

1 **Sensitivity of serum concentration of cartilage biomarkers**
2 **to 21-days of bed rest**
3

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2 study design and data interpretation; SA performed biochemical analysis of the samples; MH
3 and JM contributed to study design and data interpretation. All authors have read and
4 approved the final submitted manuscript.

1 **Abstract**

2 The objective of the study was to test the hypothesis that serum levels of cartilage oligomeric
3 matrix protein (COMP) would decrease and serum levels of tumor-necrosis factor alpha
4 (TNF- α) and selected matrix metalloproteinases (MMPs) would increase in response to bed
5 rest (BR) and that these changes are unaffected by the intake of potassium bicarbonate or
6 whey protein. Seven and 9 healthy male subjects participated in two 21-day 6° head down tilt
7 crossover BR-studies with nutrition interventions. Serum samples were taken before, during,
8 and after BR and biomarker concentrations were measured using commercial enzyme-linked
9 immunosorbent assays. MMP-3 during BR was significantly lower than at baseline (reduction
10 greater 20%; $P < 0.001$). MMP-3 increased significantly from 14 to 21 days of BR (+7%;
11 $P = 0.049$). COMP during BR was significantly lower than at baseline (reduction greater 20%;
12 $P < 0.001$). MMP-3 and COMP returned to baseline within one day after BR. MMP-9 on day 3
13 of BR was significantly lower than at baseline (-31%; $P < 0.033$) and on days 3, 5 and 14 of
14 BR significantly lower than at the end of and after BR (reduction greater 35%; $P < 0.030$). The
15 nutritional countermeasures did not affect these results. The observed changes in cartilage
16 biomarkers may be caused by altered cartilage metabolism in response to the lack of
17 mechanical stimulus during BR and inflammatory biomarkers may play a role in changes in
18 biomarker levels. **Clinical relevance:** Immobilization independently from injury can cause
19 altered cartilage biomarker concentration.

20

21 **Key words:** immobilization, cartilage health, cartilage biomarkers, metalloproteinases, tumor
22 necrosis factor, nutritional supplements,

23

1 **Introduction**

2 Research in the space flight context enables us to use unique unloading models in
3 healthy individuals to better understand the effect of physical activity or inactivity on
4 cartilage health especially because anticipated times for tissue adaptation for bradytrophic
5 tissue such as cartilage are longer than for instance for muscle or bone. Bed rest in a 6°-head-
6 down-tilt (6°HDT BR) position is commonly used as a gold standard to simulate the effects of
7 microgravity on the human body.¹ This model simulates many physiological effects of space
8 flight including a reduced mechanical stimulus of the lower extremity and a fluid shift
9 towards the head.² Previous studies have investigated the effects of nutritional supplements
10 such as different proteins or bicarbonate on bone and muscle during bed rest.^{3,4} The only
11 nutritional supplements discussed in osteoarthritis research are chondroitin and glucosamine
12 but their effects on cartilage are inconclusive.⁵ To date, the effects of protein or bicarbonate
13 supplements on articular cartilage have not been explored.

14
15 Potential cartilage biomarkers include structural proteins or enzymes that reflect
16 cartilage metabolism. MMPs are a multi-member family of proteases with a wide range of
17 substrates including extracellular components, cytokines, receptors, and cell motility
18 factors.^{6,7} For instance, MMP-1 (or stromelysin-1) is produced by chondrocytes, osteoblasts
19 and synovial cells that degrades collagen types I, II, and III in the extracellular matrix, and
20 mediates cartilage destruction.^{8,9} In pathological conditions such as osteoarthritis (OA),
21 MMP-1 is expressed at higher levels by OA chondrocytes than by normal chondrocytes
22 suggesting a predominant role of MMP-1 in OA pathogenesis.^{10,11} MMP-3 is in part
23 responsible for the degradation of non-collagen matrix proteins in cartilage in rheumatoid
24 arthritis (RA) and OA⁸ and increased levels of MMP-3 and MMP-10 are found in articular
25 cartilage and synovium of these patients.¹²⁻¹⁴ Cartilage collagen breakdown and aggrecan is
26 coordinated by MMP-9 and MMP-13. Serum MMP-9 levels are increased in several

1 rheumatic diseases such as RA¹⁵ and systemic sclerosis.¹⁶ Native Collagen 2 (Coll-2) is
2 degraded by matrix metalloproteinases (MMPs)-1, -8, -13 and -14 and partially degraded
3 Coll-2 is then further degraded by gelatinases, namely MMP-2 and MMP-9 and stromelysin-1
4 (MMP-3).¹⁷ These enzymes represent the main proteolytic enzyme group involved in
5 remodeling the extracellular matrix and modifying cell-cell and cell-matrix interactions.

6
7 Tumor necrosis factor alpha (TNF- α) is an inflammatory factor and serves as one of
8 the cytokines that can activate other cells to produce MMPs.¹⁸⁻²⁰ It is also involved in the
9 proteoglycan release from cartilage tissue²¹ and thus contributes to catabolic processes in
10 osteoarthritis. Serum levels of TNF- α have been associated with the prevalence of joint space
11 narrowing and prediction of knee cartilage loss.^{22,23} Cartilage oligomeric matrix protein
12 (COMP), also known as thrombospondin 5, is one of the non-collagenous, non-proteoglycan
13 proteins of cartilage and has been associated with cartilage degradation in OA.²⁴ Serum
14 COMP concentration is sensitive to physiological loading which has been shown in different
15 settings such a running and walking.²⁵⁻²⁷ A previous bed rest study has shown that serum
16 COMP concentration decreases during 14 days of immobilization.²⁸ However, to date the
17 effects of bed rest on other cartilage biomarkers and the effect of bed rest exceeding 14 days
18 on COMP is unknown.

19
20 The purpose of this study was to determine the effect of 21-days of unloading and
21 nutritional supplements using the HDT-bed rest model on serum concentrations of MMP-1,
22 MMP-3, MMP-9, TNF- α , and COMP in healthy male individuals. We hypothesized that
23 serum levels of COMP would decrease and serum levels of TNF- α and selected MMPs would
24 increase in response to bed rest and that these changes are unaffected by the intake of
25 potassium bicarbonate or whey protein.

26

1 **Materials and Methods**

2 We conducted two 21-days 6°HDT bed rest studies, the Nutritional Countermeasure
3 Study (NUC-Study (Medium-term bed rest, potassium bicarbonate [KHCO₃]
4 supplementation: Study 1; ClinicalTrials.gov Identifier: NCT01509456) and the Medium-
5 term-bed rest Whey Protein study 2011/2012: Evaluation of whey protein and potassium
6 bicarbonate to counteract effects of bed rest (MEP-Study) (Medium-term bed rest, whey
7 protein and KHCO₃ supplementation: Study 2; ClinicalTrials.gov Identifier: NCT01655979),
8 at the clinical research center of the Institute of Aerospace Medicine, German Aerospace
9 Center (DLR) (Cologne, Germany) in compliance with the Declaration of Helsinki and after
10 approval of the Ärztekammer Nordrhein (Düsseldorf, Germany). A detailed description of the
11 study designs can be found elsewhere.³ Briefly, participants were included in the respective
12 studies after medical and psychological screening with the following exclusion criteria:
13 history of hypertension, diabetes, obesity, rheumatic disease, hyperlipidemia, hepatic disease,
14 bone disease, physical exercise more than four times per week, smoking, consumption of
15 drugs, or alcohol excess, inconspicuous thrombophilia screening panel (AT III, S-Akt, Lupus-
16 PTT, ferritin, Factor V Leiden, Factor IV, and Factor II). All participants provided written
17 informed consent prior to participation. The studies had randomized cross-over designs with
18 two 34-day phases, each divided into 7 days adaptation (baseline data collection, BDC), 21
19 days HDT bed rest and 6 days recovery (R). Environmental conditions, daylight conditions,
20 study protocol and diet were controlled and identical in all phases. Participants were allowed
21 to freely move around the ward in the adaptation and the recovery periods but physical
22 exercise during these periods was omitted. During the intervention periods, participants were
23 in 6°HDT bed rest for 24 h every day and not allowed to elevate their upper body. All
24 activities, including eating, showering, and weighing, were carried out in the 6°HDT position.
25 Physiotherapy sessions consisting of passive treatment were scheduled every 3 to 5 days.
26 During the immobilization period in both studies, participants were quasi randomized

1 alternatively by enrolment (1:1) to receive the standard meal plus the countermeasure (Study
2 1, potassium bicarbonate [KHCO₃]; Study 2, 0.6 g whey protein/kg body weight/d) +
3 potassium bicarbonate (90 mmolKHCO₃/d) supplementation) in phase 1 and only the
4 standard meal in phase 2, or vice versa.

5

6 Study specific details: Study 1

7 The NUC-Study investigated the effect of a balanced acid-base ratio by potassium
8 bicarbonate treatment (nutritional countermeasure, daily dose: 90 mmol potassium
9 bicarbonate) on the effects of bed rest on bone turnover, protein metabolism and the immune
10 system in seven healthy male participants (Table1). In addition, the effect of the nutrition
11 countermeasure on body composition, muscle performance and the cardiovascular- and
12 neurovestibular systems were tested. NUC phase 1 was conducted from February to April
13 2010 followed by a wash out period of 4 months before NUC phase 2 (August to October
14 2010). In both phases, blood samples were taken on days BDC-6, BDC-2, HDT2, HDT6,
15 HDT10, HDT14, HDT21, R+2 and R+5. Serum was analyzed for COMP concentration.

16

17 Study specific details: Study 2

18 The MEP-Study examined the influence of 0.6 g whey protein (WP)/kg body mass
19 (BM) and alkaline salt administration (90 mmol potassium bicarbonate (KHCO₃))
20 supplementation as a potential nutritional countermeasure in multiple physiological systems
21 in 10 healthy male subjects (Table 1). MEP phase 1 was conducted in September/October
22 2011 followed by a wash out period of 4 months before MEP phase 2 (February/March 2012).
23 In both phases, blood samples were taken on days BDC-2, BDC-1, HDT2, HDT3, HDT5,
24 HDT7, HDT14, HDT21, R+1 and R+5. Serum was analyzed for COMP, MMP-1, MMP-3,
25 MMP-9 and TNF- α concentration.

26

1 Blood samples and biomarker analysis

2 In both studies, fasting (9 h) blood samples were collected shortly after waking up (~
3 6:30 am) with the subject in the supine 6°HDT position over night (at least 8 h in the
4 ambulatory study periods), according to the study period. Samples were drawn from the
5 antecubital vein by a physician using a short catheter and serum monovettes (Sarstedt,
6 Germany). 150 ml serum was aliquoted in 200µl volumes into Eppendorf tubes and frozen at -
7 80°C until analysis. Serum MMP-1, MMP-3, MMP-9, TNF-α, and COMP concentrations
8 were analyzed using commercial enzyme-linked immunosorbent assays (ELISA) (MMP-1:
9 Quantikine[®], Human Pro-MMP-1 Immunoassay; MMP-3: Quantikine[®], Human Total MMP-3
10 Immunoassay; MMP-9: Quantikine[®], Human MMP-9 Immunoassay; TNF-α: Quantikine[®]
11 HS, Human TNF-α Immunoassay, all Quantikine[®] Assays from R&D Systems INC.,
12 Minneapolis, MN, US, COMP[®] ELISA; AnaMar Medical AB, Lund Sweden;). Biomarker
13 analysis was performed according to the manufacturer's manuals and on ELISA plates from
14 the same batch of each respective marker in order to avoid inter-assay variation. All analysis
15 was done in duplicates with all samples of both phases for each participant on the same assay
16 plate.

17

18 Strict standardization of bed rest studies within the European Space Agency (ESA)
19 program allows for pooling data from different studies with the same study length and sample
20 collection on identical study days. Thus, COMP concentration data from study 1 and 2 were
21 pooled resulting in a greater sample size for the analysis of changes in COMP concentration
22 during the bed rest periods. Overall, COMP concentration values of 16 subjects were pooled
23 for study days BDC -2, HDT 2, HDT7 (HDT6), HDT 14, HDT21, R+1.

24

25 Statistical analysis

1 All statistical analyses were performed in SPSS version 21.0 (IBM Corporation,
2 Amonk, NY, USA). Descriptive statistics were calculated for all parameters (mean, standard
3 deviation, and 95% confidence interval (CI)). Data were tested for normality using Shapiro
4 Wilks tests. Linear mixed models were used to detect significant changes in biomarker
5 concentrations with time and intervention as within subject factors and paired least square
6 distance tests for posthoc analysis. Linear regression analysis was used to detect significant
7 associations among bed rest-induced changes in biomarkers. The significance level was set a
8 priori to .05.

9

10 **Results**

11 Demographic data for participants in Study 1 and Study 2 are provided in Table 1.
12 Eight healthy young male participants were included in Study 1. One participant dropped out
13 after phase 1 for personal reasons, thus blood samples of seven participants were analyzed.
14 Ten healthy young male participants were included in Study 2. One subject dropped out after
15 phase 1 for personal reasons, and thus blood samples of nine participants were analyzed. The
16 two populations did not differ in age, height or body mass (Table 1).

17

18 Table 1

19

20 MMP-1, MMP-3, MMP-9 and TNF- α concentrations

21 Biomarker data at all time points were normally distributed. MMP-3 and MMP-9
22 concentrations differed significantly between time points (repeated measures ANOVA:
23 $P < .001$ and $P = .005$, respectively; Figure 1). There was no significant time effect for MMP-1
24 and no significant time \times intervention interaction was observed for MMP-1, MMP-3, MMP-9
25 or TNF- α . MMP-3 levels during bed rest were significantly lower than at baseline (95% CI
26 HDT-BDC: HDT2 [-3.48; -1.49] ng/mL; HDT7 [-3.90; -1.90] ng/mL; HDT14 [-4.34; -1.61]

1 ng/mL; HDT21 [-3.8; -0.61] ng/mL; all $P < .012$; Figure 1). MMP-9 levels on day 3 during bed
2 rest were significantly lower than at baseline (95% CI HDT-BDC: HDT-3 [-9.42; -172.16]
3 ng/mL). TNF- α levels on days 14 and 21 during bed rest and on day 1 after bed rest were
4 significantly higher than at baseline (95% CI HDT-BDC: HDT-14 [0.083; 0.392] pg/mL;
5 HDT-21 [0.064; 0.512] pg/mL; R+1-BDC [0.170; 0.593] pg/mL).

6

7 Figure 1

8

9 MMP-3 levels increased significantly from 14 to 21 days of bed rest (95% CI HDT21-
10 HDT14: [0.00; 1.76] ng/mL; $P = 0.50$). MMP-9 levels on days 3, 7, and 14 were significantly
11 lower than at the end of bed rest and after bed rest (95% CI HDT-R+1: HDT2 [-176.86; -
12 40.55] ng/mL; HDT7 [-204.44; -32.88] ng/mL; HDT14 [-164.10; -35.47] ng/mL; HDT21-
13 HDT14: [-163.54; -14.14]; all $P < .025$). MMP-3 levels returned to baseline levels within one
14 day after bed rest (Table 2).

15

16 Table 2

17

18 COMP concentrations

19 COMP data at all time points in both studies were normally distributed. COMP
20 concentrations differed significantly between time points (repeated measures ANOVA:
21 $P < .001$; Figure 2). No significant time \times study interaction was observed. Hence, COMP data
22 from both studies were pooled for further analysis. COMP levels during bed rest were
23 significantly lower than at baseline (95% confidence interval (CI) HDT-BDC: HDT2 [-1.31; -
24 0.69] U/L; HDT7 [-1.64; -0.63] U/L; HDT14 [-1.63; -0.54] U/L; HDT21 [-1.59; -0.84] U/L;
25 all $P < .002$) and returned to baseline levels within one day after bed rest (Table 3).

26

1 Table 3

2
3 Associations among bed rest-induced changes in biomarkers

4 Changes in MMP-9 in response to bed rest correlated with changes in COMP at HDT2
5 (R=0.482; P=0.043), HDT3 (R=0.583; P=0.011) and HDT5 (R=0.468; P=0.049). Changes in
6 MMP-1, MMP-3 and TNF- α in response to bed rest did not correlate with changes in COMP.

7
8 **Discussion**

9 The purpose of this study was to determine the effect of 21-days of unloading using
10 the HDT-bed rest model on serum concentrations of MMP-1, MMP-3, MMP-9, TNF- α , and
11 COMP in healthy male individuals. We observed reduced COMP levels in both bed rest
12 studies. Moreover, MMP-3 but not MMP-1 or TNF- α were lower during bed rest than at
13 baseline independent on the intervention, and MMP-9 was lower at 3 days into bed rest
14 independent on the intervention. These results partially confirmed our hypothesis that serum
15 levels of COMP would decrease and serum levels of TNF- α and selected MMPs would
16 increase in response to bed rest and that the nutritional supplements included in this study are
17 irrelevant for cartilage metabolism.

18
19 The results of our study support previous findings²⁸ that HDT-BR leads to an
20 immediate reduction in serum COMP concentration. COMP levels recovered to baseline
21 immediately after the unloading period during 21 days of HDT-BR. As suggested
22 previously²⁸, this finding may reflect a change in diffusion patterns due to reduced mechanical
23 loading of the lower limb in HDT-BR. Previous studies²⁹ have shown that serum COMP
24 levels are sensitive to the loading mode of physical activity and that, for instance, slow but
25 deep knee bends do not affect COMP levels. Moreover, COMP levels remain elevated after
26 intense and prolonged physical activities such as a marathon³⁰ or ultramarathon.³¹ In situ

1 experiments have shown that moderate cyclic tensile strain alters the extracellular matrix
2 organization and composition.³² Such adaptations have been observed in animal models
3 during 6 weeks of running training³³ and in obese patients with knee osteoarthritis after a 16-
4 week weight loss.³⁴ These results suggest that COMP is sensitive to the magnitude of load and
5 that adaptations may occur within the time frame of the current bed rest study.

6

7 Contrary to our hypothesis, we observed a decrease in MMP-3 and MMP-9 levels
8 during bed rest. MMPs degrade the cartilage matrix. For instance, native collagen II is
9 degraded by matrix MMP-1, -8, -13 and -14 and partially degraded collagen II is then further
10 degraded by gelatinases, namely MMP-2 and MMP-9 and stromelysin (MMP-3)¹⁷. A
11 presumably detrimental effect of immobilization on articular cartilage would be expected to
12 be associated with increased levels of catabolic MMPs. The observed decrease in MMP-3 and
13 MMP-9 levels at or close to the beginning of the HDT-BR period may be – similar to COMP
14 – explained by a reduced diffusion of molecules from the joint because of the lack of
15 movement³⁵ or by generally lower metabolic activity of chondrocytes during bed rest.
16 Moreover, MMP-3 gene-expression is reduced in osteoarthritic hip joints.³⁶

17

18 Interestingly, MMP-9 increased significantly on day 5 of HDT-BR, then returned to
19 levels below baseline and showed a second increase above baseline on day 21 of HDT-BR.
20 TNF- α increased above baseline on day 14 of HDT-BR and remained elevated into the
21 recovery phase. These changes were observed in both study phases and hence were consistent
22 for the nutrition intervention and the control interventions suggesting that these changes are
23 reproducible and did not occur by chance. Moreover, all participants completed exactly the
24 same daily routine on all days of the HDT-BR. This phenomenon has not been previously
25 reported for cartilage. This change in MMP-9 levels during the immobilization period may
26 indicate a delayed effect of immobilization on tissue metabolism potentially representing

1 increased cartilage degradation after a prolonged time of bed rest that may be triggered by
2 inflammatory component³⁷ indicated by elevated TNF- α levels as observed in the second half
3 of bed rest in our study. This result is particularly relevant for space flight because monitoring
4 inflammatory markers during space flight may be a potent measure for detecting initiation of
5 degenerative processes.

6

7 MMP-1 levels were unaffected by bed rest and by the nutrition interventions. This
8 result suggests that 21-day HDT-BR did not initiate cartilage changes similar to osteoarthritic
9 changes because in pathological conditions such as OA, MMP-1 is expressed at higher levels
10 by OA chondrocytes than by normal chondrocytes suggesting a predominant role of MMP-1
11 in OA pathogenesis.^{10,11} Moreover, MMP-1 appears not to be sensitive to loading suggesting
12 that pathways unrelated to mechanical load may affect MMP-1 expression.

13

14 The finding that MMP-3 and MMP-9 but not MMP-1 or TNF- α were lower during bed
15 rest than at baseline suggests that these biomarkers reflect different physiological pathways
16 affecting cartilage with a possible influence of microgravity on other tissues. Bed rest is
17 associated with different physiological changes including redistribution of fluids, lack of load
18 of the cardiovascular system and lack of gravitational load and movement associated cyclic
19 loads on musculoskeletal structures. It appears that the effects of these factors on a specific
20 tissue such as cartilage are complex. Nonetheless, the results of this study clearly showed that
21 bed rest does affect cartilage biomarkers, although the long-term consequences of these
22 changes remain unknown. Although the investigated enzymes and cytokines are not specific
23 to cartilage, altered loading may affect cartilage metabolism directly by their production by
24 chondrocytes or indirectly by systemic presence by production of other cells. These different
25 mechanisms cannot be elucidated from blood samples. However, the correlation of bed rest-
26 induced changes in MMP-9 and COMP levels and evidence of cartilage adaptation in studies

1 on extreme loading shown by changes in the same biomarkers³¹ and changes in the T2
2 relaxation times in magnetic resonance image sequences of articular cartilage³⁸ strongly
3 suggest cartilage adaptation through altered metabolism during periods of bed rest. These
4 results also support the theory that pathological pathways of degenerative disease are
5 multifaceted and cannot be attributed solely to mechanical factors.

6

7 Similar to other physiological aspects, the response of cartilage biomarkers to bed rest
8 was not affected by the nutrition interventions. For instance, in the NUC study the nutrition
9 intervention did not affect glucose intolerance³⁹, intervertebral disc properties and spine
10 muscle performance⁴ while potassium bicarbonate supplementation led to higher levels of
11 interleukin (IL)-2⁴⁰. In the MEP study, the nutrition countermeasure did not affect atrophy
12 and fiber type transition of the soleus and vastus lateralis muscles.³ Hence, the benefit of these
13 nutritional interventions on muscle and cartilage must be questioned.

14

15 The observed reduction in COMP, MMP-3 and MMP-9 but not in TNF- α and MMP-1
16 may be caused by altered cartilage metabolism in response to the lack of mechanical stimulus
17 during HDT-BR. Increases during bed rest in MMP-9 but not in MMP-3 or COMP suggest
18 that serum biomarker concentrations are not only affected by the lack of diffusion caused by
19 the immobilization but that the tissue metabolism changes with prolonged immobilization
20 shifting the balance between anabolic and catabolic processing towards net tissue loss. This
21 may reflect an inflammatory component independent of the nutrition intervention. Future
22 research should investigate the effects of exercise countermeasures on cartilage metabolism
23 with the ultimate goal to minimize negative effects of bed rest or spaceflight on cartilage
24 health.

25

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8

9 **Competing interests**

10 The authors declare no competing interest.

11

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1 **Figure Legends**

2 Figure 1. Changes in MMP-1, MMP-3, MMP-9 and TNF- α with and after 21-day bed rest in
3 the MEP study (N=9). MEP – medium-term bed rest whey protein; BDC – baseline data
4 collection; HDT – head down tilt; R – recovery; MMP – metalloproteinases; TNF – tumor
5 necrosis factor. ^a – significantly different from BDC; ^b – significantly different from R+1; ^c –
6 significantly different from HDT7; ^d – significantly different from HDT14; ^e – significantly
7 different from HDT21.

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9 Figure 2. Changes in serum COMP with and after 21-day bed rest in the NUC (N=7) and
10 MEP (N=9) studies. NUC – nutritional countermeasures; MEP – medium-term bed rest whey
11 protein; BDC – baseline data collection; HDT – head down tilt; R – recovery; COMP –
12 cartilage oligomeric matrix protein. ^a – significantly different from BDC; ^b – significantly
13 different from R+1; ^c – significantly different from HDT7; ^d – significantly different from
14 HDT14.

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Table 3. Mean (1 standard deviation) absolute serum concentrations of the biomarkers in the NUC study.

Parameter	Phase	BDC-2	HDT2	HDT6	HDT10	HDT14	HDT21	R+2
COMP (U/L)	control	4.41 (1.18)	3.81 ^{a,b} (0.90)	3.79 ^{a,b} (0.89)	3.84 ^{a,b} (1.01)	3.71 ^{a,b} (1.15)	3.43 ^{a,b} (1.11)	4.54 (1.13)
	intervention	4.73 (0.66)	3.93 ^{a,b} (0.49)	4.43 ^{a,b} (1.47)	4.16 ^{a,b} (1.37)	4.23 ^{a,b} (2.08)	3.81 ^{a,b} (1.73)	5.13 (1.62)

NUC – nutritional countermeasures; ^a – significantly different from BDC-2; ^b – significantly different from R+1; BDC – baseline data collection; HDT – head down tilt; R – recovery; COMP – cartilage oligomeric matrix protein; MMP – metalloproteinases; TNF – tumor necrosis factor.

Table 2. Mean (1 standard deviation) absolute serum concentrations of the biomarkers in the MEP study.

Parameter	Phase	BDC-1	HDT2	HDT3	HDT5	HDT7	HDT14	HDT21	R+1
MMP-1 (ng/mL)	control	4.08 (2.43)	3.80 (1.96)	3.76 (2.00)	4.56 (2.71)	4.25 (2.57)	4.21 (2.68)	3.66 (1.92)	3.85 (2.51)
	intervention	3.33 (1.74)	3.39 (1.93)	3.31 (2.29)	3.68 (1.80)	3.61 (1.91)	3.76 (1.80)	4.22 (2.42)	3.29 (1.59)
MMP-3 (ng/mL)	control	13.3 (5.86)	10.66 ^{ab} (4.15)	9.90 ^{ab} (3.67)	9.66 ^{ab} (3.67)	9.98 ^{ab} (4.28)	9.86 ^{ab} (3.81)	10.55 ^{ab} (5.18)	12.55 (6.01)
	intervention	12.69 (5.28)	10.38 ^{ab} (4.53)	10.45 ^{ab} (4.91)	9.96 ^{ab} (4.40)	10.23 ^{ab} (4.87)	10.20 ^{ab} (5.30)	11.27 ^{ab} (5.75)	13.51 (7.14)
MMP-9 (ng/mL)	control	278.57 (153.31)	210.55 (103.31)	193.48 ^{ab} (71.59)	269.22 ^{b,c,d} (116.40)	197.63 ^b (81.32)	232.86 ^b (100.70)	330.03 (188.10)	321.45 (196.60)
	intervention	285.03 (204.29)	205.78 (88.61)	188.55 ^{ab} (102.83)	240.78 ^{b,c,d} (137.76)	201.79 ^b (85.67)	204.30 ^b (126.38)	284.81 (199.20)	315.28 (154.92)
TNF- α (pg/mL)	control	1.61 ^b (0.42)	1.73 ^b (0.44)	1.63 (0.43)	1.67 ^b (0.54)	1.67 ^b (0.53)	1.95 ^a (0.51)	2.00 ^a (0.66)	1.98 ^a (0.37)
	intervention	1.52 ^b (0.27)	1.67 ^b (0.48)	1.87 (0.95)	1.54 ^{b,d} (0.31)	1.53 ^{b,d} (0.28)	1.66 ^a (0.38)	1.71 ^a (0.49)	1.92 ^a (0.57)
COMP (U/L)	control	5.98 (0.92)	4.88 ^{ab} (0.95)	4.33 ^{ab} (0.95)	4.51 ^{ab} (1.04)	4.51 ^{ab} (0.92)	4.78 ^{ab} (0.91)	4.75 ^{ab} (0.57)	6.11 (0.63)
	intervention	6.69 (1.25)	5.32 ^{ab} (1.07)	4.72 ^{ab} (0.96)	5.10 ^{ab} (1.24)	4.84 ^{ab} (1.13)	4.96 ^{ab} (1.18)	5.07 ^{ab} (1.13)	6.59 (1.14)

MEP – medium-term bed rest whey protein; ^a – significantly different from BDC; ^b – significantly different from R+1; ^c – significantly different from HDT7; ^d – significantly different from HDT14; BDC – baseline data collection; HDT – head down tilt; R – recovery; COMP – cartilage oligomeric matrix protein; MMP – metalloproteinases; TNF – tumor necrosis factor.

Table 3. Mean (1 standard deviation) absolute serum concentrations of the biomarkers in the NUC study.

Parameter	Phase	BDC-2	HDT2	HDT6	HDT10	HDT14	HDT21	R+2
COMP (U/L)	control	4.41 (1.18)	3.81 ^{a,b} (0.90)	3.79 ^{a,b} (0.89)	3.84 ^{a,b} (1.01)	3.71 ^{a,b} (1.15)	3.43 ^{a,b} (1.11)	4.54 (1.13)
	intervention	4.73 (0.66)	3.93 ^{a,b} (0.49)	4.43 ^{a,b} (1.47)	4.16 ^{a,b} (1.37)	4.23 ^{a,b} (2.08)	3.81 ^{a,b} (1.73)	5.13 (1.62)

NUC – nutritional countermeasures; ^a – significantly different from BDC-2; ^b – significantly different from R+1; BDC – baseline data collection; HDT – head down tilt; R – recovery; COMP – cartilage oligomeric matrix protein; MMP – metalloproteinases; TNF – tumor necrosis factor.

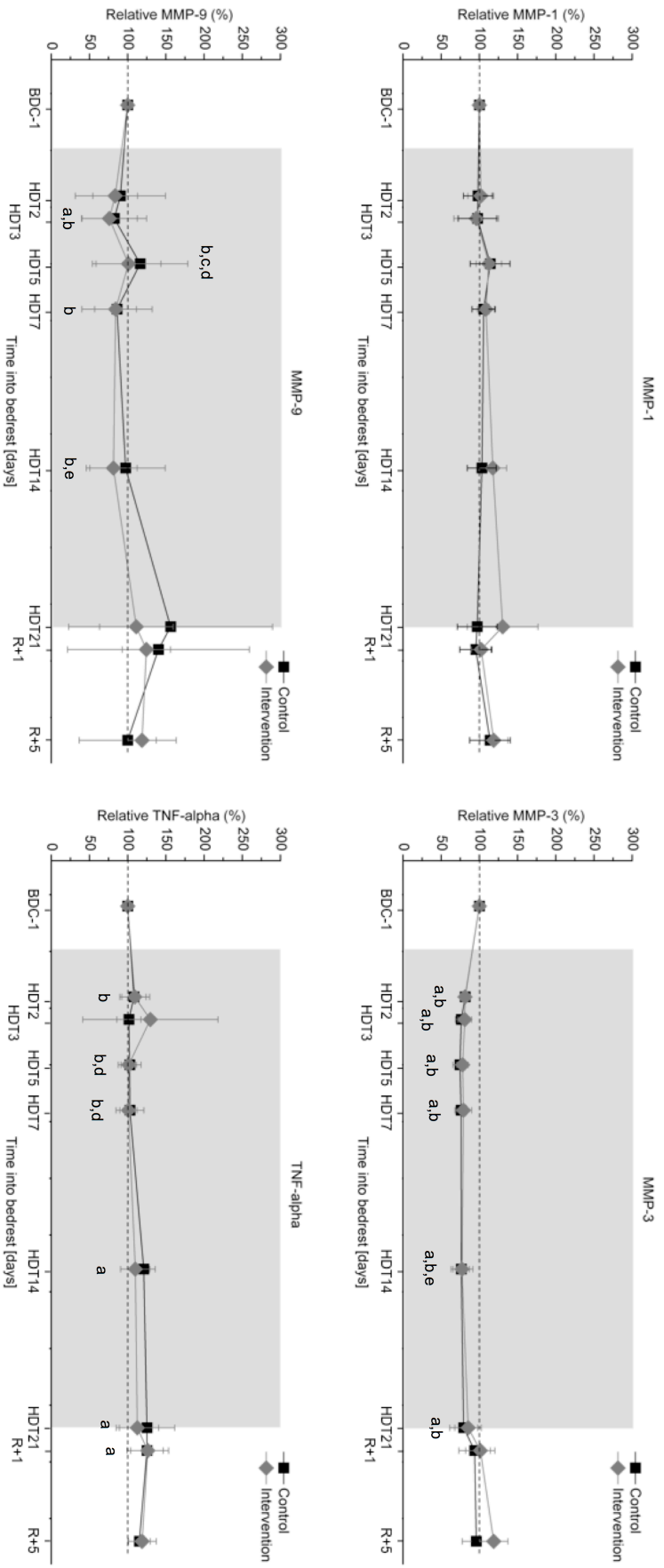


Figure 1

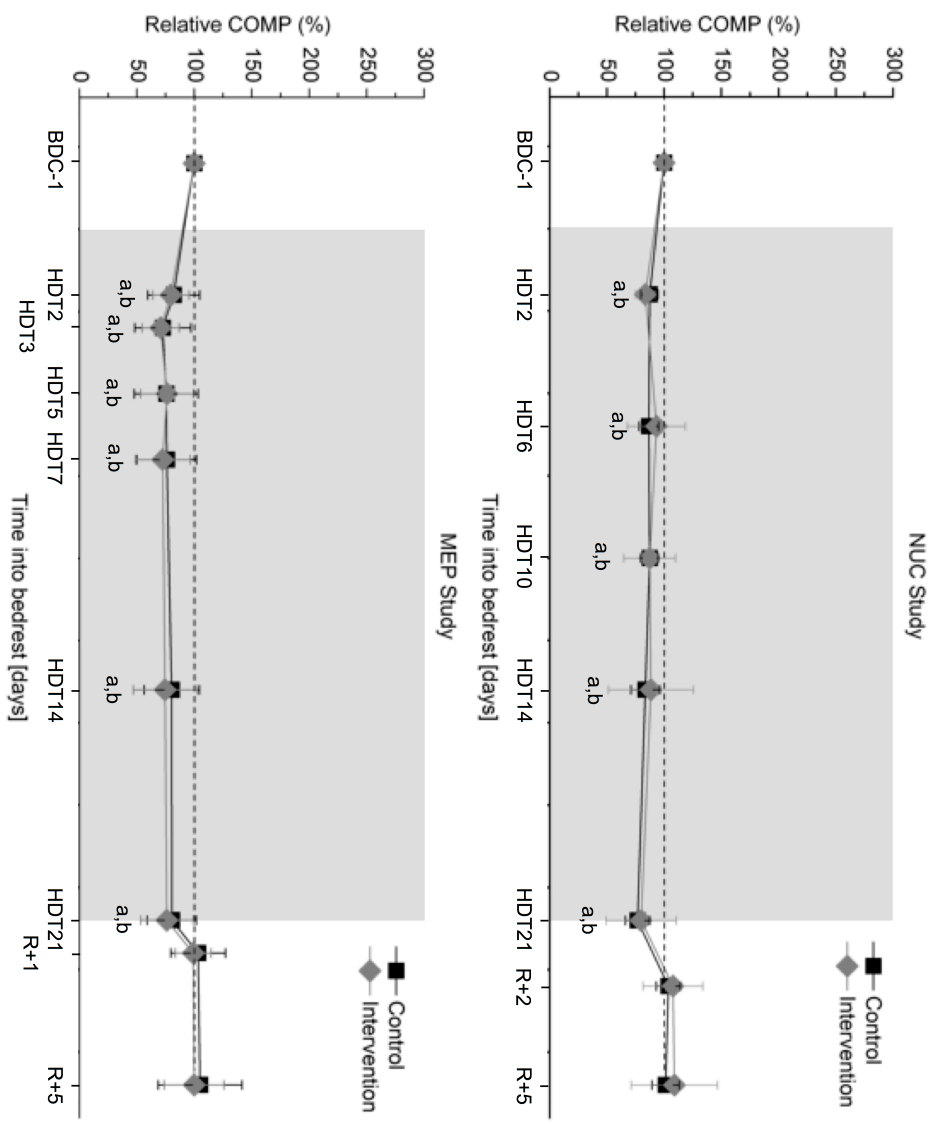


Figure 2