68 ± 3	69 ± 13	71 ± 4
%	31 ± 0	32 ± 1	31 ± 1	32 ± 1	33 ± 1	33 ± 1
Protein						
g/day	114 ± 2	109 ± 2	109 ± 3	79 ± 6	85 ± 5	81 ± 5
%	19 ± 0	18 ± 0	18 ± 0	16 ± 1	18 ± 1	16 ± 1

%, Percentage of daily energy intake.

TABLE 3. Daily hormones and metabolites

	Units	Men (n = 8)			Women (n = 8)		
		Control	HDBR	Recovery	Control	HDBR	Recovery
Plasma							
Insulin	pmol/L	36.2 ± 4.1	48.5 ± 6.3[a]	48.2 ± 6.8[a]	55.6 ± 14.8	70.3 ± 15.3[a]	68.9 ± 9.3
Glucose	mmol/L	5.02 ± 0.08	4.92 ± 0.07	4.86 ± 0.07	4.69 ± 0.08	4.38 ± 0.10[a]	4.41 ± 0.06[a]
Ratio		7.40 ± 0.83	9.65 ± 1.35[a]	9.73 ± 1.42	11.95 ± 2.38	14.92 ± 2.63[a]	13.59 ± 2.41
24-h urinary							
Normetanephrine	μg/day	271 ± 30	191 ± 16[a]	251 ± 19[b]	256 ± 26	215 ± 16[a]	279 ± 25[b]
Metanephrine	μg/day	200 ± 22	183 ± 13	190 ± 16	151 ± 29	147 ± 30	149 ± 25
Cortisol	nmol/day	89 ± 14	61 ± 20	67 ± 15	74 ± 16	65 ± 14	50 ± 8[a]

Significance was determined by RM-ANOVA PLSD Fisher's test.
[a] $P < 0.05$ *vs.* control period.
[b] $P < 0.05$ *vs.* HDBR period.

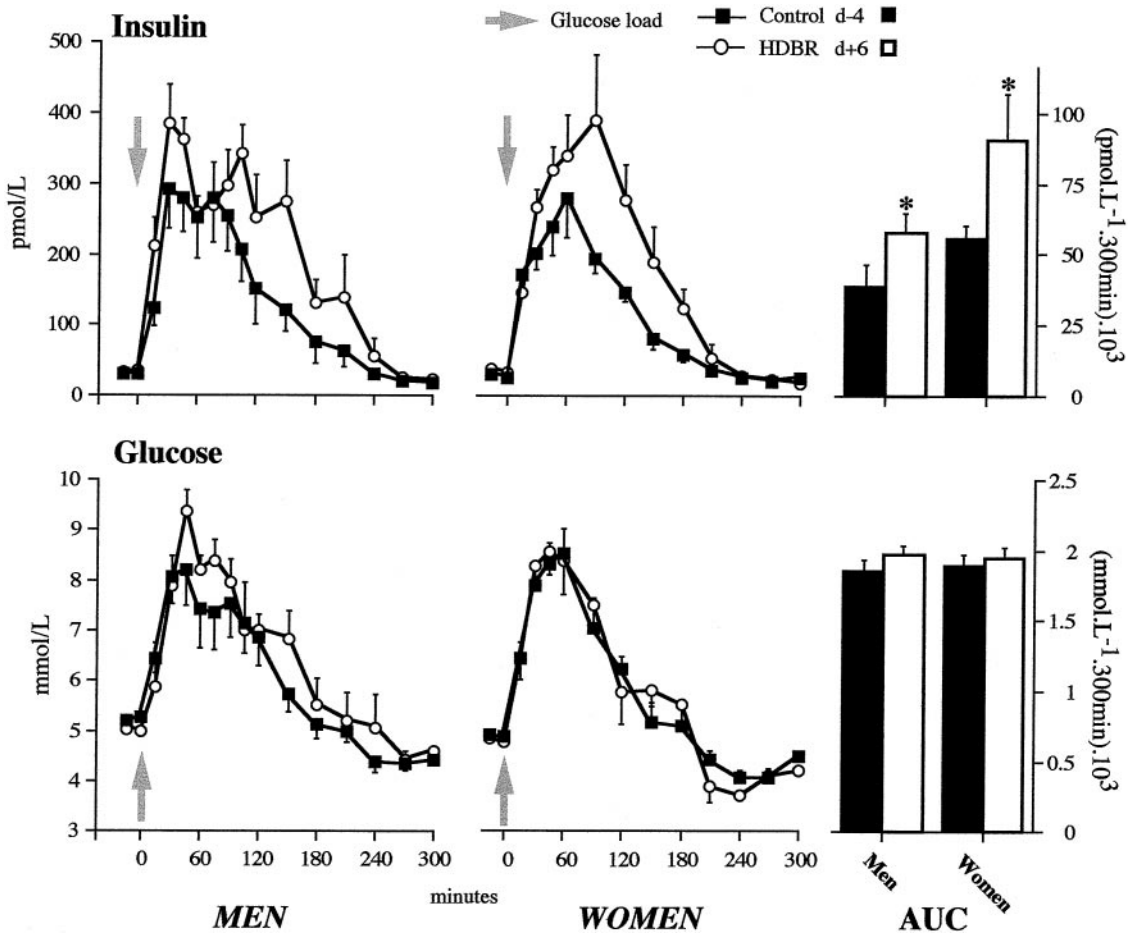

FIG. 2. Time course of insulin and glucose during the OGTTs. RM-ANOVA protected least significant difference Fisher's test results: *, $P < 0.05$ *vs.* control day −4 OGTT; $, $P < 0.05$ *vs.* AUC in men on the same day. n = 8 in each group.

8,439 ± 578 (control day −4) to 14,421 ± 3,195 (HDBR day 6); F = 26.988; $P = 0.0020$].

NEFA and β-hydroxybutyrate: NEFA AUCs decreased in both sexes after 6 days of HDBR (men: 41%; F = 18.255; $P = 0.0037$; women: 23%; F = 6.134; $P = 0.0424$; Fig. 3). β-Hydroxybutyrate decreased in both sexes, but the level of significance was only reached in men (47%; F = 7.069; $P = 0.0325$).

NE and E: Integration of NE and E AUCs showed scattered changes due to HDBR. The NE response to the glucose load decreased in men (from 94 ± 6 ng/mL·300 min on control day −4 to 77 ± 4 ng/mL·300 min on HDBR day 6; F = 9.114; $P = 0.0194$), but not in women (from 103 ± 6 ng/mL·300 min on control day −4 to 101 ± 10 ng/mL·300 min on HDBR day 6). Conversely, E was unchanged in both sexes: from 12 ± 1 to 13 ± 1 ng/mL·300 min in women and from 20 ± 2 to 18 ± 2 ng/mL·300 min in men.

GH: GH responses to the glucose load were similar on the control day −4 and HDBR day 6 in both men (respectively, 1.3 ± 0.5 and 0.9 ± 0.4 mg/mL·300 min) and women (respectively, 1.4 ± 0.3 and 0.8 ± 0.2 mg/mL·300 min).

24-h urinary excretion of urea and creatinine: Bed rest induced

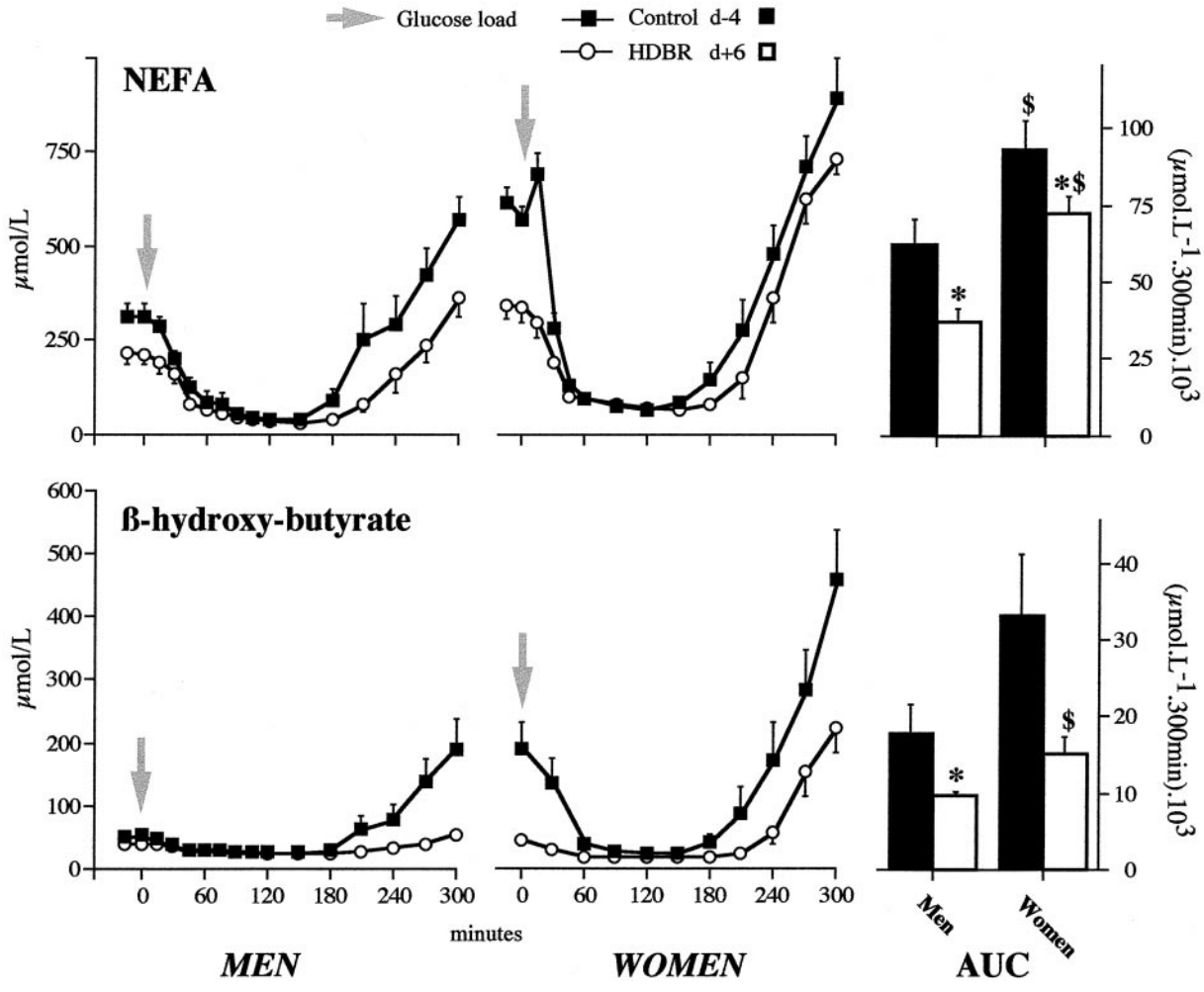

FIG. 3. Time course of NEFA and β-hydroxybutyrate during the OGTTs. RM-ANOVA protected least significant difference Fisher's test results: *, $P < 0.05$ *vs.* control day −4 OGTT; $, $P < 0.05$ *vs.* AUC in men on the same day. n = 8 in each group.

an increase in the urinary excretion rate of creatinine in both men [1205 ± 77 mg/day (control day −4) to 2395 ± 98 mg/day (HDBR day 6); F = 190.836; $P < 0.0001$] and women [1050 ± 119 mg/day (control day −4) to 1436 ± 56 mg/day (HDBR day 6); F = 13.377; $P = 0.0106$]. Conversely, urea excretion was unaltered by HDBR in men [27 ± 8 g/day (control day −4) to 36 ± 3 g/day (HDBR day 6)], but was significantly increased in women [16 ± 3 g/day (control day −4) to 27 ± 2 g/day (HDBR day 6); F = 23.389; $P = 0.0029$].

Resting energy expenditure, diet-induced thermogenesis (DIT), and NPRQ. The resting energy expenditures (Fig. 4) were unchanged by HDBR in both sexes during the glucose load despite being close to the level of significance in women (F = 3.442; $P = 0.0600$). Therefore, DIT was increased after HDBR in women [4.1 ± 1.2% (control day −4); 7.6 ± 1.6% (HDBR day 6); F = 5.591; $P = 0.0500$], but not in men (2.9 ± 1.7% on control day −4; 7.1 ± 2.7% on HDBR day 6). The NPRQ (Fig. 4) was higher after HDBR under basal conditions in both sexes [men: from 0.906 on control day −4 to 1.004 on HDBR day 6 (F = 24.681; $P = 0.0016$); women: from 0.969 on control day −4 to 1.101 on HDBR day 6 (F = 16.579; $P = 0.0046$)]. This difference was maintained during the OGTT.

Substrate oxidation. The results related to the substrate oxidation were compared both at the basal level and during the OGTTs from the AUCs integrated over the 300 min of the test (Fig. 5).

Carbohydrates: The basal levels of carbohydrate oxidation were increased in men after bed rest (40%; F = 6.840; $P = 0.346$), but not in women. This gender difference was maintained during the OGTT after simulated microgravity: carbohydrate oxidation increased in men (25%; F = 12.559; $P = 0.0094$), but was unchanged in women.

Lipids: After bed rest, the basal levels of lipid oxidation were strongly reduced in both sexes (men: 90%; F = 12.503; $P = 0.0095$; women: 93%; F = 9.082; $P = 0.0192$) as during the OGTTs (men: 84%; F = 11.014; $P = 0.0128$; women: 80%; F = 10.046; $P = 0.0157$). The basal levels of lipogenesis were higher after bed rest in women (570%; F = 11.791; $P = 0.0109$), but not in men. However, in response to the HDBR OGTT, lipogenesis increased significantly in men (304%; F = 28.992; $P = 0.0010$) and women (74%; F = 17.240; $P = 0.0043$).

Proteins: In women, protein oxidation (Table 4) increased during the HDBR day 6 OGTT (22%; F = 5.598; $P = 0.0499$), but was unchanged in men.

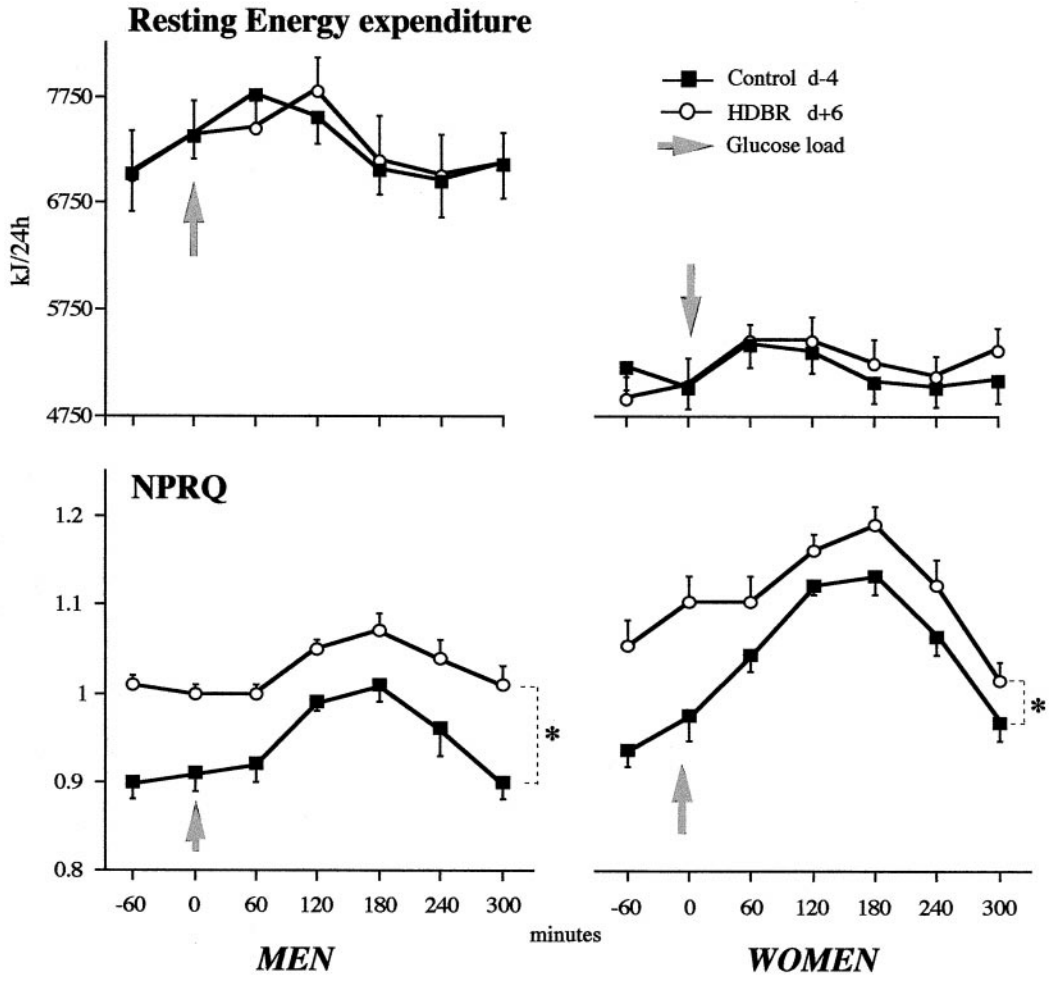

FIG. 4. Time course of resting energy expenditure and NPRQ during the OGTTs. RM-ANOVA results: *, $P < 0.05$ *vs.* control day −4 OGTT. n = 8 in each group.

Glucose metabolism. The effects of HDBR on glucose turnover are presented in Table 4. The results are expressed as grams of substrate appearing into, disappearing from, or oxidized into the system during the 5-h OGTTs. Bed rest-induced changes in glucose turnover were only observed in men. Exogenous glucose oxidation was increased (39%; F = 8.505; $P = 0.0225$) during the OGTT performed after HDBR compared to the OGTT conducted during the control period, as was the total glucose oxidation above detailed. The endogenous glucose production was more suppressed after HDBR in men (28%; F = 19.940; $P = 0.0029$) than in women, in whom no change was noted.

Discussion

In this study we used 7 days of HDBR as a model of enforced physical inactivity and simulated weightlessness. Keeping in mind that simulated weightlessness induces alterations in body composition in a state of energy balance, we determined the implication of an altered substrate oxidation balance in these changes, with special attention to endocrine status and gastrointestinal function. The purposes of the study were evaluated during an OGTT using stable molecules of glucose, indirect calorimetry, and breath tests.

Body mass and composition

Body composition was similar before and after the bed rest in both sexes. During longer bed rest, our group (4) observed a decrease in LBM without an increase in FM. We specifically demonstrated using dual x-ray absorptiometry that LBM was lost from and FM was redistributed into trunk and legs. However, the isotope dilution technique measured the whole body composition, and such regional changes cannot be excluded. Effectively, for the same HDBR duration and using magnetic resonance imaging, Shangraw *et al.* (7) observed that protein mass was lost from calves and thighs, but FM was unchanged.

Daily hormonal responses

The insulin to glucose ratio, an indirect index of the development of a resistance to the metabolic effect of insulin, increased in both sexes. Such changes have been previously observed. Vernikos-Danellis *et al.* (26) reported insulin and glucose patterns in five normal subjects during 56 days of bed rest. They found no changes in fasting glucose, but basal insulin secretion doubled. Similarly, Acheson *et al.* (11) observed an increased level of fasting insulin after 3 days of

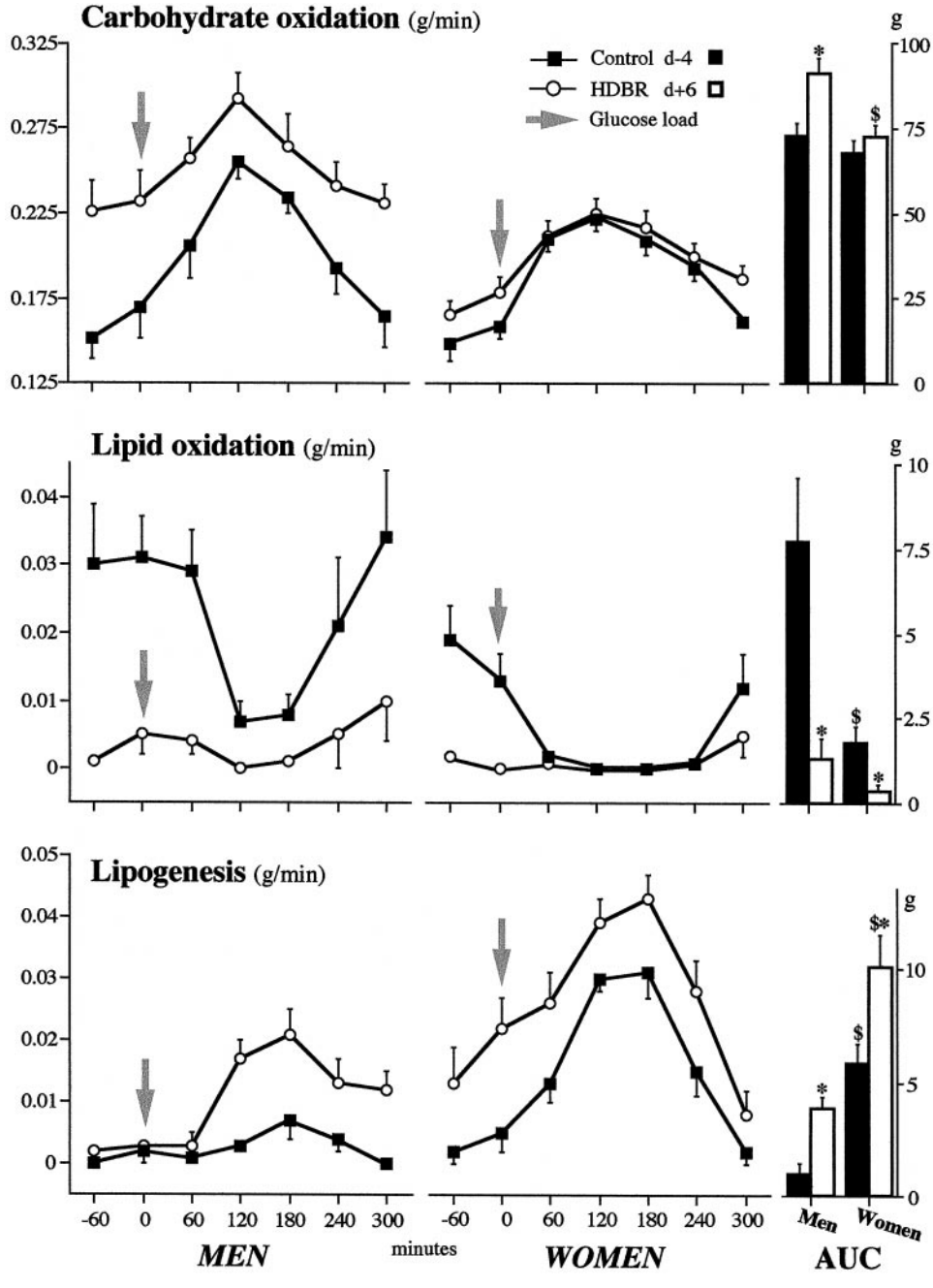

FIG. 5. Time course of carbohydrate and lipid oxidation and lipogenesis during the OGTTs. RM-ANOVA protected least Fisher's test results: *, $P < 0.05$ *vs.* control day −4 OGTT; $, $P < 0.05$ *vs.* AUC in men on the same day. n = 8 in each group.

HDBR. Some studies determined the levels of insulin counterregulatory hormones during HDBR. Vernikos-Danellis *et al.* (26) found a triphasic response of GH during extended bed rest; it first decreased, then increased during the 56-day protocol and later declined to below control levels. These researchers also found increased T_3 and decreased daily plasma cortisol during 56 days of bed rest (27). During a 42-day bed rest, we observed that both cortisol and GH urinary excretion increased (4). Urinary excretion of cortisol, like that of GH, did not change during the present study. The data reported by Stuart *et al.* (28) during a 7-day bed rest showed an augmentation of glucagon and cortisol, but little or no change in epinephrine or GH. As the urinary excretion rate of norepinephrine can be considered an indirect index of sympathetic nervous system activity, we actually found a reduction in activity during this study. Therefore, OGTTs were performed in a state of lower sympathetic activity, as previously observed during HDBR (4). Adrenomedullary system activity, assessed by the urinary excretion of MN, was not modified in either sex throughout the experiment, suggesting that the subjects were not stressed.

In conclusion, there have been scattered reports of bed rest-induced hormonal changes, but data other than those for insulin and NMN are scant. However, it is possible that these bed rest-associated hormonal changes might contribute to the observed insulin resistance, but the changes may also be a compensatory response to the hyperinsulinemia and insulin resistance.

TABLE 4. Glucose turnover and substrate oxidation after 1 g/kg [^{13}C]glucose load

Variables (g/5 h)	Men (n = 8)		Women (n = 8)	
	Control d-4	HDBR d+6	Control d-4	HDBR d+6
Glucose ingested	74.5 ± 3.1	75.1 ± 2.9	56.2 ± 2.2	55.7 ± 2.1
Total glucose appearance	94.3 ± 6.7	91.4 ± 2.9	70.6 ± 4.5	65.6 ± 3.9
Exogenous glucose appearance	64.1 ± 4.7	76.7 ± 3.6	52.4 ± 4.2	52.3 ± 4.0
Endogenous glucose production	32.0 ± 3.4	23.1 ± 2.7[a]	19.3 ± 2.4	14.1 ± 2.3
Total glucose disappearance	95.9 ± 6.6	92.6 ± 2.7	71.2 ± 4.4	66.6 ± 3.9
Exogenous glucose disappearance	55.9 ± 4.6	65.4 ± 3.5	45.6 ± 3.5	45.7 ± 3.6
Exogenous glucose oxidation	34.6 ± 2.9	47.6 ± 3.7[a]	32.3 ± 1.4	36.7 ± 3.1
Total carbohydrate oxidation	66.4 ± 3.5	83.8 ± 4.2[a]	62.5 ± 1.3	66.5 ± 2.9
Total lipid oxidation	9.6 ± 1.9	1.3 ± 0.8[a]	1.2 ± 0.4	0.4 ± 0.2[a]
Total protein oxidation	19.3 ± 1.8	20.0 ± 1.4	10.9 ± 0.9	13.4 ± 0.7[a]

[a] $P < 0.05$ *vs.* control d-4, by RM-ANOVA.

Glucose biodispondibility

Very few data are available concerning gastrointestinal function during actual or simulated weightlessness. However, this is of importance because overall nutritional success involves gastrointestinal function. If ingested nutrient digestion and absorption are altered, nutrition may be inadequate. There appear to be significant chronological changes in gastrointestinal function early during flight, some of which appear to be related to space motion sickness. Thornton *et al.* (referenced in Ref. 29) reported that gastric motility decreased early during spaceflight. This change in motility may, in turn, modify gastric emptying and rate of absorption. The protocol used in this study using a glucose load naturally enriched in ^{13}C allowed us to determine the glucose biodisponibility after HDBR. We did not observe any change in the kinetics of the exogenous glucose appearance, thus suggesting that gastric emptying is not altered during bed rest. Similarly, the quantity of [^{13}C]glucose appearing in blood was similar before and after bed rest in both sexes. Thus, gastrointestinal function is normal during simulated microgravity conditions.

Glucose turnover

There is clear evidence from the OGTT results that both men and women develop insulin resistance during HDBR. There was a 48% increase in men and a 63% increase in women in the insulin response to the glucose load. Interestingly, in men, there was no alteration by HDBR of insulin suppression of hepatic glucose output, indicating that the insulin resistance occurs primarily in musculature. Conversely, in women, endogenous glucose production suppression was not changed despite higher insulin levels, suggesting that insulin resistance occurred at both hepatic and muscle levels. In support of the idea of muscle insulin resistance, a recent study demonstrated that 20 days of bed rest induced a decrease in muscle GLUT-4 transporters (30). However, the mechanisms underlying such gender differences are not clear and warrant further analysis. Men have greater muscle mass than women, but we do not think that such a difference is implied in our results, firstly because the glucose load was corrected for body mass (1 g/kg) and secondly because Davis *et al.* (31) did not observe gender effects of exercise on glucose turnover.

There is no previous report of glucose metabolism response to inactivity in women. Glucose intolerance has only been observed during prolonged bed rest in men. Lipman *et al.* (32) showed that a 50% decrease in forearm glucose uptake could be demonstrated as early as after 3 days of bed rest and found that bed rest resulted in higher glucose and insulin secretion after an oral glucose challenge. Conversely, after 3 days of bed rest Acheson *et al.* (11) did not observe changes in insulin and glucose concentrations after a 1 g/kg glucose load. Two euglycemic hyperinsulinemic clamp studies conducted during 7–10 days of bed rest but in the horizontal position demonstrated that the decrease in physical muscular activity during bed rest resulted in resistance to the effects of insulin on whole body glucose utilization and that insulin resistance occurred primarily in muscle without alterations in insulin regulation of hepatic production (28, 33). Dolkas *et al.* (34) demonstrated that the increased glucose and insulin responses induced by bed rest after an oral glucose load were returned to control values by physical exercise in excess of 1000 Cal/day, suggesting that physical conditioning can ameliorate the insulin resistance induced by bed rest. Although physical exercise prevents the abnormal glucose tolerance to bed rest, it is also possible that these observations are related to blood flow and muscle perfusion induced by exercise. As one of the principal mechanisms of glucose disposal, after ingestion of a glucose load, is peripheral muscle uptake, anything interfering with muscle perfusion might also alter glucose uptake. Interestingly, resistance to insulin's action to stimulate glucose metabolism, which characterizes the clinical state of obesity, type 2 diabetes, and hypertension, is associated with impaired insulin-mediated vasodilatation (35). HDBR and detraining are known to reduce leg blood flow, and athletes are characterized by enhanced muscle flow and glucose uptake (36). Therefore, the insulin resistance developed during bed rest may alter the insulin-induced increase in leg blood flow and, in turn, perturb the glucose uptake. This warrants further analysis.

Thus, available evidence from this inactivity study supports and extends the view derived from studies of acute exercise (37), training (38), and detraining (39) that physical activity increases insulin action in the whole body by enhancing its effect on muscle. However, although it has been suggested that the insulin sensitivity of hepatic glucose production varies directly with the level of inactivity (37–39), according to the present study it does not change during HDBR, at least in part in men.

Substrate utilization

For a condition of energy balance, the macronutrient of the diet must equal the fuel mix oxidized by the body (10). The body's regulatory mechanisms are capable of efficiently adjusting carbohydrate oxidation to intake, whereas the capacity to regulate short-term fat balance is limited. Adjustment of fat oxidation to fat intake may only occur over extended periods by changes in body fat and subsequent changes in circulating NEFA and fat oxidation. Short-term fat oxidation is merely dependent on factors related to carbohydrate metabolism, such as glycogen stores, rather than on fat intake (10). This is why we chose the paradigm to study the substrate oxidation balance after an oral glucose load. The main results were strongly gender dependent. After the HDBR and in the basal state, *i.e.* before the OGTTs, carbohydrate oxidation was 50% increased, whereas it was unchanged in women, and lipid oxidation dropped to zero in both sexes. Bed rest did not modify the basal level of lipogenesis in men, but induced a 570% increase in women. By comparing the OGTTs performed before and at the end of HDBR, we observed a 25% net increase in carbohydrate oxidation in men. Lipid oxidation was 80% reduced after HDBR in both sexes. The reduction was greater in men, but after HDBR levels of fat oxidation were approximately zero. Lipogenesis was observed during the control OGTT in women. After HDBR, it was enhanced in both sexes, but levels in women were always superior. These results were confirmed by the plasma β-hydroxybutyrate and NEFA responses that were strongly reduced after HDBR in both sexes. Interestingly, the exogenous glucose oxidation increased in men after bed rest, but not in women. Overall, HDBR induced both insulin resistance and reduction in sympathetic activity and led to major changes in the oxidative substrate balance in the absence of changes in energy intake or in the macronutrient composition of the diet. During inactivity and in response to a glucose load, men increase their carbohydrate oxidation without lipogenesis in a context where lipid oxidation is suppressed. This may be 1) an adaptive process to maintain fat balance in a context of reduced total energy expenditure, and this suggests that for longer inactivity periods, women (increasing only lipogenesis) may be more subject to fat accumulation than men; or 2) the consequence of muscle atrophy processes, because hepatic neoglucogenesis is the main pathway of amino acid catabolism (40). This latter hypothesis seems unlikely, because we did not observe a modification in basal endogenous glucose production (data not shown).

The substrate oxidation balance during physical inactivity or simulated microgravity has been poorly studied. Two studies, conducted in men, are available, and the findings are inconsistent. After 3 days of HDBR, Acheson *et al.* (11) showed that glucose oxidation was unchanged after a 1 g/kg glucose load. However, fat oxidation was higher in HDBR conditions. Conversely, Ritz *et al.* (6) during a 42-day bed rest detected an increase in carbohydrate oxidation but a decrease in fat oxidation both in the basal state and after a standard meal. However, the different durations of the studies limit the interpretations. Our findings support a sexual dimorphism in basal rates of fat oxidation that has been previously reported. Nagy *et al.* (41) examined several determinants of basal fat oxidation in 720 healthy Caucasian volunteers in a retrospective study. Women showed a lower rate of basal fat oxidation (both absolute and adjusted for confounded factors) than men. Long duration exercise experiments were conducted in fuel metabolism to study the gender differences (42). During exercise, but not before or after, women derived proportionally more energy from fat and men from carbohydrate, respectively. These data support the view that different priorities are placed on lipid and carbohydrate oxidation during exercise and that these gender-based differences extend to the lower response of catecholamines observed in women. Therefore, the dramatic reduction in lipid oxidation after HDBR may be driven by an overall decrease in the lipolytic action of the sympathetic tonus expressed by the low urinary NMN excretion rate. Effectively, autonomous responses after an OGTT indicate sympathoneural and parasympathetic stimulation combined with adrenomedullary inhibition (43). There is a growing body of data suggesting that OGTT or a meal triggers sympathetic activation through hyperinsulinemia, and it is tempting to partly attribute the changes in sympathovagal balance to the insulin resistance. In support of this, it has been recently demonstrated that the sympathetic influence on the heart is directly linked with the level of insulin sensitivity in men, but not in women (44). These considerations strongly suggest that HDBR induces low sympathetic nervous activity and insulin resistance that triggers lipogenesis, which may, in turn, alter body composition, and that women may be more susceptible to such changes.

Shangraw *et al.* (7) showed that insulin resistance occurring during bed rest did not modify the insulin responsiveness of protein breakdown in active men. Interestingly, protein oxidation was unchanged in men during the two OGTTs, whereas it was 22% increased in women. The 24-h urea and creatinine urinary excretions support the nitrogen loss in both sexes. However, urea was higher only in women. Such results were previously reported in men, but the reason why protein oxidation increased in women is unknown and warrants further analysis. This may suggest that the insulin responsiveness of protein breakdown is maintained in men, but not in women.

Resting energy expenditure and NPRQ

We did not observe any change in the resting metabolic rate in either men or women. Resting energy expenditure increased (8%) and decreased (6%) after, respectively, 3 and 42 days of bed rest (6, 11). The decrease during longer periods of HDBR has been attributed to the loss of LBM. Taken together, these results suggest that in short-term HDBR, resting energy expenditure increases and then decreases for a longer duration. In this study the NPRQ increased significantly in men and women both in the fasting state and after the glucose load. Similarly, the two above-mentioned studies (6, 11) noted higher fasting NPRQ. The present increase can be related to 1) a shift toward carbohydrate oxidation in men, as lipogenesis is not increased in the basal state; and 2) lipogenesis in women, as carbohydrate oxidation is not modified.

Conclusions

This is the first study that has investigated the gender differences in fuel homeostasis during enforced inactivity and simulated weightlessness. Effectively in this field only scattered studies were conducted in men, and none is available in women. However, a better understanding of the deleterious consequences of a sedentary lifestyle implicated in numerous pathologies is needed. In the context of reduced sympathetic activity, our results have suggested that bed rest induces insulin resistance at the muscle level in men, but at both the muscle and liver levels in women. Moreover, despite no measurable changes in body composition, the fuel mix oxidized is altered gender dependently. At rest, both sexes triggered a drop in lipid oxidation, but men increased carbohydrate oxidation and women increased lipogenesis. During an oral [^{13}C]glucose load, lipid oxidation was suppressed, and lipogenesis was increased in both sexes. However, at the same time in men, but not in women, lipogenesis was limited by concomitantly increasing the exogenous glucose oxidation. These changes were not due to gastrointestinal dysfunction. Further studies are now necessary to delineate the gender mechanisms involved in these adaptations.

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