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Marrow adipose tissue gradient is preserved through high protein diet and bed rest. A randomized crossover study

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ABSTRACT

Context: Marrow adipose tissue (MAT) has a peripheral to central distribution in adults, higher in peripheral bones. Similarly, the spine has a caudal to cephalad MAT distribution, higher in lumbar vertebras. Diet and the level of physical activities are known modulators of MAT with significant impact on bone; however, whether these can modulate the MAT gradient is unknown.
Objective: To measure the effect of high protein diet and bed rest interventions on the lumbar MAT gradient.
Design, participants, intervention: In a prospective randomized crossover trial, 10 healthy men participated in 2 consecutive campaigns of 21days head-down-tilt-bed-rest (HDTBR). They received either whey protein and potassium bicarbonate-supplemented or control diet separated by a 4-month washout period.
Main outcome measure(s): Ten serial MRI measures of lumbar vertebral fat fraction (VFF) were performed at baseline, 10days and 20days of HDTBR and 3 and 28days after HDTBR of each bed rest campaign.
Results: The mean L5-L1 VFF difference of 4.2 ± 1.2 percentage point higher at L5 ($p = 0.008$) constituted a caudal to cephalad lumbar MAT gradient. High protein diet did not alter the lumbar VFF differences during both HDTBR campaigns (all time points $p > 0.05$). Similarly, 2 campaigns of 21days of HDTBR did not change the lumbar VFF differences (all time points $p > 0.05$).
Conclusions: This pilot study established that the lumbar vertebral MAT gradient was not altered by a high protein nor by 2 × 21days bed rest interventions. These findings demonstrated that this lack of mechanical stimulus was not an important modulator of the lumbar MAT gradient. The highly preserved MAT gradient needs to be measured in more situations of health and disease and may potentially serve to detect pathological situations.

1. Introduction

Bone marrow adipose tissue (MAT) is a large endocrine organ interacting with multiple systems from skeletal to hematological to cardiovascular (Rosen and Spiegelman, 2014; Cawthorn et al., 2014). Bone marrow conversion, the progressive replacement of hemopoietic marrow with marrow adipose tissue (MAT) proceeds throughout the human lifespan (Dunnill et al., 1967; Liney et al., 2007; Justesen et al., 2001; Tuljapurkar et al., 2011). Marrow adipose conversion progresses in a peculiar peripheral to central direction with bones of the hands and feet progressively losing hemopoietic function and only central bones remaining hemopoietically active in adulthood (skull, vertebras, sternum, ribs, pelvis, proximal humeri and femora) (Emery and Follett, 1964; Moore and Dawson, 1990; Waitches et al., 1994; Taccone et al., 1995). Even in these central bones, marrow conversion has been described to continue with aging in men and in women (Chan et al., 2016; Kugel et al., 2001). In the human spine, a similar caudal to cephalad MAT gradient from lumbar to cervical has consistently been reported

Abbreviations: MAT, marrow adipose tissue; HDTBR, head-down-tilt-bed-rest; VFF, vertebral fat fraction; MEP, whey protein study; DLR, German Aerospace Center; MR, magnetic resonance; BDC, baseline data collection; HDT, head-down tilt; R, recovery; IOP, in-phase and out-phase imaging; TR, repetition time; in-phase, echo time 1 (TE1); out-phase, echo time 2 (TE2); FOV, field of view; ROI, region of interest; PDFF, proton-density fat fraction

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over the past 10 years in cross-sectional studies (Liney et al., 2007; Ruschke et al., 2017; Li et al., 2011, 2014; Martin et al., 2014; Baum et al., 2015; Belavy et al., 2018). However, little is known about the role and modulation of the spinal MAT gradient in humans.

Besides age, a number of factors have been associated with altered rates of MAT conversion, the most prominent being dietary changes (Styner et al., 2014; Doucette et al., 2015; Adler et al., 2014; McCabe et al., 2019), physical activity (Belavy et al., 2018; Styner et al., 2014; McCabe et al., 2019; David et al., 2007; Menuki et al., 2008; Hu et al., 2014; Pagnotti and Styner, 2016), hormonal/metabolic changes (Cawthorn et al., 2014; Bredella et al., 2011), diabetes and antidiabetic medication (Kim and Schafer, 2016; Crossno et al., 2006; Harsløf et al., 2011), obesity (Bredella et al., 2011) and osteoporosis (Justesen et al., 2001; Meunier et al., 1971a; Griffith et al., 2006; Shen et al., 2007, 2012; Cohen et al., 2012; Patsch et al., 2013). Anorexia nervosa with extreme caloric restriction increases MAT conversion (Geiser et al., 2001; Bredella et al., 2009; Ecklund et al., 2010; Fazeli et al., 2010). Opposite to physical activity, immobility was found to increase MAT in various models and clinical situations (Wronski and Morey, 1982; Ahdjoudj et al., 2002; Devlin and Rosen, 2015; Trudel et al., 2009, 2012). The combination of 2 factors led to spectacular results: ovariectomized rats in a 14-day spaceflight increased the lumbar bone marrow adipose signal by 306% (Keune et al., 2016). However, we found no datum on the effects of the various modulators of MAT conversion on the lumbar MAT gradient in animal models or humans.

High protein and specifically whey protein supplementation effects on bone have been controversial (Kerstetter et al., 2015; Cuenca-Sánchez et al., 2015). Whey protein and $KHCO_3$ have successfully prevented muscle wasting during periods of bed rest and disuse (Stein et al., 1999; Buehlmeier et al., 2014). While caloric restriction and high fat diet accelerated bone marrow conversion, the effect of a high whey protein and $KHCO_3$ supplemented diet is unknown. Long durations of bed rest (60 days) have significantly accelerated bone marrow adipose conversion (Trudel et al., 2009, 2012). Bed rest not only decreases the axial loading on the lumbar spine but it also homogenizes the load over each vertebra (Supplemental Fig. 1). Consequently, should the lumbar vertebrae MAT gradient be modulated by mechanical forces, bed rest may equalize the MAT content of lumbar vertebrae and lead to a decrease or disappearance of the lumbar MAT gradient; this remains to be tested. The effect of shorter durations of bed rest (21 days), more common in clinical practice than 60 days, and the effect of two consecutive courses of bedrest on lumbar vertebrae MAT have never been measured. Finally, the effect of a combination of high protein and bed rest on the spinal MAT gradient is unknown.

MAT is increasingly associated with detrimental effects not only on bone but also systemically with conditions such as obesity, diabetes, aging, anemia, paraplegia and anorexia. Locally in the bone marrow, MAT can be detrimental to bone production (Ambrosi et al., 2017) and its expansion in mice decreased trabecular bone mass by 29% and cortical thickness by 5% (Tencerova et al., 2018). Since MAT and osteoblasts share a common stem cell, adipogenesis may be reciprocally linked to osteoblastogenesis (Rharass and Lucas, 2018; Turner et al., 2018).

Historically, marrow adipose content was studied using bone biopsies (Meunier et al., 1971b; Minaire et al., 1984; Burkhardt et al., 1987; Verma et al., 2002). In 1984, Dixon proposed a non-invasive method for quantifying MAT through magnetic resonance (MR) imaging, which corroborated histological measures (Dixon, 1984). Today, chemical shift-encoded imaging has demonstrated short- and long-term reproducibility to quantify the fat signal in the vertebral bone marrow (Maas et al., 2001; Li et al., 2016).

We therefore conducted a prospective clinical study on the effect of 2 interventions that can potentially affect MAT conversion, namely high protein diet and bed rest, on the lumbar MAT gradient. Our objectives were to measure the effects of 1) a whey protein and $KHCO_3$ nutritional intervention; and of 2) two consecutive periods of 21 days of head down tilt bed rest (HDTBR) on the lumbar vertebral fat fraction (VFF) of ten healthy men using serial MR in a crossover design. Our hypotheses were that 1) a whey protein and $KHCO_3$ diet, in isolation, will not alter the caudal to cephalad gradient of MAT across the lumbar spine and 2) 2 consecutive periods of HDTBR will decrease the MAT gradient.

2. Methods

2.1. Participants, inclusion and exclusion

10 healthy male participants responded to mailings and internet advertisements and were selected to participate in the whey protein study (MEP) at the German Aerospace Center (DLR) in Cologne, Germany (Buehlmeier et al., 2014). The study was approved by the local ethics committee (Ärztekammer Nordrhein; Düsseldorf, Germany) and the Ottawa Health Science Network Research Ethics Board (Ottawa, Canada). It was registered in ClinicalTrials.gov as Identifier: NCT01655979. The subjects' rights were protected in accordance with the Declaration of Helsinki. All subjects provided written informed consent. The primary objective of the MEP study was to prevent bone demineralization and muscle deconditioning during bed rest using a nutritional countermeasure. Within the confines of the bed rest study, multiple additional international investigations were selected, including ours as explained in the protocol publication (Buehlmeier et al., 2014). Extensive inclusion and exclusion criteria were previously described (Buehlmeier et al., 2014). Briefly, 10 participants were included from a general prescreening by telephone with n = 195 if they were males aged between 20–45 yr, with a body mass index 20–25 kg/m^2, and underwent physical, medical, and psychological screening. They were excluded if they had a history of smoking, drug consumption, alcohol excess, anemia, nutrient deficiency, and any muscle/cartilage/joint or psychiatric diseases.

2.2. Nutritional intervention

The nutritional intervention consisted of a high protein intake (1.2 g/kg body weight/d) plus whey protein (0.6 g/kg body weight/d; Diaprotein, Dr. Streudle, Linden, Germany) with alkaline salts (90 mmol potassium bicarbonate/d; Kreuger GmbH, Bergisch-Gladbach, Germany). Carbohydrates and fat were isocalorically replaced in a 1:1 ratio (Buehlmeier et al., 2014). The control group received 1.2 g/kg body weight/day of protein, which is higher than the recommended daily amount of 0.8 g/kg body weight/day (Buehlmeier et al., 2014). The participants were randomized 1:1 to nutritional intervention (n = 5) or control (n = 5) for a first campaign of 21 days of bed rest. The assignments were switched for the second campaign.

2.3. Inactivity intervention

The 10 participants laid in a bed inclined to -6° with their head down. Subjects were not allowed to sit or stand; at least one shoulder had to remain on the bed at all times. Compliance was ensured by study personnel, and 24/7 video surveillance (Buehlmeier et al., 2014). Following a randomized crossover design, each of the two bed rest campaigns consisted of 7 days of baseline data collection (BDC), 21 days of head-down tilt (HDT), and 6 days of recovery (R). A four-month washout separated the 2 campaigns. There was no change to trial design after trial commencement.

2.4. Measurement of Vertebral Fat Fraction (VFF)

A 1.5-T Siemens Symphony scanner (equipped with the syngo MR VB17A software) and the table built-in spine coil array was used to acquire MRI data from all lumbar vertebrae (L5, L4, L3, L2, & L1) in sagittal orientation (Hospital Porz am Rhein, Cologne, Germany). Data was obtained at five points in time for each campaign: prior to the start

of bed rest during baseline data collection (BDC), twice during head down tilt bed rest after 10 (HDT10) and 20 (HDT20) days, and twice in the recovery period: 3 (R + 3) and 28 (R + 28) days after bed rest. The data for lumbar VFF was acquired using a T_1-weighted 3D FLASH sequence (two echoes for in-phase and out-phase imaging (IOP)) with the following parameters: Repetition time (TR) = 10.60 ms, echo time 1 (TE1) = 4.76 ms (in-phase), echo time 2 (TE2) = 7.14 ms (out-phase), field of view (FOV) = 320 × 320mm^2, matrix = 256 × 256, in-plane resolution = 1.25 × 1.25mm^2, slice thickness = 2 mm, 30 slices, flip angle = 10°, averages = 2.

Both, the in-phase and the out-phase dataset were imported into ImageJ (64-bit Java 1.8.0_112, NIH, Bethesda, MD) for post-processing. The operator was blinded to the group assignment. For each vertebra, five central slices through the vertebrae were selected. Using ImageJ "ROI Manager", a standardized rectangular region of interest (ROI) was manually drawn into the cancellous bone of each of the lumbar vertebrae, avoiding the surrounding endplates, cortical bone and vascular artifacts. ImageJ function "Measure" provided the mean pixel intensity of each ROI in both datasets. The ROI mean pixel intensities were used to calculate the fat fraction of each vertebra (Cassidy et al., 2009; Peng et al., 2011). The VFF was used as a surrogate measure of vertebral MAT to assess changes between lumbar vertebras.

2.5. Data and statistical analyses

Data were analyzed using SPSS 25.0 (SPSS-IBM, Armonk, NY, USA). Aggregate data were reported as mean ± 1sem. The convenience sample size was decided by the study leaders, the European Space Agency and DLR. The sample size needed to detect a 1 percentage point difference between 2 adjacent vertebrae at significance level 0.05 with power 82% is n = 10 (Anon, 2019). The presence of a lumbar MAT gradient was tested by comparing the lumbar VFF distribution between the 5 lumbar vertebrae for both campaigns (1 and 2) and groups (diet, control) using oneway ANOVA with post-hoc Bonferroni. While our primary outcome was the L5-L1 VFF difference, secondary outcomes included the VFF difference between all other lumbar vertebrae (L5-L2, L5-L3, L5-L4, L4-L1, L4-L2, L4-L3, L3-L1, L3-L2 and L2-L1). The effect of diet on the lumbar VFF differences for each campaign was tested using unpaired t-tests at each time point. The effect of bed rest on the lumbar VFF differences was tested using repeated measures ANOVA with post-hoc Bonferroni. Finally, the effect of bed rest on VFF combining all lumbar levels from both groups were tested for each campaign using repeated measures ANOVA with post-hoc Bonferroni. Corrected p-values below 0.05 were considered statistically significant.

3. Results

In the first campaign, one subject missed MRI at HDT20, and the MRI data of four patients were lost at R + 28. On 3 scans, artifacts did not allow measuring the L1 VFF. In the second campaign, one participant allocated to the diet intervention did not return. The final number of participants and MR studies are found in Fig. 1 and Supplemental Table 1, respectively. Baseline demographic and clinical characteristics are found in Buehlmeier et al (Buehlmeier et al., 2014).

3.1. Identification of a lumbar MAT gradient

There was a statistically significant lumbar distribution of VFF (ANOVA, p = 0.008). Averaging data from the 10 MR measuring times of the study, the 10 participants maintained a mean L5-L1 VFF difference of 4.2 ± 1.2 percentage point higher at L5 (p = 0.008; Fig. 2; Supplemental Table 1). The VFF was progressively lower in a caudal to cephalad direction constituting a lumbar MAT gradient (Fig. 2). The L3-L1 VFF difference was also statistically different (3.5 ± 1.2 percentage points, p = 0.046; Fig. 2).

3.2. Effect of high protein diet on the lumbar spine VFF gradient

In both the first and second campaigns, the $KHCO_3$ / whey protein supplemented diet had no statistically significant effect on the L5-L1 VFF difference nor on any other lumbar vertebral VFF difference; all p > 0.05 (Fig. 3).

3.3. Effect of bed rest on the lumbar spine VFF gradient

In both the first and second campaigns, 10 and 20 days of bed rest and 3 and 28 days of recovery after bed rest had no statistically significant effect on the L5-L1 VFF difference nor any other lumbar vertebral VFF differences across the 10 time points; all p > 0.05 (Fig. 4).

3.4. Effect of bed rest on lumbar spine VFF

In the first campaign, the mean VFF for all lumbar vertebras (L5 to L1) and for both groups (diet and control) did not significantly change throughout the 21 days of bed rest and 28 days of recovery (Fig. 5). In the second campaign, similarly, the lumbar VFF did not significantly change compared to the baseline VFF of Campaign 2. However, the Campaign 2 lumbar VFF HDT20 (34.4 ± 2.5 percentage points) was significantly lower than the Campaign 1 BDC and HDT10 VFF (respectively 38.6 ± 2.6 and 37.2 ± 2.6 percentage points; both p < 0.05; Fig. 5).

4. Discussion

This prospective human crossover study demonstrated a highly preserved MAT gradient in the lumbar spine unaffected by 2 previously recognized modulators of MAT content: diet and inactivity.

MAT conversion in humans has been described that progresses from peripheral to central bones (Li et al., 2011, 2014; Martin et al., 2014; Baum et al., 2015). Liney et al. (2007) first reported the peculiar feature of a L5-L1 gradient of MAT in a 57 years old subject (Liney et al., 2007). Li et al (2011) and later Li et al. (2014) measured a L4-L1 MAT gradient in osteoporotic as well as control patients (Li et al., 2011, 2014). Martin et al. measured a linear gradient from L5 to T11 in 5 patients; and from S4 to T10 in one of these patients (Martin et al., 2014). Baum et al (2015) reported in 38 subjects a spinal MAT gradient from L5 up to C3 (Baum et al., 2015). Finally, Ruschke showed that a lumbar to cervical gradient was established early, at median age 4.5 in 93 children (Ruschke et al., 2017). In the current trial, we confirm the results of previous studies and report a lumbar MAT gradient from L5 to L1 in healthy participants at the outset of the study.

There is currently no explanation for the existence of a spinal MAT gradient. Li (2014) had suggested that the lumbar MAT gradient may be part of the "peripheral to axial" conversion from red to yellow marrow with aging (Li et al., 2014). Or could it reflect the distribution of mechanical forces in the spine? In the erect position, mechanical loads decrease from the feet to the skull according to the share of body weight born by each bone. While mechanical stimulation was shown to modulate MAT conversion, this question is problematic for both the peripheral to central and for the caudal to cephalad MAT gradients (Belavy et al., 2018; Styner et al., 2014; McCabe et al., 2019; David et al., 2007; Menuki et al., 2008; Hu et al., 2014; Pagnotti and Styner, 2016). Distal bones bearing higher loads than proximal would predict highest MAT at the skull and lowest at the feet. As per the literature and the current study, the reverse has been systematically reported (Li et al., 2011, 2014; Martin et al., 2014; Baum et al., 2015); defying a primarily mechanobiology mechanism. There is currently no valid explanation for the MAT gradient phenotype. And little do we know of its modulation by mechanical forces. The human literature thus far has consisted of cross-sectional studies that prevented from commenting on the evolution of the MAT gradient over time or on the effect of interventions. We conducted the first prospective analysis of the effect of high protein diet

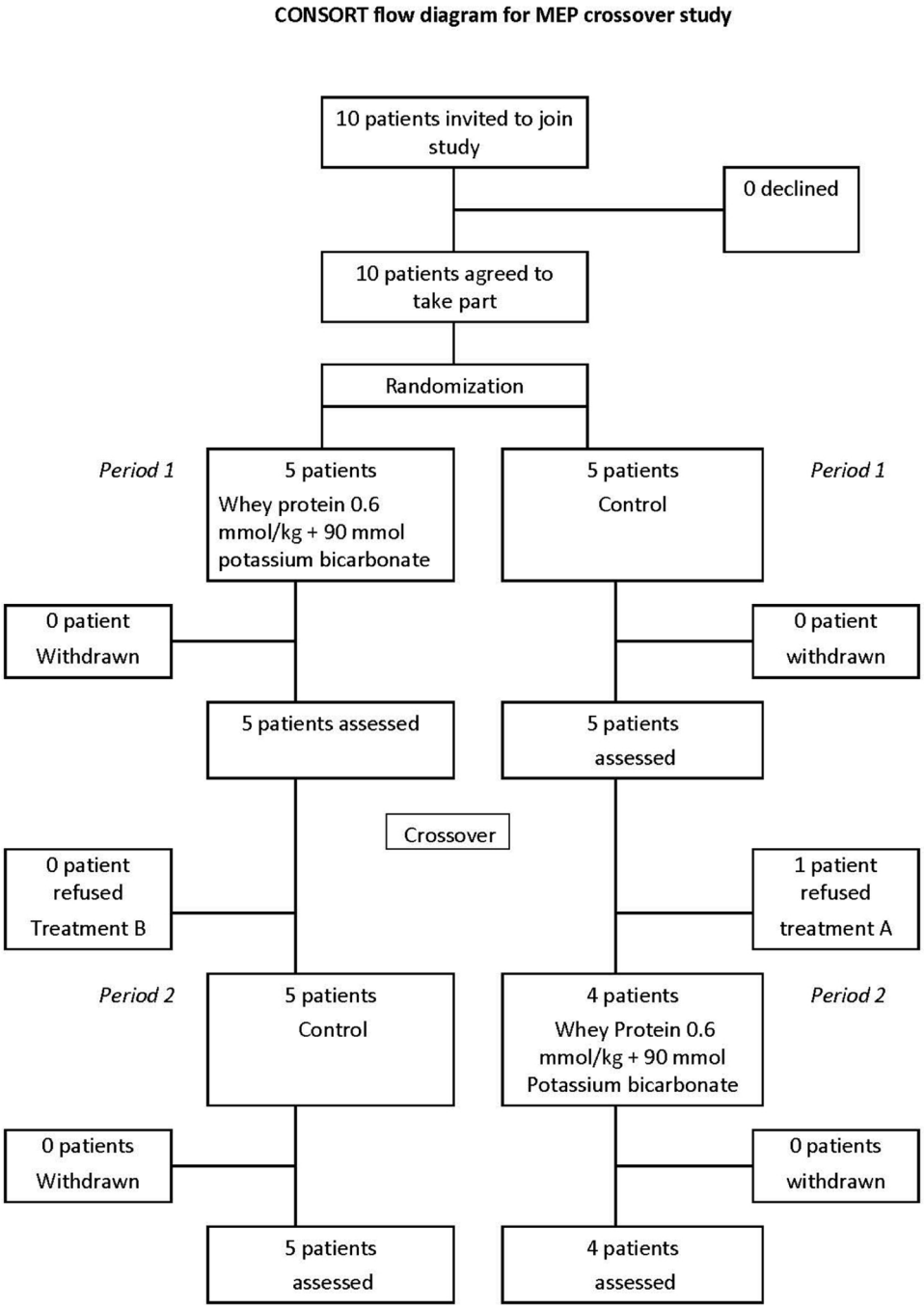

Fig. 1. Consort flow diagram.

and bed rest on the lumbar MAT gradient.

4.1. Effect of high protein diet on the MAT gradient

Dietary modifications leading to severe weight loss have been associated with high bone MAT (Geiser et al., 2001; Bredella et al., 2009; Ecklund et al., 2010). Curiously, experimental studies feeding a high lipid diet also rapidly accrued the bone MAT (Styner et al., 2014; Doucette et al., 2015; Adler et al., 2014; McCabe et al., 2019). These experimental studies were all conducted in mice. In humans, women with high body mass index and high visceral fat also had high spinal MAT; although their dietary intake was not specified (Bredella et al., 2011). Those studies support the influence of dramatically lower and higher caloric intakes to increase MAT. High protein diets may benefit bones through increased calcium absorption, bone turnover, and production of insulin-like growth factor 1 or may be harmful through bone acid buffering (Cuenca-Sánchez et al., 2015). However, no change was found in the lumbar spine bone mineral density in 208 older women on a whey protein supplement for 18 months (Kerstetter et al., 2015). In a different study combining bedrest and a high protein, leucine-supplemented diet, 8 healthy women aged 25–40, showed no change in lumbar VFF (Trudel et al., 2009). In the current study in healthy men a diet enriched in whey protein and supplemented with $KHCO_3$ did not alter the highly preserved caudal to cephalad gradient of MAT across the lumbar spine. These results confirmed our first hypothesis. The efforts made to ensure that the high protein diet remained isocaloric may explain the absent modulation compared to caloric-restricted or high lipid diets. MAT modulation arose from high lipid or weight-reducing

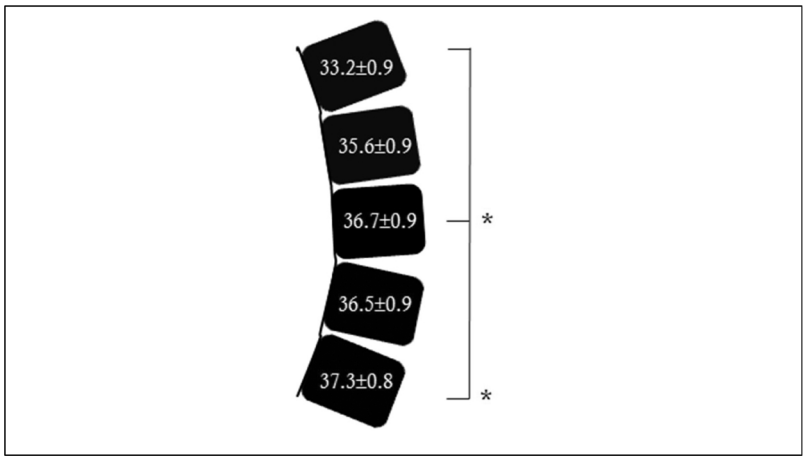

Fig. 2. Identification of a MAT gradient in the lumbar spine. 10 male participants had their lumbar spine imaged 10 times over 7 months. The average ± 1sem of the 10 measures are illustrated. The VFF decreased from L5 to L1 in caudal to cephalad direction creating a MAT gradient. The L5-L1 and the L3-L1 VFF differences reached statistical significance. *p < 0.05 after Bonferroni correction of all possible lumbar VFF differences.

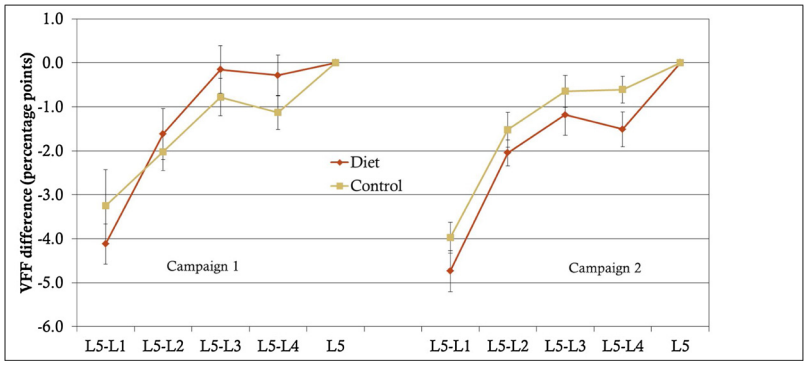

Fig. 3. Effect of a high protein diet on the lumbar MAT gradient. VFF decreased from L5 to L1 in both the diet and control groups, and in both campaigns. The high protein diet intervention had no effect on all lumbar vertebras VFF differences. n = 10 in the first campaign and n = 9 in the second campaign. Error bars = 1 sem.

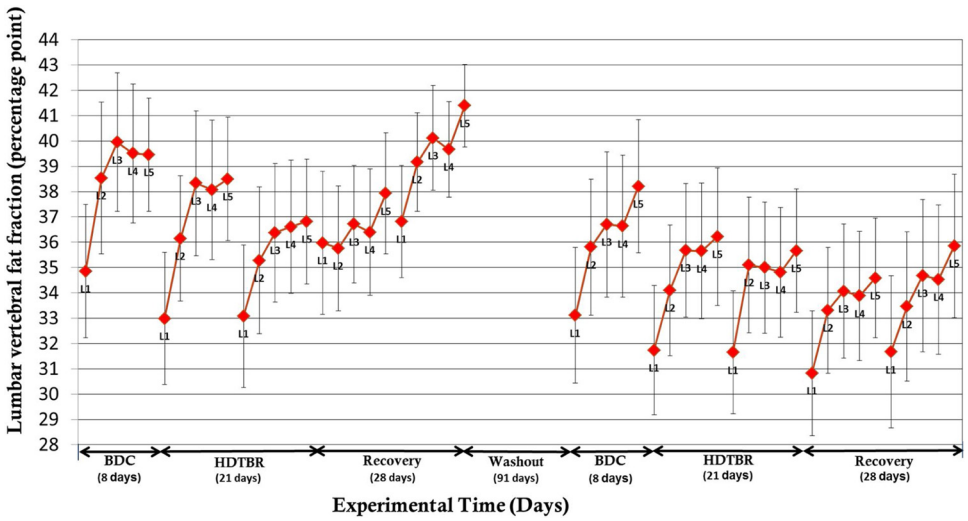

Fig. 4. Effect of two campaigns of 21 days of bed rest on the lumbar MAT gradient. At study onset, the 10 participants had a L5-L1 VFF difference of 4.5 ± 0.7 percentage point higher at L5. The lumbar VFF difference from L5 to L1 was preserved throughout 2 consecutive campaigns of 21 days of bed rest as were all other lumbar vertebral VFF differences across the 10 time points. Data includes participants receiving the high protein and the control diet; n = 10 in the first campaign and n = 9 in the second campaign. Error bars = 1 sem.

diets that were not isocaloric (Styner et al., 2014; Doucette et al., 2015; Adler et al., 2014; McCabe et al., 2019; Geiser et al., 2001; Bredella et al., 2009; Ecklund et al., 2010; Fazeli et al., 2010). In addition, the combination of the 2 interventions high protein diet and bed rest did not have a detectable effect on the lumbar MAT gradient, indicating a high preservation.

4.2. Effect of bed rest on the MAT gradient

Inactivity in the form of HDTBR increased lumbar MAT in 2 previous studies (Trudel et al., 2009, 2012). With 60days of bed rest both women and men increased lumbar VFF by 2.5 and 3.3 percentage points respectively. These studies support that spinal unloading from prolonged bed rest modulated MAT. In athletes, higher VFF was reported in non-load bearing (cyclists) compared to loadbearing athletes (joggers and long-distance runners) (Belavy et al., 2018). 21 consecutive days of 24 h/day in the 6° antiorthostatic position constituted a draconian change in loadbearing for the spine. Removing 8 h of sleeping time, active individuals spend approximately 16 h/day sitting or standing and undergo repeated spinal cyclical loads during walking or physical activity. During 6° head down tilt bed rest, axial loading forces on the spine are minimized and slightly inverted. Redirecting

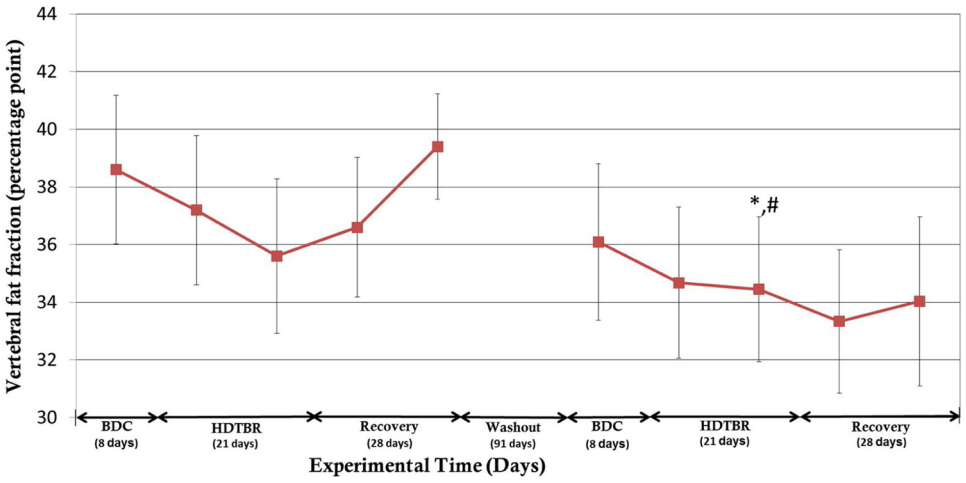

Fig. 5. Effect of 2 campaigns of 21 days of bed rest on the lumbar VFF. In Campaign 1, 21 days of bed rest did not change significantly the mean lumbar VFF for participants in both groups (diet and control) and all vertebral levels (L5 to L1). Campaign 2 started at a lower VFF level than Campaign 1. In Campaign 2, similarly, 21 days of bed rest did not change significantly the lumbar VFF compared to the baseline of Campaign 2. *$p < 0.05$ compared to BDC of Campaign 1; #$p < 0.05$ compared to HDT10 of Campaign 1; $n = 10$ in the first campaign and $n = 9$ in the second campaign. Error bars = 1 sem.

gravity perpendicular to the long axis of the spine not only decreased the overall spinal loading but also equalized the loads borne by individual lumbar vertebrae (Supplemental Fig. 1). The bed rest model appears therefore valid for studying the effect of unloading on lumbar MAT gradient. We reported that 21 days of 6° antiorthostatic bed rest did not alter the L5-L1 VFF difference or the VFF difference between any 2 lumbar vertebra, our primary and secondary outcomes, respectively. These results did not confirm our second hypothesis that 2 campaigns of 21 days of bed rest would decrease the caudal to cephalad gradient of MAT across the lumbar spine. The L5-L1 VFF difference before the first bed rest campaign began was 4.5 percentage points. 28 days after the second bed rest campaign was terminated, it was 4.2 percentage points, with no significant change throughout both campaigns (Fig. 4). These data are comparable to previously reported lumbar VFF and MAT gradients in healthy adult men (Ruschke et al., 2017; Belavy et al., 2018). These results do not support a mechanical loading hypothesis for the establishment nor for the modulation of the lumbar MAT gradient.

4.3. Effect of bed rest on VFF

There was no statistically significant change in lumbar VFF after a first campaign of 21 days of bed rest. A second 21 days of bed rest closely reproduced the effects of the first campaign. However, a 4 month washout during which participants returned to habitual activities saw Campaign 2 start at a significantly lower VFF. Two studies showed increased VFF after 60 days of HDTBR (Trudel et al., 2009, 2012). The shorter duration of HDTBR in the current study may have been insufficient to cause a measurable change in lumbar MAT. Second, the crossover design is rare among bed rest studies and could have affected the results; the unknown level of activity during the 4-month washout period between the 2 legs of the crossover study may explain the lower Campaign 2 baseline VFF (Fig. 5). Third, a large amount of blood is drawn during HDTBR studies for various investigations. While the blood drawn per campaign compares to other studies, the two campaigns in this crossover study doubled the blood drawn per participant. In the current study, 1.262 liters of blood were drawn from each participant over 7 months. Large blood losses can trigger hematopoietic hyperplasia, described in the lumbar spine of endurance athletes with high hemopoietic demand (Caldemeyer et al., 1996; Altehoefer et al., 2002). Importantly, even a putative hematopoietic hyperplasia had no effect on the MAT gradient.

4.4. Clinical implications

The clinical importance of MAT is increasingly recognized because of its close association with multiple common acute and chronic conditions such as osteoporosis, obesity, diabetes, aging, anemia, paraplegia, anorexia and metabolic syndrome. Locally in the bone marrow, MAT can be detrimental to bone and hemopoietic production (Ambrosi et al., 2017; Naveiras et al., 2009). Systemically, MAT contributes to serum levels of adiponectin which plays salient roles in obesity, hypertension, glucose metabolism, longevity and cancer (Cawthorn et al., 2014; Ambrosi et al., 2017; Reagan and Rosen, 2016; Holland et al., 2011; Ohashi et al., 2011; Yamauchi and Kadowaki, 2013; Lin et al., 2013; Tilg and Moschen, 2006). Beyond MAT quantity, more attention is being devoted to the distinction and overlap between constitutive (more peripheral) and regulated MAT (more central) (Yu et al., 2019; Scheller et al., 2015). Constitutive MAT was found to have higher unsaturation index that increased with age and to be unaffected by physical activity (Scheller et al., 2015; Huovinen et al., 2015). Here we demonstrate a highly preserved distribution gradient of MAT in the lumbar spine. Similar to the peripheral to central MAT gradient, the spine has a caudal to cephalad distribution gradient. Given how robust the MAT gradient was to combined inactivity and dietary interventions, finding a decreased or reversed lumbar MAT gradient may indicate disease. More attention will need to be devoted to measuring the spinal MAT gradient in various conditions of health and disease.

4.5. Limitations

The sample size of this study was small but the power adequate to detect between-vertebrae VFF differences. There was no exercise countermeasure involved in this study, which has previously countered the increased MAT caused by bed rest (Trudel et al., 2012). No datum was collected past 28 days of recovery in the second campaign therefore the study does not inform on long term effects of the high protein diet or bed rest. Newer sequences often referred to as proton-density fat fraction (PDFF) are measuring fat fraction unbiased by different water and fat relaxation times (Reeder et al., 2012). The PDFF helps making VFF data more comparable between studies acquired at different field strength and sites. In this trial, we corrected the VFF according to T1 and T2* calculations from the literature (Le Ster et al., 2016) and found identical outcomes. Men have higher MAT than women in the range 24–54 years old (Ishijima et al., 1996) and only men were tested in this study; the results may not apply to women.

5. Conclusions

These results confirmed the MAT gradient in the lumbar spine of healthy men. This first interventional prospective pilot study adds that the combined effect of a high protein diet and two consecutive periods of strict bedrest, did not alter the lumbar MAT gradient. This trial did not support that mechanical stimuli were important in establishing or

modulating the lumbar MAT gradient. The highly preserved MAT gradient needs to be measured in more situations of health and disease and may potentially serve to detect pathological situations.

Disclosures

The authors have nothing to disclose.

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Authors' roles

Study design: GT, AS, OL; Data collection: GT, AS; Data analysis: GT, GM, AS, OL, TR; Data interpretation: GT, AS, OL; Drafting manuscript: GT; Approval and final version of manuscript: GT, GM, AS, TR OL.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bonr.2019.100229.

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