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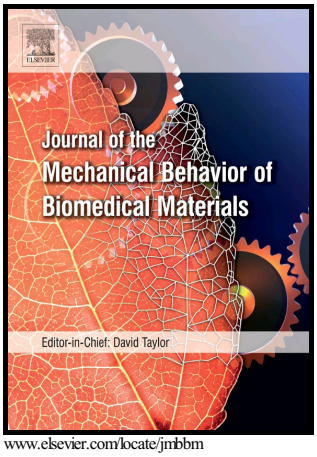

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Cytokine IL1α and lactate as markers for tissue damage in spineboard immobilisation. A prospective, randomised open-label crossover trial

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Abstract

Background

Spinal immobilisation using a rigid long spineboard is a well-established procedure in trauma care. During immobilisation, the body is exposed to high tissue-interface pressures. This may lead to a localised inflammatory response of the skin, which may be used to monitor the body's response to different types of immobilisation device.

Aim

In this study we compared the standard rigid spineboard with a new soft-layered spineboard regarding tissue-interface pressures, skin redness as an indicator of reactive hyperaemia and cutaneous IL1α and lactate release.

Methods

Twelve healthy male participants were asked to lie supine on both a rigid and a soft-layered spineboard, loading the sacrum for one hour, followed by one hour in unloaded position. Tissue-interface pressures on the buttocks during loading were measured continuously using a pressure mapping mat. Cutaneous IL1α and lactate concentrations were assessed using Sebutapes, during 20-minute periods. After each

20-minute period, a photo of the buttocks was taken, which was later assessed for redness by two observers.

Results
Significant differences in tissue-interface pressure and reactive hyperaemia were found between the two types of spineboard. Release of IL1α and lactate were found to increase with prolonged exposure to pressure, and to decrease in the unloaded prone position. A significant relationship was found between tissue-interface pressure and reactive hyperaemia, but not with IL1α nor lactate release. Time course of IL1α and lactate release was similar for both types of spineboard.

Conclusions
IL1α and lactate both have a strong relationship with pressure exposure time, but not with pressure magnitude. Furthermore, IL1α was measured even in the absence of visible redness of the skin. The study offers the potention of biomarkers , reflecting inflammation and/or tissue metabolism, for use in assessing the effects of prolonged spineboard support.

Introduction

The use of spinal immobilisation with long spineboards and cervical collars in the extrication and transfer of trauma patients is a well-established standard precaution in both pre-hospital and in-hospital protocols [1, 2]. These methods are considered particularly essential for unconscious trauma patients, who are unable to maintain spinal alignment by muscle tone and thus need to be protected from any subsequent spinal injury. Although there is a general consensus that the patient should be removed from the spineboard as soon as reasonably possible [1, 3, 4], the average time they are reported to be supported on a spineboard has been estimated to be approximately one hour [5-7] but in some cases may be markedly longer [6, 8]. Prolonged immobilisation on the spineboard causes significant discomfort and pain [9] and, on occasions, may cause pressure ulcers to develop adjacent to bony prominences [8]. These ulcers are painful [10-12] and debilitating for the patient [13, 14] and take a long time to heal [15-17], resulting in prolonged hospitalisation [18, 19] and reduced quality of life [20-23].

Pressure ulcers can be caused by a number of mechanisms including oxygen and/or nutrient deprivation and cell damage caused by pressure [24-26]. They can be initiated either superficially at the skin surface and progress downwards or in deeper

soft tissue layers adjacent to bone prominences and progress up towards the the skin surface. Lying on a rigid surface compromises the ability of the soft tissues (skin, muscles, fat) to adapt to the shape of the surface, resulting in high tissue-interface pressures [27, 28], which can lead to high compressive and shear strains within the tissues [29-31]. Although the relation between (high) tissue-interface pressures and the onset of pressure ulcers remains debated, whether it is either the superficial or deep-tissue injury [30], high tissue-interface pressures may lead to closure of capillaries, thereby depriving tissues of oxygen and other vital nutrients, which will eventually lead to cell damage. Furthermore, the high strains themselves may cause cell damage directly [32, 33], adding to the risk of developing pressure ulcers. This cell damage triggers an inflammatory response involving the release of cytokines, such as IL1α, IL1RA and IL-8, into the skin. Previous studies [34, 35] showed that IL1α can be detected after relatively short periods of loading time (<2 hours) with its release related to the magnitude of pressure, and its release is up-regulated over sacral sites at which pressure ulcers are observed [36, 37]. In addition, high pressures and high tissue strains can disturb the balance between oxygen delivery and consumption, thereby increasing lactate production [38]. Cytokines and lactate can be collected non-invasively in humans with the use of Sebutapes [35, 39, 40], self-adhesive patches specifically developed to absorb sebum, which can yield a number of proteins including cytokines. Previous research by our group showed that lying on a rigid spineboard results in high tissue-interface pressures [27], increased reactive hyperaemia [28] and increased tissue strains at the sacrum [29]. The hypothesis for the present study was that lying on a rigid spineboard would result in an elevated release of IL1α and lactate as a result of the increased tissue-interface pressures when compared to lying on a soft-layered spineboard. In addition, we hypothesised that there would be a relationship between the pressure-induced reactive hyperaemia and the IL1α and lactate concentrations.

Methods

Study design

We performed a prospective, randomised open-label crossover study on healthy participants to evaluate a number of parameters including tissue-interface pressures, redness of the skin, and IL1α and lactate concentrations as measured on both a standard rigid long spineboard and a soft-layered long spineboard. The study was approved by the Medical Research Ethics Committee of the Maastricht University Medical Centre and registered as NTR4537 in the Dutch Primary Registry.

Participants

Following the rationale presented by Julious [41] for pilot studies where no prior information is available, a sample size of 12 was selected. The participants were recruited through advertisements and word of mouth. Inclusion criteria were male sex, age 20-30 years, BMI 19-25 kg/m^2 and Caucasian ethnicity. Exclusion criteria included individuals with a history of pressure ulcers and skin conditions such as eczema, rashes or psoriasis with local expression on the buttocks/sacrum. Written informed consent was obtained from all participants before enrolment in the study.

Materials and interventions

Participants were randomly assigned to the order in which the rigid and soft-layered spineboard were to be tested. Testing took place in two separate sessions. Two different support devices designed for prehospital trauma care were used: a standard long spineboard (Ferno-Washington, Wilmington, OH), and a prototype soft-layered long spineboard [27-29]. All tests were done in the same climate-controlled room, which was kept at a temperature of 23.2 ± 0.4 °C. Each session started with a 15-minute acclimatisation period, during which the buttock remained completely unloaded.

The area of interest surrounding the bony prominence of the sacrum was marked with a surgical pen. At the end of the acclimatisation period, four Sebutapes were placed on the skin in the marked-out area, defined as top left, top right, bottom left and bottom right (Figure 1), and fixed in place using a custom-made pressure-roller, specifically designed to minimise the pressure onto the skin while applying the Sebutpaes [36]. After one minute, each Sebutape was retrieved, placed in a coded vial and stored on dry-ice. The Sebutapes were then replaced and the participant lay supine on the spineboard for another 20 minutes, after which the Sebutapes were once again replaced. This procedure was repeated three times, allowing for a total loaded period of 1 hour. It was then repeated three more times at 20-minute intervals while the buttocks remained unloaded, to monitor the reperfusion during which the volunteers were allowed to either stand or lie prone. At the end of the test session, the vials with Sebutapes were placed in a -80°C freezer and stored until further processing. The second test session was planned a week later, and at this session the other device was tested in a manner identical to that used in the first session.

The Sebutape extraction process was based on the protocol reported by Perkins and colleagues [40]. Briefly, the vials with tapes were thawed to room temperature, after which 2 mL phosphate buffered saline was added to each vial. After 1 hour, the tapes were sonicated for 10 min at 20°C, vigorously vortexed for 2 min and additionally mixed with a pipette tip. IL1α concentration in the samples was measured using a

commercially available human IL1α/ IL1F1 enzyme-linked immunosorbent assay (ELISA) kit (DuoSet R&D system) with a detection range of 3.9-250 pg/mL. IL1α levels below the detection threshold were recorded as zero for statistical testing. The amount of total protein (TP) collected on each Sebutape was measured using a Thermo Scientific TM Micro BCA Protein Assay Kit (Pierce Biotechnology) with a detection range of 0.5-20 µg/mL.

Tissue-interface pressures were continuously measured using a Tekscan Conformat model 5330 pressure-mapping device (Tekscan Inc, Boston, MA, USA). This system consists of a thin, easily foldable seat-sized pressure-mapping mat, equipped with 1024 capacitive sensors. This mat was placed between the participant and the spineboard at the level of the buttocks. The mat was connected to a laptop computer with special Tekscan software (version 7.20), for real-time pressure monitoring.

Lactate concentrations in the Sebutape samples were enzymatically analysed using the Cobas Fara Centrifugal Spectrophotometer (Roche Diagnostica, Basel, Switserland).

After each 20-minute period, the back of each participant was visually inspected and the skin redness was semi-quantitatively graded. Any redness was tested for being blanchable using a clear plastic disc, so as to distinguish between blanchable and non-blanchable erythema [36]. Photographs of the buttocks were made to permit an independent second reviewer to quantify and qualify the presence of redness.

Outcome measures

IL1α in pg/mL and lactate in mmol/L were measured at baseline and subsequently after every 20-minute period. Total protein (TP) was measured in µg/mL at the same time points. To correct for sebum uptake by the Sebutapes, IL1α/(TP)- ratio was calculated as IL1α concentration (pg/mL) divided by the total protein (TP) concentration (µg/mL). IL1α /TP ratio was used as a standardised measure to compensate for inter-individual excretion differences.

Pressures in mmHg were recorded continuously during the loaded period for each sensor, as described in previous studies [27, 28]. Peak pressure (in mmHg) was defined as the sensor showing the highest pressure in the sacral area in the first recording of every 20-minute period. Peak Pressure Index (PPI) in mmHg was defined as the area of 3x3 sensors which included the sensor showing the peak pressure and which had the highest average pressure including this sensor (figure 2a and 2b). The area was identified based on the first recording of every 20-minute period in the loaded condition. By assessing the values over a larger area (1950 mm^2) than that of a single sensor 216 mm^2), the PPI parameter provides a more reliable indicator of the pressure distribution behaviour of support surfaces than peak pressures alone [42]. Pressures were analysed for the areas covered by the Sebutapes, as well as for the sacral location

with the highest pressure, regardless of the location on the buttocks (top left, top right, bottom left, bottom right).

As a clinical measure of tissue response, redness of the skin was scored after every 20-minute period, and recorded as absent, diffuse or clearly defined, and further categorised in terms of blanchable or non-blanchable. Redness of the skin was assessed independently by two of the authors (BH and PB) blinded for the spineboard allocation. Inter-rater reliability was tested using Cohen's kappa.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, IBM), version 20.0.0.1. Pressures are presented as mean ± SD for each 20-minute time period and for each Sebutape location; lactate concentrations and IL1α/TP ratio are presented as mean ± SD for each 20-minute time period; redness scores are presented in categories for each 20-minute time period. Statistical analysis of tissue-interface pressures, IL1α/TP ratio and lactate concentrations was performed using repeated-measures analysis of variance with correction of the degrees of freedom using the Greenhouse–Geisser estimates of sphericity when Mauchly's test indicated that the assumption of sphericity had been violated. The analysis used time as the within-group factor and type of spineboard as the between-group factor. Post-hoc group comparisons at the different time points were only performed when the overall repeated-measures tests were statistically significant. All scores were tested for normality using the Kolmogorov–Smirnov test. Parametric variables were compared using Student's t-test, while non-parametric variables were compared using the Mann–Whitney U statistic, Wilcoxon signed-rank test and Spearman rank correlation. Nominal variables were compared across independent groups using the chi-squared test or Fisher's exact test. Homogeneity of variance was assessed using Levene's test. Level of significance was set at $P < 0.05$.

Results

Tissue-interface pressures

Both mean peak tissue-interface pressures and peak pressure indexes were higher on the rigid spineboard than on the soft-layered spineboard, for the majority of locations and time points (*P*s < 0.05; Tables 1 and 2).

Table 1. Mean ± standard deviation peak pressure and Peak Pressure Index in mmHg for the rigid spineboard, in relation to location of measurement (relative part of the buttocks) and duration of loading

Rigid spineboard	**Peak pressure**			**Peak pressure index**		
Location	20 mins loaded	40 mins loaded	60 mins loaded	20 mins loaded	40 mins loaded	60 mins loaded
Top left	215 ± 144	208 ± 134	251 ± 157	234 ± 128	212 ± 126	252 ± 138
Bottom left	138 ± 62	163 ± 135	127 ± 76	191 ± 96	202 ± 106	180 ± 84
Top right	78 ± 89	84 ± 48	138 ± 93	105 ± 82	102 ± 53	161 ± 91
Bottom right	117 ± 98	78 ± 46	93 ± 73	144 ± 99	117 ± 64	144 ± 71
Max sacrum	337 ± 110	314 ± 122	344 ± 124	284 ± 104	254 ± 102	282 ± 117

Table 2. Mean ± standard deviation peak pressure and Peak Pressure Index in mmHg for the soft-layered spineboard, in relation to location of measurement (relative part of the buttocks) and duration of loading

Soft-layered spineboard	**Peak pressure**			**Peak pressure index**		
Location	20 mins loaded	40 mins loaded	60 mins loaded	20 mins loaded	40 mins loaded	60 mins loaded
Top left	28 ± 11	23 ± 5	28 ± 11	34 ± 10	30 ± 7	35 ± 8
Bottom left	35 ± 8	34 ± 10	34 ± 10	39 ± 11	36 ± 10	38 ± 7
Top right	33 ± 8	34 ± 25	27 ± 10	35 ± 6	34 ± 9	31 ± 5
Bottom right	32 ± 10	36 ± 8	37 ± 10	35 ± 7	37 ± 5	37 ± 7
Max sacrum	66 ± 15	66 ± 15	62 ± 12	42 ± 9	45 ± 9	44 ± 7

On the rigid spineboard, mean tissue-interface pressures and pressure indexes differed significantly between the Sebutape locations at all time point ($P < 0.05$), but remained constant over time. On the soft-layered spineboard, pressures and pressures indexes were similar across locations and time points ($P > 0.05$ in all cases).

On the rigid spineboard, mean tissue-interface pressures and pressure indexes at the sacral area were higher than those to the right of the sacrum ($P < 0.05$). On the soft-layered spineboard, maximum pressures at the sacrum were higher than those measured at all four Sebutape locations ($P < 0.01$) [1].

[1] All tests performed using Friedmans ANOVA for non-parametric related data

IL1α /TP ratio

The IL1α /TP ratio showed a similar pattern over time on both the soft-layered and the rigid spineboard, for both the three loading and subsequent unloading periods (Figure 3; Table 3 (Appendix)). On both spineboards, the ratio increased significantly between values at the baseline and the first loading period ($P < 0.01$) and between the first and second loaded periods ($P < 0.05$). The decrease in the ratio after the first period of unloading was also significant ($P < 0.05$) for both spineboards. For the rigid spineboard, a significant decrease in IL1α /TP ratio was also seen between the first and second unloaded periods ($P < 0.05$). Except for 40-minutes unloaded period, there were no significant differences in IL1α /TP ratio between the two spineboard for the various loaded and unloaded periods (Ps > 0.05).[2]

Lactate

Figure 4 illustrates the corresponding lactate concentrations for each of the loading/unloading periods for both spineboards (see also Appendix, Table 3) . These values were clearly higher during the loaded periods compared to the corresponding baseline values. Indeed lactate concentrations increased significantly for both spineboards for the first loaded period ($P < 0.05$) and decreased significantly for the first unloaded period when compared to the value after 60 minutes of loading (Ps < 0.01). Lactate concentrations remained stable over both the loaded period and the unloaded period. No differences between the two spineboards were seen in lactate levels for the various periods ($P > 0.05$).[3]

Skin redness

The results of the redness categorisation is shown in Figure 5. Log-linear regression analysis revealed a significant difference between the two spineboards in both the intensity and course of pressure-induced redness. In particular, the soft-layered spineboard generally caused no or only diffuse redness after several periods of loading. By contrast, the rigid spineboard produced clearly defined redness even after the first period of loading ($\chi 2(1)=23.24$, $P < 0.01$).

Complete agreement between the two observers with regards to level of redness was found in 77% of the cases, resulting in a kappa value of 0.61. Disagreement was mostly

[2] All tests performed using paired samples t-test

[3] All tests performed using Wilcoxon matched-pairs test

seen in the assessment of no redness versus diffuse redness (13%). All redness was blanchable.

Interactions between variables

Table 3 shows the interactions (or lack thereof) found between tissue-interface pressure and skin redness, IL1α/TP ratio and lactate.

A significant relation was found between tissue-interface pressure and skin redness, with higher pressures correlating with higher levels of skin redness.

There was no significant correlation between tissue-interface pressures and IL1α/TP ratio for either the rigid or the soft-layered spineboard. No significant relation between pressure and lactate level was seen for the soft-layered spineboard but there was a significant relation between pressure and lactate levels for the rigid spineboard .

For both the rigid spineboard and the soft-layered spineboard, a significant correlation was found between redness and IL1α /TP ratio , and between redness and lactate levels.

Table 3. Correlations between tissue-interface pressure and skin redness, IL1 α/TP ratio and lactate

	Tissue-interface pressure	**Skin redness**	**IL1α/TP ratio**	**Lactate**
Tissue-interface pressure	-	$R = 0.707$, $P < 0.01$	$R = 0.017$, $P = 1.0$ (RSB); $R = 0.070$; $P = 0.4$ (SLSB)	R = 0.2, $P < 0.05$ (RSB); R < 0.01, $P = 1.0$ (SLSB)
Skin redness		-	$R = 0.634$, $P < 0.01$ (RSB); R = 0.286, $P < 0.05$ (SLSB)	$Rr = 0.264$, $P < 0.05$ (RSB); R = 0.243, $P < 0.05$ (SLSB)
IL1α/TP ratio			-	n.a.
Lactate				-

* RSB: rigid spineboard; SLSB: soft-layered spineboard; n.a. not applicable

Discussion

This prospective, randomised open label crossover study examined the relationship between tissue-interface pressure, and the response of the skin in healthy participants

lying supine on two types of spineboards. More specifically, we tested the hypothesis that lying on a rigid spineboard would result in an elevated release of IL1α and lactate as a result of the increased tissue-interface pressures when compared to lying on a soft-layered spineboard. In addition, we hypothesised that there would be a relationship between the pressure-induced reactive hyperaemia and the IL1α and lactate concentrations. We found marked differences between the effects of the rigid spineboard and the soft-layered spineboard with regard to tissue-interface pressure and redness of the skin. In addition, IL1α /TP ratios increased during the loaded period on the spineboards and remained elevated during the unloaded period when compared to the baseline measurements. By contrast, the elevated lactate concentrations remained stable during the loaded period but were restored to baseline values levels within the first unloaded period. Furthermore, IL1α /TP ratios and lactate concentrations, which were similar between the two spineboards, correlated with skin redness, but not with the magnitude of tissue-interface pressures.

To our knowledge, this is one of the first studies to relate IL1α /TP ratio and lactate concentration to tissue-interface pressures in a clinically relevant scenario [43]. Earlier studies have applied pressures to engineered skin constructs [34-36] and controlled mechanical loading of the forearm of healthy volunteers [36]. In accordance with our earlier study [28], we found a clear relationship between tissue-interface pressure and redness of the skin on both types of spineboard. The traditional rigid spineboard showed mean tissue-interface pressures significantly higher than the corresponding values measured on the soft-layered spineboard. Pressures were measured across the entire sacral area, including the four sites where the Sebutapes were placed. These local pressures were recorded in order to identify any local correlations between pressures and IL1α /TP ratio and lactate concentrations. In addition, we determined the peak pressure in the sacral area, to test for global effects of pressure. No clear correlations were found between either the local or the global tissue-interface pressures and IL1α /TP ratio or lactate concentration. However, IL1α/TP ratio revealed a significant increase during the first 40 minutes in the loaded conditions for both spineboards (Figure 2), but remained stable during the last 20 minute loaded period. Lactate remained stable over the three loaded periods for both spineboards (Figure 3). This reflects the nature of the response of these markers, where IL1α release is part of a complex tissue regeneration process, while lactate concentration, a marker of anaerobic metabolism, is associated with the availability of oxygen and other nutrients within the tissues. By contrast, a clear relationship was found between the level of skin redness and both the IL1α /TP ratios and lactate concentration. It appears that either IL1α and lactate are released at a lower pressure threshold than the pressure needed to induce reactive hyperaemia, or that they are upregulated as an epiphenomenon. This uncertainty about the regulating mechanism

underlying our findings may abnegate the clinical applicability of using these biomarkers for the assessment of pressure-related skin damage. Further research concerning whether abolishing the inflammatory response directly, reduces the risk of pressure-related skin damage are warranted.

A few remarks should be made about the methods used in this study. First, the study population is quite homogenous in terms of age, sex and ethnicity, and therefore probably not representative of the population at highest risk of pressure ulcer onset. Also, although tissue-interface pressures are an easy way to get a first indication of the effect of the surface on the body, they may not be accurate in predicting the onset of pressure ulcers [30] Furthermore, we restricted the investigation to measuring IL1α and lactate concentrations, as both have been implicated as biomarkers related to tissue damage. However, other markers may be more specific for the pressure-induced changes in skin physiology [43]. In addition, measurements in the loaded condition were performed in three periods of 20 minutes, with a short unloaded period of two minutes in between to replace the Sebutapes and photograph the buttock area. Although unlikely [35], these brief reperfusion periods may have affected the local expressions of cytokines.

As shown in an earlier study from our group [27-29], the rigid spineboard induces high levels of stresses and strains on the soft tissues, whereas these effects are significantly less on the soft-layered spineboard. The real danger of the rigid spineboard may therefore be to damage the deeper tissue layers, whereas the using soft-layered spineboard avoids the fast deformation damage [29]. Although IL1α and lactate concentrations in the skin may not be the appropriate biomarkers to indicate deep tissue injury, other markers such muscular fatty-acid binding protein may be released in response to ischemic muscle damage [44-46], and could be used as indicators of this specific type of tissue damage. Finally, skin hyperaemia was assessed semi-quantitatively. While other, more sensitive methods [47], could have been used, the agreement between the two assessors was good.

Conclusion

Cutaneous IL1α and lactate both have a strong relationship with pressure exposure time, but not with pressure magnitude. Furthermore, IL1α was measured even in the absence of visible redness of the skin. The study may offers the potention of biomarkers, reflecting inflammation and/or tissue metabolism, for use in assessing the effects of prolonged spineboard support, but further studies are warranted.

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Figure legends

Figure 1. Four Sebutapes in place in the marked-out area of interest on the sacrum/buttocks for biomarker measurement.

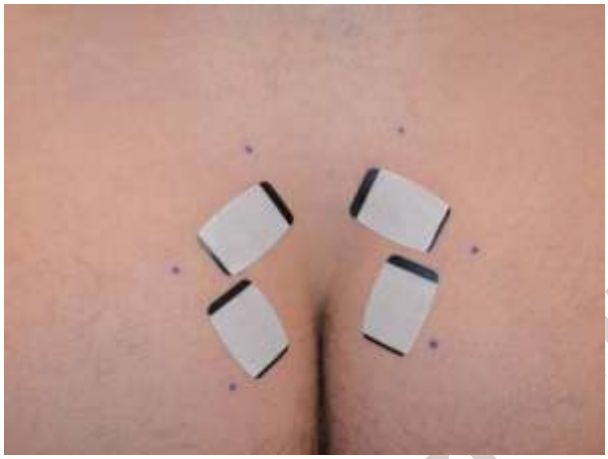

Figure 2a. Sample of pressure measurement; red square outlines sacral area

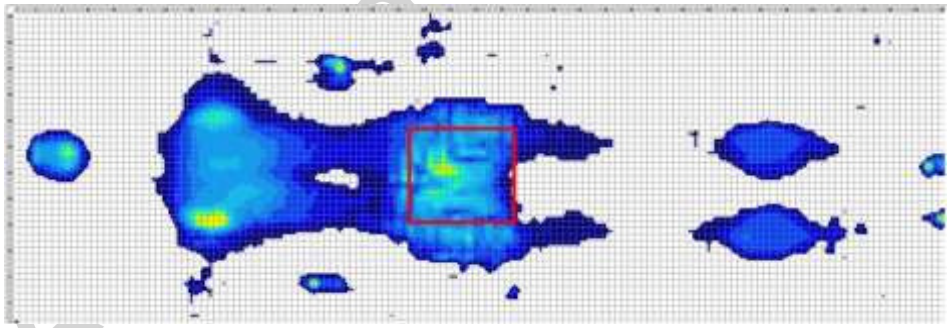

Figure 2b. zoomed-in picture of pressures measured in sacral area; sensor with solid red line indicates sensor with Peak Pressure (in this case: PP=117 mmHg); area of 3x3 sensors indicates area for Peak Pressure Index calculation (in this case: PPI=102.6 mmHg).

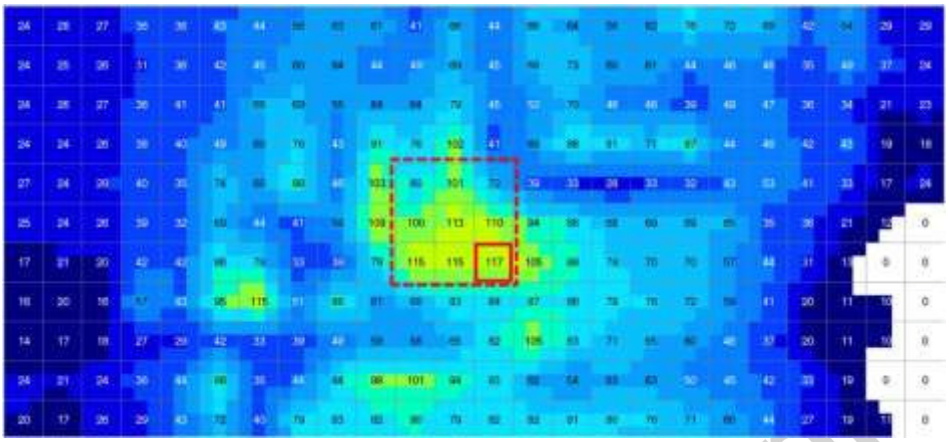

Figure 3. IL1α /TP ratio, average of the four locations of measurement (relative part of the buttocks), comparing the two devices over time.

Presented data are means + standard deviation

* significant difference between devices for this timepoint

significant difference compared to previous timepoint, within device

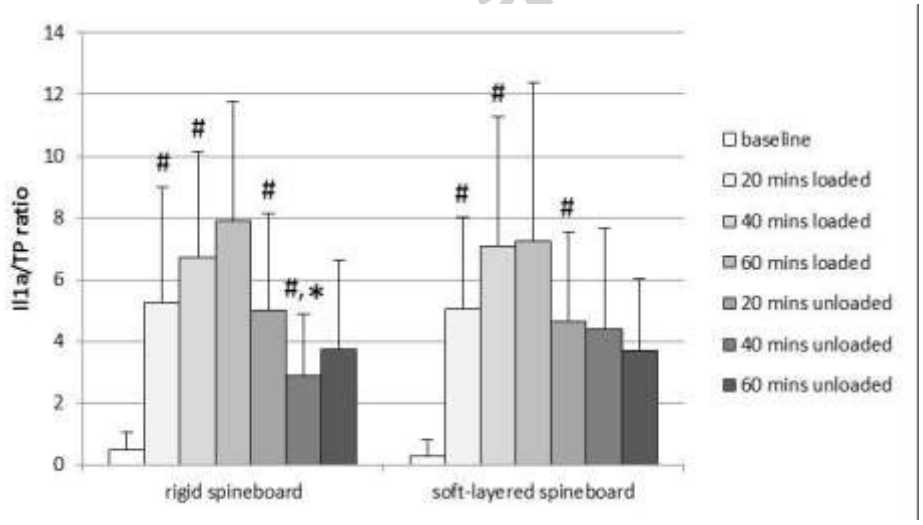

Figure 4. Lactate levels, average of the four locations of measurement (relative part of the buttocks), comparing the two devices over time.

Presented data are means + standard deviation

significant difference compared to previous timepoint, within device

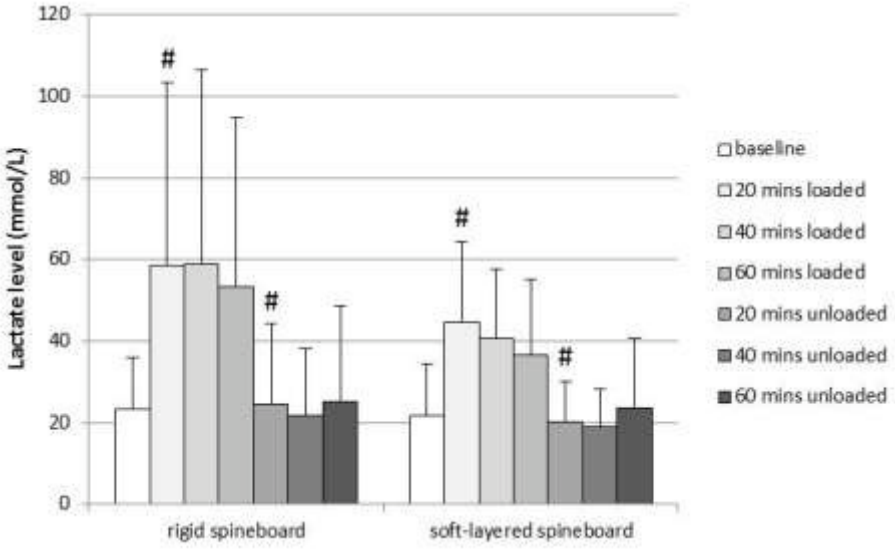

Figure 5. Redness of the buttocks in relation to time point and device; results after resolving differences between the observers. Vertical axis shows percentage of participants showing the specified kind of redness on the buttocks at the end of each specified time period.

+: loaded: -: unloaded.

Other: red streak within the intergluteal cleft, no redness on the buttocks

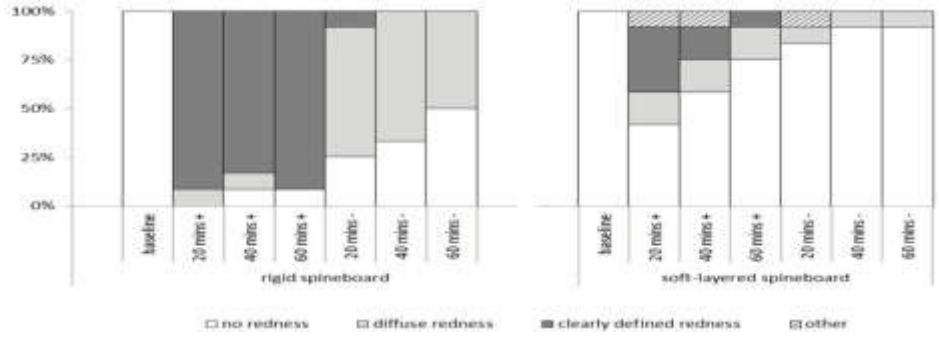

Table 3. Mean IL1a/TP ratio and lactate levels for the rigid and the soft-layered spineboard, in relation to duration of loading

		Baseline	20 mins loaded	40 mins loaded	60 mins loaded	20 mins unloaded	40 mins unloaded	60 mins unloaded
Rigid spineboard	IL1a/TP ratio	0,48	5,24	6,73	7,91	5,00	2,91	3,75
	Lactate (mmol/L)	23,2	58,6	58,7	53,3	24,5	21,7	25,3
Soft-layered spineboard	IL1a/TP ratio	0,27	5,05	7,09	7,26	4,64	4,38	3,69
	Lactate (mmol/L)	21,7	44,5	40,5	36,6	20,3	19,0	23,6