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Original article

Alkalinization with potassium bicarbonate improves glutathione status and protein kinetics in young volunteers during 21-day bed rest

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SUMMARY

Background & aims: Physical inactivity is associated with lean body mass wasting, oxidative stress and pro-inflammatory changes of cell membrane lipids. Alkalinization may potentially counteract these alterations. We evaluated the effects of potassium bicarbonate supplementation on protein kinetics, glutathione status and pro- and anti-inflammatory polyunsaturated fatty acids (PUFA) in erythrocyte membranes in humans, during experimental bed rest.

Methods: Healthy, young, male volunteers were investigated at the end of two 21-day bed rest periods, one with, and the other without, daily potassium bicarbonate supplementation (90 mmol $\times$ d^{-1}), according to a cross-over design. Oxidative stress in erythrocytes was evaluated by determining the ratio between reduced (GSH) and oxidized glutathione (GSSG). Glutathione turnover and phenylalanine kinetics, a marker of whole body protein metabolism, were determined by stable isotope infusions. Erythrocyte membranes PUFA composition was analyzed by gas-chromatography.

Results: At the end of the two study periods, urinary pH was 10 ± 3% greater in subjects receiving potassium bicarbonate supplementation (7.23 ± 0.15 vs. 6.68 ± 0.11, $p < 0.001$). Alkalinization increased total glutathione concentrations by 5 ± 2% ($p < 0.05$) and decreased its rate of clearance by 38 ± 13% ($p < 0.05$), without significantly changing GSH-to-GSSG ratio. After alkalinization, net protein balance in the postabsorptive state improved significantly by 17 ± 5% ($p < 0.05$) as well as the sum of n-3 PUFA and the n-3-to-n-6 PUFA ratio in erythrocyte membranes ($p < 0.05$).

Conclusions: Alkalinization during long-term inactivity is associated with improved glutathione status, anti-inflammatory lipid pattern in cell membranes and reduction in protein catabolism at whole body level. This study suggests that, in clinical conditions characterized by inactivity, oxidative stress and inflammation, alkalinization could be a useful adjuvant therapeutic strategy.

1. Introduction

Acid-base balance has important regulatory effects on body protein kinetics. The consequences of alkalosis and acidosis on protein metabolism have been extensively described in health and disease conditions [1,2]. In experimental models in vitro, increasing extracellular pH enhanced protein anabolism and inhibited protein catabolism [3–5]. In hyperventilated head trauma patients, respiratory alkalosis increased muscle protein synthesis up to 33% [6]. On the contrary, metabolic acidosis observed in chronic kidney

Abbreviations: absolute synthesis rate (ASR), ambulatory period (AMB); area-under-the-curve (AUC), bed rest with standard nutrition (BR); bed rest with standard nutrition plus potassium bicarbonate (BRKB), chronic kidney disease (CKD); fat mass (FM), fractional synthesis rate (FSR); reduced glutathione (GSH), glutathione clearance (GSH clearance); oxidized glutathione (GSSG), high density lipoprotein (HDL); lean body mass (LBM), low density lipoprotein (LDL); polyunsaturated fatty acid (PUFA), reactive oxygen species (ROS).

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disease (CKD), or experimentally induced by ammonium chloride administration, was associated with body protein wasting [7]. The mechanism involved are inhibition of protein synthesis, activation of the ubiquitin-proteasome proteolytic pathway and stimulation of amino acid oxidation [8,9].

Anti-catabolic effects of alkalinizing interventions have been investigated in patients with CKD and in elderly subjects. In patients with CKD and metabolic acidosis, a long-term sodium bicarbonate supplementation improved muscle mass and function [10,11]. Oral administration of potassium bicarbonate (60–120 mmol × d-1) in elderly men and women reduced bone resorption rates and improved muscle mass and function [12–14].

Changes in intracellular pH can also affect redox balance. Glutathione (a glycine-cysteine-glutamic acid tripeptide) is the most important non-enzymatic, intracellular antioxidant. Its concentration is particularly abundant in the liver and erythrocytes where it acts as local and systemic antioxidant agent [15]. Models of hyperlactacidemia and hyperketonemia showed that acidosis can induce reactive oxygen species (ROS) formation [16], leading to glutathione depletion [17,18]. Conversely, alkalinization may attenuate oxidative damage and prevent glutathione reduction (depletion) [19,20]. The impact of alkalosis and/or acidosis on oxidative stress has been scarcely investigated, in humans.

Physical inactivity induces alterations in protein kinetics and lean body mass loss [21,22]. Experimental bed rest is also associated with oxidative stress and activation of the glutathione system [22,23]. A cause–effect relationship between oxidative stress and protein catabolism during inactivity is possibly present, since antioxidant supplementation prevented disuse atrophy in animal models [24–26].

Polyunsaturated fatty acids (PUFA) play a key role in modulating inflammation, being n-3 and n-6 PUFA anti- and pro-inflammatory compounds, respectively. We have recently shown that the n3/n6 PUFA ratio significantly decreases in bed rest conditions [27]. In addition, changes in acid base balance may also affect the inflammatory pattern of cell membrane lipids [28,29].

In the present work, we have hypothesized that alkalinization during 21 days of experimental bed rest could improve protein metabolism and redox balance in healthy young volunteers. We have therefore investigated the effects of daily administration of potassium bicarbonate on whole body protein kinetics and the glutathione system, assessed by stable isotope infusion [30,31]. The ratios between n6 and n3 PUFA (n3/n6 PUFA) in erythrocyte membranes [27] were determined as markers of systemic inflammatory response.

2. Materials and methods

Eight young male volunteers (age 27 ± 3 years; BMI 24.4 ± 0.7 kg/m^2) participated to the interventional, randomized crossover trial “Integrative Physiology in Space Flight: Nutritional Countermeasures (NUC)” [33]. The experimental protocol of the study was approved by the Ethical Committee of the Aerztekammer Nordrhein (Duesseldorf, Germany), in accordance to the local ethical standards and the Declaration of Helsinki and following amendments. A written informed consent was obtained from each volunteer upon enrolment. The study was performed at the Simulation Facility for Occupational Medicine Research (AMSAN) of the German Aerospace Center (DLR) in Cologne, Germany.

2.1. Experimental protocol

The experimental design has been previously published [32]. Briefly, each volunteer was studied twice according to a randomized crossover design (Fig. 1). Each study period (campaign) consisted in 1-week of dietary and environmental adaptation (ambulatory period, AMB); 21 days of 6°-head-down-tilt bed rest (bed rest period); and 1 week of recovery. Before the bed rest period of the first campaign (March 2010), subjects were randomized 1:1, either to bed rest with standard nutrition (BR) or to bed rest with standard nutrition plus potassium bicarbonate (BRKB). Volunteers assigned to the BRKB group (n = 4) received daily a potassium bicarbonate supplementation, while the other group (BR, n = 4) had no supplementation. During the bed rest period of the second campaign (August–September 2010), the same volunteers were further investigated according to a crossover

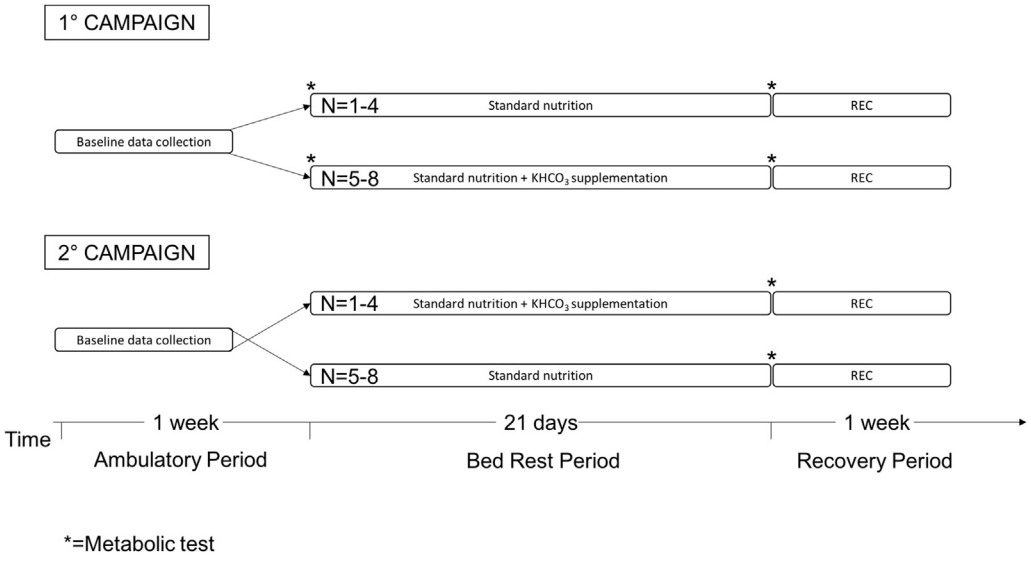

Fig. 1. Study design. Each volunteer was studied twice according to a randomized crossover design. Each study period (campaign) consisted in 1-week of dietary and environmental adaptation (ambulatory period); 21 days of 6°-head-down-tilt bed rest (bed rest period); and 1 week of recovery. Before the bed rest period of the first campaign (March 2010), subjects were randomized 1:1, either to a bed rest with standard nutrition (BR) or to a bed rest with standard nutrition plus potassium bicarbonate (BRKB). Volunteers assigned to the BRKB group (n = 1–4) received daily a potassium bicarbonate supplementation, while the other group (BR, n = 5–8) had no supplementation. During the bed rest period of the second campaign (August–September 2010), the same volunteers were further investigated according to a crossover design. The metabolic test consisted in the evaluation of whole body protein kinetics, red blood cell glutathione kinetics, red cell membrane fatty acid content and other analysis in the postabsorptive state.

design. In this second investigation, one subject withdrew because of medical problem unrelated to the protocol. In all volunteers, energy intake, calculated from resting energy expenditure, measured by indirect calorimetry (Deltatrac II, Datex, Finland), was tailored to maintain a near-eucaloric condition. Total energy intake was 1.4 and 1.1 times the resting energy expenditure through both the ambulatory and bed rest periods, respectively [32]. Carbohydrate and lipid intakes were approximately 50–55% and 28–31% of total energy, respectively. Protein intake was maintained at 1.2 g $\times$ kg^{-1} $\times$ d^{-1} throughout the study. Water intake was 50 mL $\times$ kg^{-1} $\times$ d^{-1}, sodium 2.5 mmol $\times$ kg^{-1} $\times$ d^{-1}, calcium 1000 mg $\times$ d^{-1} and vitamin D 1000 IU $\times$ d^{-1}. All volunteers received six meals per day (i.e., 3 main meals and 3 snacks). The actual food intake has been monitored daily by a dietitian, who weighed the food before and after each meal. Subjects in the BRKB treatment group received 90 mmol $\times$ d^{-1} of potassium bicarbonate (3 $\times$ 30 mmol at each main meal) as soluble tablets dissolved in 200 mL tap water, whereas the BR group received only 200 mL of plain tap water. 24-hour urinary pH was monitored daily throughout the study (i.e., ambulatory period, bed rest period, with or without supplementation and recovery period) using a pH electrode (inoLab pH 720, WTM, Weilheim, Germany). Body composition [i.e. fat mass (FM) and lean body mass (LBM) as well as left and right leg and arm lean mass] was assessed by DXA four times in each subject, i.e. at the beginning and at the end of each campaign. Body weight and composition did not differ at the beginning of the two campaigns (body weight: 78.3 ± 2.5 and 78.3 ± 2.4 kg, $p = 0.90$; lean mass: 62.5 ± 1.3 and 62.6 ± 1.1 kg, $p = 0.80$). Total leg lean mass was calculated as sum of lean mass of the right and left legs (right + left leg lean mass). To assess the effects of potassium bicarbonate administration on inactivity-induced modifications of whole body protein turnover and glutathione kinetics, a stable isotope infusion was performed (metabolic test) [29,30]. For technical reasons, the metabolic and biochemical basal data were obtained only once at the beginning of the ambulatory period (AMB) of the first campaign in subjects #1–4 and of the second campaign in subjects #5–8, considering the crossover design of the study. Metabolic and biochemical tests were performed again at the end of the first and second campaign in all subjects (Fig. 1).

2.2. Infusion protocol

The infusion protocol started early in the morning (07.00 AM), after an overnight fast. In each volunteer, two polyethylene catheters were inserted: one into a forearm vein, for isotope infusion, and another into a wrist vein of the opposite arm, heated at 50 °C, to obtain arterialized venous blood. After basal blood sample collection, a single bolus of [ring-$^2H_4$]-tyrosine (1.1 μmol $\times$ kg^{-1}) was administered, in parallel with 6-h primed-continuous infusions of: $^2H_2$-glycine (26.5 μmol $\times$ kg^{-1} $\times$ h^{-1}), [ring-$^2H_5$]-phenylalanine (3.3 μmol $\times$ kg^{-1} $\times$ h^{-1}) and $^2H_2$-tyrosine (1.0 μmol $\times$ kg^{-1} $\times$ h^{-1}) (Cambridge Isotope Laboratories, Andover, MA). Basal blood samples were collected to assess natural isotopic enrichments of [ring-$^2H_5$]-phenylalanine, [ring-$^2H_4$]-tyrosine, $^2H_2$-tyrosine in plasma and $^2H_2$-glycine and $^2H_2$-glutathione in erythrocytes. The same basal blood sample were used to measure: red blood cells membrane fatty acid composition, plasma concentrations of triglycerides and high density lipoprotein (HDL) cholesterol as well as total glutathione concentration and the ratio between reduced (GSH) and oxidized (GSSG) glutathione (i.e. GSH/GSSG) in erythrocytes. During the metabolic test, blood samples were drawn at 180, 270 and 360 min to assess steady state enrichments of [ring-$^2H_4$]-tyrosine and [ring-$^2H_5$]-phenylalanine in plasma as well as of free $^2H_2$-glycine in red blood cells. The increments of glutathione-bound [$^2H_2$-glycine] were assessed at 180, 270 and 360 min. Total glutathione concentrations were determined in erythrocytes also at the end of tracer infusions to assess metabolic steady state during the tracer incorporation period. All blood samples were collected in EDTA tubes and immediately centrifuged at 3000 g at 4 °C for 10 min; plasma and erythrocytes were separated and stored at −80 °C. Volume and number of red blood cells were assessed at the end of bed rest period in both BR and BRKB groups.

2.3. Analyses

Isotopic enrichments of phenylalanine and tyrosine, in plasma, as well as of glycine and glutathione, in red blood cells, were determined by gas chromatography–mass spectrometry (GC–MS) (HP 5890; Agilent Technologies, Santa Clara, CA) as t-butyldimethylsilyl derivatives [23,30–32]. Enrichments were expressed as tracer/tracee ratios. Plasma concentrations of glutamate and pyroglutamic acids were assessed by GC–MS, using the internal standard technique, as previously described [15,23]. Fatty acid compositions of red blood cell membranes were analyzed by gas-chromatography flame-ionization-detection (GC6850 Agilent Technologies), as previously described [27]. Fatty acid compositions of red blood cell membranes were expressed as percent of total fatty acid content. The GSH/GSSG ratio was assessed in red blood cells by a commercially available kit (Oxford Biomedical Research Inc., MI, USA). Triglycerides and HDL cholesterol concentration and the volume and the number of red blood cells were measured by standard methods by a certified external laboratory (Synlab Italia Srl, Italy). Urinary pH was calculated daily as mean value of 24-h urinary pH in ambulatory, bed rest (with or without supplementation) and recovery periods.

2.4. Calculations

Whole body protein kinetics were determined in plasma by the tracer model of phenylalanine and tyrosine metabolism in steady state (average enrichments at 180, 210, as previously described [31]. Briefly, phenylalanine rate of appearance from protein degradation (Protein Degradation) was calculated as ratio between the infusion rate of [ring-$^2H_5$]-phenylalanine ($IR_{D5\text{-}Phe}$) and the enrichment of [ring-$^2H_5$]- phenylalanine ($E_{D5\text{-}Phe}$) expressed as tracer/tracee ratio.

$$\text{Protein Degradation} = IR_{D5-Phe}/E_{D5-Phe}.$$

The rate of phenylalanine hydroxylation to tyrosine was directly determined as marker of Protein Balance in the postabsorptive state. This figure was expressed as negative value as follows:

$$\text{Protein Balance} = -\left(IR_{D2-Tyr} \times E_{D2-Tyr^{-1}}\right) \times \left(E_{D4-Tyr} \times E_{D5-Phe^{-1}}\right)$$

where $IR_{D2\text{-}Tyr}$ is the infusion rate of $^2H_2$-tyrosine and $E_{D2\text{-}Tyr}$, $E_{D4\text{-}Tyr}$ and $E_{D5\text{-}Phe}$ are the steady state enrichments (tracer/tracee ratios) of $^2H_2$-tyrosine, [ring-$^2H_4$]-tyrosine and [ring-$^2H_5$]-phenylalanine, respectively.

The rate of phenylalanine disappearance into protein synthesis (Protein Synthesis) was calculated as follows:

$$\text{Protein Synthesis} = \text{Protein Degradation} + \text{Protein Balance}$$

All kinetic parameters were normalized by kg whole body lean mass and expressed as mmol $\times$ kg^{-1} $\times$ h^{-1}.

Glutathione fractional synthesis rate (FSR; % $\times$ day^{-1}) was calculated as:

$$FSR = \left[E_{D2-glu} \times t^{-1}\right] \times E_{D2-gly^{-1}} \times 24h \times 100$$

where $[E_{D2\text{-}glu} \times t^{-1}]$ is the slope of the regression line describing the rise of $^2H_2$-glutathione enrichment in erythrocyte as a function of time (hours), and $E_{D2\text{-}gly}$ is the mean glycine enrichment in erythrocytes at steady state [23]. The absolute synthesis rate (ASR) was calculated as product of mean glutathione concentrations and FSR over the tracer incorporation period ($\mu mol \times L^{-1} \times day^{-1}$). During the tracer infusion period, in steady state conditions of glutathione concentration, the absolute rates of glutathione synthesis is equivalent to the absolute rates of glutathione degradation. Thus, the rate of glutathione clearance in erythrocytes ($L \times day^{-1}$) can be calculated as ratio between ASR and glutathione concentration. This figure is equivalent of the FSR divided by 100.

The relative fatty acid contents in erythrocyte membranes were evaluated as previously described [27]. Fatty acids concentration in red blood cell membrane were expressed as percent ratio between the area-under-the-curve (AUC) of each fatty acid peak and the sum of all fatty acid peaks.

Plasma low density lipoprotein (LDL) cholesterol was calculated by the Friedewald's formula.

2.5. Data presentation and statistics

Data are expressed as mean ± SEM. AMB data of subject #1–4 were collected at the beginning of the first campaign. AMB data of subject #5–8 were collected at the beginning of the second campaign. Differences among AMB, BR and BRKB periods were evaluated by repeated measures ANOVA, using the volunteer randomization sequences to test between-subjects homogeneity. Post hoc analysis evaluated specific differences between AMB and BR as well as between BR and BRKB using paired Student t test and Bonferroni correction. Pearson's test was used to assess bivariate correlation between variables. In order to use parametric statistics, data were log-transformed, when appropriate. $P \leq 0.05$ was considered statistically significant. $P \leq 0.10$ was considered as tendency. Statistical analysis was performed using SPSS statistical software (version 12; SPSS, Inc., Chicago, IL).

3. Results

Results of body weight and composition, as assessed by DXA, are shown in Table 1. Body weight significantly decreased after 21 days of bed rest in all volunteers, with no significant differences between BR and BRKB. Lean body mass significantly decreased following BR by about 6%, with no significant effects of BRKB. Body fat mass and arm lean mass were no significantly affects by BR and BRKB. BR significantly decreased right and left leg lean mass with no significant effects of BRKB. Changes from AMB of right + left leg lean mass following BRKB tended to be lower than those following BR (−2.0 ± 0.6% versus −4.1 ± 0.9%, respectively, $p = 0.09$).

Table 1
Effects of bed rest with and without potassium bicarbonate supplementation on body weight and composition as assessed by DXA.

Body Composition	AMB	BR	BRKB	p[a]
Body weight (kg)	79 ± 2	77 ± 2[#]	77 ± 3	0.001
Fat body mass (kg)	15.5 ± 1.5	17.2 ± 2.1	17.4 ± 1.0	0.07
Lean body mass (kg)	63.1 ± 1.2	59.5 ± 1.2[#]	59.3 ± 1.8	0.001
Right arm lean mass (kg)	3.8 ± 0.1	3.6 ± 0.1	3.8 ± 0.1	0.09
Left arm lean mass (kg)	3.7 ± 0.1	3.5 ± 0.1	3.6 ± 0.2	0.19
Right leg lean mass (kg)	10.8 ± 0.3	9.9 ± 0.3[#]	9.9 ± 0.3	0.002
Left leg lean mass (kg)	10.2 ± 0.2	9.4 ± 0.3[#]	9.5 ± 0.4	0.003
Right + left leg lean mass (kg)	20.9 ± 0.5	19.2 ± 0.6[#]	19.4 ± 0.6	0.001

n = 7. AMB, ambulatory period; BR, bed rest with standard nutrition; BRKB, bed rest with standard nutrition plus potassium bicarbonate. Data are expressed as mean ± SEM.
[#]significant difference between AMB and BR, $p < 0.05$.
[a] Differences among groups (AMB, BR and BRKB) were evaluated by repeated measures ANOVA, using the volunteers randomization sequence to test between-subjects homogeneity. Post hoc analysis evaluated specific differences between AMB and BR as well as between BR and BRKB using paired Student t test and Bonferroni correction.

Daily 24-h urinary pH, assessed throughout the study periods (i.e. ambulatory, bed rest and recovery) is showed in Fig. 2. Mean values of urinary pH in the BR group did not change significantly as compared to the mean values of the last three days of ambulatory period. As expected, mean values of urinary pH were significantly higher in BRKB than in BR group (7.22 ± 0.13 and 6.59 ± 0.09 respectively, $p < 0.01$), while no significant differences were observed between treatments during the last three days of either ambulatory (BRKB, 6.56 ± 0.08 vs BR, 6.58 ± 0.06) or recovery period (BRKB, 6.48 ± 0.08 vs BR, 6.38 ± 0.10).

The effects of potassium bicarbonate supplementation on phenylalanine kinetics in the postabsorptive state, as marker of whole body protein kinetics, at the end of the ambulatory and bed rest periods, with or without alkalinization, are shown in Table 2. The rates of protein degradation and synthesis were not significantly affected by BR or BRKB. Nonetheless, there was a significant decrease (29 ± 5%) in protein balance in BR. Protein balance was significantly less negative in BRKB as compared to BR, by about 17 ± 5%.

The effects of potassium bicarbonate supplementation on the erythrocyte glutathione system after 21 days of bed rest are showed in Table 3. BR with no alkalinization did not change significantly glutathione concentrations, FSR, ASR and clearance, as compared to AMB. In contrast, BRKB significantly increased total glutathione concentrations by about 5 ± 2%, while decreasing glutathione FSR, ASR and clearance, as compared to BR. This suggests a cause–effect relationship between a lower FSR, ASR and clearance of glutathione and its higher concentrations. Moreover, there was a significant inverse correlation between glutathione clearance and changes in urinary-pH induced by BRKB from AMB (Fig. 3). Potassium bicarbonate supplementation did not affect erythrocyte count [$(5.2 \pm 0.1) \cdot 10^6 \times mm^{-3}$ and $(5.1 \pm 0.1) \cdot 10^6 \times mm^{-3}$ for BR and BRKB, respectively] or volume (86.4 ± 1.1 μm^3 and 86.1 ± 1.1 μm^3 for BR and BRKB, respectively).

The ratio between plasma pyroglutamic acid (i.e. 5-oxoproline, the precursor of glutamic acid in the γ-glutamyl cycle) and glutamic acid (an index of the γ-glutamyl cycle activity and of glutathione turnover) tended to decrease in the BRKB. Furthermore, in pooled data there was a significant negative correlation between the absolute values of pyroglutamic-to-glutamic acid ratio and glutathione concentrations ($r = -0.52$; $p = 0.017$). BR and BRKB did not significantly affect the GSH-to-GSSG ratio in erythrocytes. The effects of bed rest, with or without supplementation, on fatty acid composition of erythrocyte membranes are reported in Table 4. There was a significant increase in the n-3 PUFA total content after bed rest with potassium supplementation (+3 ± 1%, $p < 0.05$), as compared to the standard nutrition group. Moreover, the n-3-to-n-6 PUFA ratio was significantly increased in BRKB group as compared to BR group (0.28 ± 0.01 and 0.26 ± 0.01, respectively, $p = 0.03$). There was no effect of BR or BRKB on plasma total, HDL and LDL cholesterol as well as on triglycerides (data not shown).

4. Discussion

In the present study, we have evaluated in healthy young male volunteers, the effects of 3 weeks of oral potassium bicarbonate supplementation during experimental bed rest, on whole body

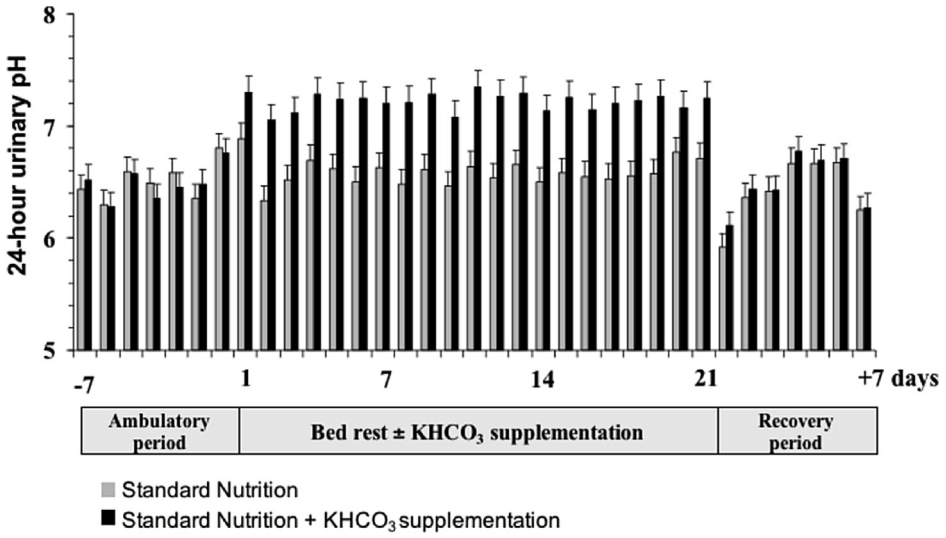

Fig. 2. Daily 24-h urinary pH, assessed during the ambulatory, bed rest and recovery periods. Data are mean ± SEM.

Table 2
Effects of bed rest with and without potassium bicarbonate supplementation on whole body protein kinetics in the postabsorptive state.

Whole body protein kinetics (μmol phenylalanine $\times$ kg LBM^{-1} $\times$ h^{-1})	AMB	BR	BRKB	p[a]
Protein Degradation	0.90 ± 0.03	0.88 ± 0.03	0.87 ± 0.03	0.34
Protein Synthesis	0.81 ± 0.03	0.77 ± 0.03	0.78 ± 0.03	0.13
Protein Balance	−0.09 ± 0.01	−0.11 ± 0.01#	−0.09 ± 0.01*	0.003
Synthesis/Degradation	0.90 ± 0.01	0.87 ± 0.01#	0.90 ± 0.01	0.002

n = 7. AMB, ambulatory period; BR, bed rest with standard nutrition; BRKB, bed rest with standard nutrition plus potassium bicarbonate; LBM, lean body mass as determined by DXA; Protein Degradation: phenylalanine rate of appearance from protein degradation; Protein Balance: phenylalanine hydroxylation into tyrosine expressed as negative value; Protein Synthesis: phenylalanine disappearance to protein synthesis. Data are expressed as mean ± SEM.
#significant difference between AMB and BR, $p < 0.05$; * significant difference between BR and BRKB, $p < 0.05$.
[a] Differences among groups (AMB, BR and BRKB) were evaluated by repeated measures ANOVA, using the volunteer randomization sequence to test between-subjects homogeneity. Post hoc analysis evaluated specific differences between AMB and BR as well as between BR and BRKB using paired Student t test and Bonferroni correction.

Table 3
Effects of bed rest with and without potassium bicarbonate supplementation on glutathione kinetics in the postabsorptive state.

Glutathione kinetics	AMB	BR	BRKB	p[a]
RBC Glutathione concentration (μmol RBC $\times$ L^{-1})	1718 ± 77	1770 ± 96	1861 ± 88*	0.05
RBC Glutathione FSR (% $\times$ day^{-1})	70 ± 13	59 ± 8	37 ± 10*	0.02
RBC Glutathione ASR (μmol $\times$ L^{-1} $\times$ d^{-1})	123 ± 24	103 ± 15	68 ± 18*	0.01
RBC Glutathione clearance (L^{-1} $\times$ d^{-1})	0.70 ± 0.13	0.59 ± 0.08	0.37 ± 0.10*	0.02
Plasma 5-oxoproline-to-glutamate ratio	0.87 ± 0.05	0.94 ± 0.08	0.77 ± 0.08	0.09
RBC GSH-to-GSSG Ratio	252 ± 55	387 ± 56	366 ± 86	0.60

n = 7. AMB, ambulatory period; ASR absolute synthesis rate; BR, bed rest with standard nutrition; BRKB, bed rest with standard nutrition plus potassium bicarbonate; FSR, fractional synthesis rate; GSH, reduced glutathione; GSSG, oxidized glutathione; RBC, Red Blood Cell. Data are expressed as mean ± SEM.
* significant difference between BR and BRKB, $p < 0.05$.
[a] Differences among groups (AMB, BR and BRKB) were evaluated by repeated measures ANOVA, using the volunteer randomization sequence to test between-subjects homogeneity. Post hoc analysis evaluated specific differences between AMB and BR as well as between BR and BRKB using paired Student t test and Bonferroni correction.

protein and erythrocyte glutathione kinetics, according to a cross-over experimental design. We found that treatment with potassium bicarbonate during bed rest, as compared to bed rest with no supplementation, improved whole body protein kinetics and increased intracellular glutathione availability by lowering the utilization of this antioxidant molecule.

Glutathione is a thiol tripeptide synthesized from glycine, glutamate and cysteine. The exchange between the monomeric (GSH) and the dimeric (GSSG) form of glutathione, catalyzed by peroxidase and reductase enzymes, is critical in maintaining intracellular redox balance. This process does not involve changes in the amount of total glutathione (i.e, GSH + GSSG). In addition to the GSH-GSSG exchange, GSH can be irreversibly utilized to directly inactivate free radicals and detoxify metabolites by donating hydrogen. The status of glutathione in cells is dependent, therefore, on the ratio between GSH and GSSG as well as on the absolute glutathione content. We have investigated the effects of alkali supplementation during bed rest on the metabolism of glutathione in red blood cells. Alkali supplementation increased glutathione content by about 5%. Because the pool of glutathione is turning over quickly, an increase in erythrocyte glutathione could stem from: 1) a higher synthesis rate, 2) a decreased utilization rate, or 3) the two process combined. In our study, alkali mediated increase in glutathione availability occurred despite decreased glutathione synthesis rate. Since glutathione concentrations did not change during the tracer infusion period, the rates of synthesis equaled the rates of

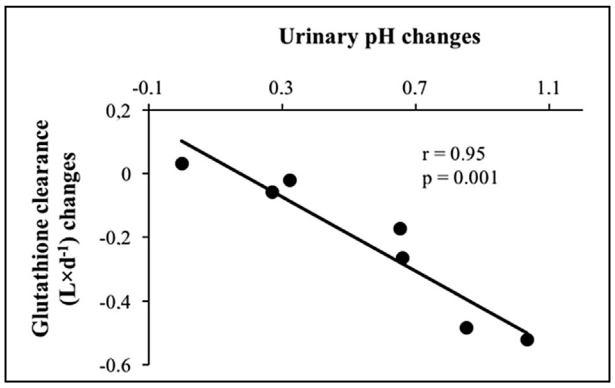

Fig. 3. Effects of potassium bicarbonate supplementation after 21 days of bed rest. Correlation between changes in urinary pH and red blood cell glutathione clearance rate induced by potassium bicarbonate supplementation. AMB, ambulatory period; BRKB, bed rest with standard nutrition plus potassium bicarbonate.

degradation. Thus, the calculated rates of glutathione utilization or clearance decreased by 35% following alkalinization and explained the higher glutathione availability. Despite such increase in total glutathione availability, the ratio between the reduced and the oxidized form, i.e., the GSH-to-GSSG ratio, was unaffected by alkalinization. These results indicate that treatment with potassium bicarbonate during bed rest causes a lower glutathione utilization, while increasing its availability as antioxidant. Although the mechanisms of treatment with potassium bicarbonate and increased antioxidant availability are not completely understood, we can speculate that mild alkalinization could down-regulate free radical production and decrease antioxidant requirement [19,20]. This hypothesis is supported by the fact that, in our study, changes of the glutathione fractional turnover rate were inversely related with modifications in urinary pH. Our results are in agreement with previous observations showing down-regulation and up-regulation of glutathione availability by acidosis and alkalosis, respectively [17–20].

Glutathione is catabolized and re-synthetized through the gamma–glutamyl cycle. Pyroglutamic acid is the product of glutathione catabolism and the immediate precursor of glutamate available for glutathione re-synthesis. The ratio between pyroglutamic acid and glutamate is therefore an index of glutathione turnover through the gamma–glutamyl cycle [15]. In agreement with kinetic results, we found that the ratio between pyroglutamic acid and glutamate tended to decrease following alkalinization. In pooled data from the AMB, BR and BRKB conditions, the ratio between pyroglutamic acid and glutamate inversely correlated with glutathione concentrations. This further suggests cause–effect relationships between decreased glutathione catabolism and turnover and increased glutathione availability following alkalinization.

In agreement with previous studies [10–14], we have observed a significant improvement of whole body protein balance in the postabsorptive state following alkali treatment. In addition, bicarbonate supplementation was associated with a trend toward a lesser decrease in leg muscle. Administration of 90 mmol × d-1 of potassium bicarbonate during bed rest improved net protein balance, as rate of phenylalanine hydroxylation to tyrosine, by 18 ± 6%. Since phenylalanine is an essential amino acid, the rate of its irreversible non-anabolic utilization is a marker of whole body protein balance, i.e., the difference between utilization to protein synthesis and release from proteolysis. The results of our study do not allow a deep understanding of the mechanisms of the anti-catabolic effects of alkali therapy on protein kinetics. In fact, the potassium bicarbonate supplementation effect on the absolute rates of phenylalanine release from proteolysis and utilization, for protein synthesis, did not achieve statistical significance. The potential protein

Table 4
Effects of potassium bicarbonate supplementation after 21-day bed rest on selected fatty acids in red blood cell membrane.

	AMB	BR	BRKB	p[a]
SATURATED FAs				
Myristic acid 14:00	0.24 ± 0.02	0.21 ± 0.02	0.21 ± 0.02	0.06
Palmitic acid 16:00	20.60 ± 0.28	20.04 ± 0.30	20.25 ± 0.25	0.19
Stearic acid 18:00	17.84 ± 0.15	17.86 ± 0.26	18.09 ± 0.22	0.31
SUM SATURATED FAs	38.68 ± 0.43	38.12 ± 0.44	38.56 ± 0.38	0.22
MONOUNSATURATED FAs				
Palmitoleic acid 16:1 n-7	0.28 ± 0.04	0.22 ± 0.03	0.22 ± 0.02	0.09
Oleic acid 18:1 n-9	14.22 ± 0.22	13.31 ± 0.26	13.56 ± 0.24	0.07
Elaidic trans 18:1n-9	1.06 ± 0.04	1.18 ± 0.03#	1.19 ± 0.03	0.02
Eicosaenoic acid 20:1n-9	0.33 ± 0.00	0.33 ± 0.01	0.34 ± 0.01	0.42
SUM MONOUNSATURATED FAs	15.89 ± 0.25	15.03 ± 0.26	15.30 ± 0.22	0.12
n-3 POLYUNSATURATED FAs				
Eicosapentaenoic acid 20:5n-3	0.89 ± 0.07	0.79 ± 0.06	0.81 ± 0.06	0.06
Docosapentaenoic acid 22:5n-3	3.08 ± 0.20	3.10 ± 0.19	3.19 ± 0.15	0.10
Docosahexaenoic acid 22:6n-3	5.39 ± 0.35	5.80 ± 0.40#	6.00 ± 0.40	0.001
SUM n-3 PUFA	9.43 ± 0.32	9.69 ± 0.36	10.02 ± 0.37*	0.02
n-6 POLYUNSATURATED FAs				
Linoleic acid 18:2 n-6	11.05 ± 0.46	10.53 ± 0.53	10.27 ± 0.50	0.20
γ-linoleic acid 18:3 n-6	0.19 ± 0.02	0.18 ± 0.02	0.18 ± 0.02	0.28
Eicosadienoic acid 20:2n-6	1.10 ± 0.48	1.53 ± 0.48	0.95 ± 0.41	0.31
Dihomo-γ-linolenic acid 20:3n-6	2.11 ± 0.14	2.22 ± 0.10	2.31 ± 0.09*	0.04
Arachidonic acid 20:4n-6	16.69 ± 0.47	17.45 ± 0.61#	17.72 ± 0.57	0.01
Adrenic acid 22:4n-6	3.55 ± 0.22	3.67 ± 0.18	3.58 ± 0.14	0.42
Docosapentaenoic acid 22:5n-6	1.21 ± 0.26	1.58 ± 0.33	1.12 ± 0.26	0.10
SUM n-6 PUFA	35.92 ± 0.68	37.16 ± 0.76	36.12 ± 0.69	0.08
n-3/n-6 PUFA ratio	0.26 ± 0.01	0.26 ± 0.01	0.28 ± 0.01*	0.001

n = 7. AMB, ambulatory period; BR, bed rest with standard nutrition; BRKB, bed rest with standard nutrition plus potassium bicarbonate. FA, fatty acid. PUFA, polyunsaturated fatty acids. Values are percent of total FAs. Data are percent of total FAs and are expressed as mean ± SEM.
*significant difference between BR and BRKB, $p < 0.05$; #significant difference between AMB and BR, $p < 0.05$.
[a] Differences among groups (AMB, BR and BRKB) were evaluated by repeated measures ANOVA, using the volunteers randomization sequence to test between-subjects homogeneity. Post hoc analysis evaluated specific differences between AMB and BR as well as between BR and BRKB using paired Student t test and Bonferroni correction.

sparing effects of alkali supplementation during bed rest can be inferred assuming 58 μmol of phenylalanine content per g of lean body mass [34]. Treatment with 90 mmol × d^{-1} of potassium bicarbonate could potentially spare 18 g × d^{-1} of lean body mass in bed resting subjects, which would amount to about 350 g over the 21 days of the study period. This estimation is in agreement with the results of a previous study obtained using the same potassium bicarbonate dose in post-menopausal women [12].

Evidence indicates that an increased production of reactive oxygen species could contribute to activation of mechanisms of muscle atrophy during unloading [22–26]. Muscle inactivity is associated with oxidative stress and compensatory activation of the glutathione and other antioxidant systems in skeletal muscle [22,23]. In our study, the evaluation of the effect of treatment with potassium bicarbonate on the glutathione system was limited to the erythrocytes. There is evidence, however, indicating that erythrocyte glutathione reflects whole body metabolism of this antioxidant [35]. This suggests a potential link between the positive effects of alkalinization on erythrocyte glutathione and the improved whole body protein kinetics shown by our study.

We have found that 3-weeks of bed rest, with or without potassium bicarbonate supplementation, can modify PUFA composition of erythrocyte membranes independently from dietary lipid intake. Strong evidence indicates that changes of n-3 PUFA (i.e., eicosapentaenoic and docosapentaenoic acids) and of n-6 PUFA (i.e., arachidonic acid) content in cell membranes modulate the systemic inflammatory response and potentially affect redox balance and protein kinetics [27,36–38]. Bed rest without bicarbonate supplementation independently increased arachidonic acid and the ratio between arachidonic and eicosapentaenoic acid leading to a proinflammatory pattern, which may have contributed to metabolic alterations associated with muscle unloading, as already shown in our previous investigation [29]. In addition, we have recently shown that a higher baseline n-3-to-n-6 PUFA ratio is associated with blunted lean body mass depletion following 5 weeks of experimental bed rest in healthy volunteers [38]. The present study showed that alkalinization during bed rest increased the sum of n-3 PUFA and the n-3-to-n-6 PUFA ratio in cell membranes leading to an anti-inflammatory pattern, as compared to bed rest with no bicarbonate supplementation.

In the present study, we demonstrated that alkali supplementation significantly improved selected aspects of protein kinetics, glutathione status and cell membrane PUFA in a human model of muscle disuse atrophy. Positive effects of alkalinization have been demonstrated in other clinical conditions of muscle catabolism such as ageing and chronic kidney disease [10–14]. Alkali diets have been associated with similar metabolic benefits to those shown with bicarbonate supplementation [39–41]. Thus, in clinical conditions characterized by inactivity, oxidative stress and inflammation, alkalinization could be a useful adjuvant therapeutic strategy.

Conflict of interest

The authors declare that there is no conflict of interests.

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Authors' contributions to manuscript

GB, research design, data analysis and statistical analysis, manuscript draft and had primary responsibility for final content; FGDG, data analysis and interpretation, statistical analysis, manuscript draft; MH, research design, research conduction; MS, research conduction and sample analysis; SM research conduction, data analysis; FA, research conduction, data analysis; RS, data analysis, manuscript draft; PV, data analysis and interpretation; MG data analysis and interpretation; JB, research conduction, data collection; PFM, research conduction, data collection; FM, data analysis and interpretation; NF, data analysis and interpretation statistical analysis. All authors have read and approved the final manuscript.

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