Physiological processes of inflammation and oedema initiated by sustained mechanical loading in subcutaneous tissues: a scoping review

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Short running title

Oedema and inflammation in deep tissue injury

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deep tissue injury, inflammation, oedema, pressure injury, pressure ulcer

ABSTRACT

Deep tissue injuries are pressure ulcers which initiate in the subcutaneous tissues and extend through a bottom-up pathway. Once deep tissue injuries are visual at skin level, serious irreversible tissue damage has already occurred. In pressure ulcer development, inflammation and oedema are coupled physiological processes associated with tissue damage arising due to sustained mechanical loading. This study aimed to provide an in-depth overview of the physiological processes of inflammation and oedema initiated by sustained mechanical loading in subcutaneous tissues, in the context of pressure ulceration. A scoping review was performed according to the framework by Arksey and O'Malley. The databases MEDLINE, EMBASE, Web of Science, and Scopus, and the reference lists of included studies were searched for in vivo (animal, human), and in vitro studies matching the study objectives (from inception to 28 May 2018). No restrictions for inclusion were applied for study design, setting, participants, and year of publication. A total of 12 studies were included, varying in study

design, sample characteristics, amount and duration of mechanical loads that were applied, follow-up time, and assessment methods. Neutrophil infiltration and oedema occur in the subcutaneous tissues nearly immediately after the application of load on soft tissues. The amount of neutrophils and oedema increase in the first days after the mechanical insult and decrease once healing has been initiated and no supplementary mechanical load was applied. One study indicated that oedema may extend up to the level of the dermo-epidermal junction. Further research should focus on how deep tissue inflammation and oedema are reflected into unique tissue changes at skin level, and how abnormal inflammatory responses manifest (e.g. when the nervous system is not functioning normally).

INTRODUCTION

The first publication on pressure ulcers identified in the Medline database, was published in 1886 (1). More recently (14 December 2018), a search strategy combining the search terms 'pressure sore', 'bed sore', 'pressure ulcer', 'pressure injury', and 'decubitus', using the PubMed search engine, revealed a total of 69786 publications. Although substantial knowledge exists on the epidemiology, aetiology, pathophysiology, associated factors, prevention and treatment, pressure ulcers persist to be a major challenge in health care, especially in patients with limited sensation and/or mobility. Recent prevalence figures in hospital settings varied between 5.9% and 16.1% (2-4). Pressure ulcers have been associated with a substantial burden for patients, health care providers, and the society, arising as a consequence of wound related pain (5, 6), a decreased quality of life (10, 11), an increased length of hospital stay (7-9), and an increased mortality rate (9, 10). In addition, the costs

associated with the time and resources required to treat pressure ulcers cause an important financial burden (9, 11-13).

The clinical practice guideline, developed by international collaboration between the National Pressure Ulcer Advisory Panel (NPUAP), the European Pressure Ulcer Advisory Panel (EPUAP), and the Pan Pacific Pressure Injury Alliance (PPPIA), defined pressure ulcers as *'a localised injury to the skin and/or subcutaneous tissue, usually over a bony prominence, caused by sustained pressure, or pressure associated with shear'* (14). Pressure ulcers were subdivided into four categories: (a) category I - non-blanchable erythema, (b) category II - partial thickness skin loss, (c) category III - full thickness skin loss, and (d) category IV - full thickness tissue loss. Unstageable and suspected deep tissue injury are characterised by a visually undeterminable depth. In unstageable injuries, slough and/or crust cover the wound bed. In suspected deep tissue injuries, damage has occurred in the underlying soft tissue whereas the skin remained intact (15). A localised purple or maroon discolouration of the skin, or a blood-filled blister can be observed (14).

Pressure ulcers can develop either by a top-down, or a bottom-up pathway (16, 17). The top-down pathway is initiated at the level of the epidermis and the dermis, and is involved in the development of superficial pressure ulcers. The latter are mainly caused by shearing stress, which results in detachment and mechanical failure of the epidermis and the dermis (16, 18). In contrast, the bottom-up pathway is initiated at a bone-soft tissue interface, typically at

weight bearing bony prominences, and it is that pathway which is responsible for the development of deep tissue injuries. The main causative factor for deep tissue injuries are sustained deformations of subcutaneous tissues in combined compression, tension and shear (18). The tissue damage progresses through skeletal muscle (if applicable), fascia, and subcutis towards the skin (16).

Four primary pathophysiological mechanisms are described which may lead to the development of pressure ulcers: (1) ischemia, (2) cell distortion/deformation, (3) reperfusion, and (4) impaired interstitial fluid flow and lymphatic drainage (19, 20). Ischemia, due to obstruction, or occlusion of the microcirculation by external loading, is associated with a reduced supply of oxygen, a reduced elimination of metabolites, and a decrease in pH (19, 21, 22). Cell deformation is associated with changes in the cell physiology, caused by stretching of the cellular plasma membrane, and reorganisation of the cytoskeleton (18, 23, 24). Reperfusion is a process associated with ischemia and describes the restoration of the blood flow following an ischemic insult, which causes the release of reactive oxygen metabolites (16). Finally, impaired interstitial fluid flow and lymphatic drainage are associated with an increase in the amount of interstitial fluid and a decrease in the drainage of interstitial fluid by lymph vessels (25, 26). All four mechanisms evoke inflammatory responses (including oedema), and tissue damage (27).

Inflammation is the first phase in the wound healing process and aims to clear cell debris, to protect against the invasion of pathogens and to promote overall healing (28, 29). The inflammatory process can be subdivided into two phases: (1) vasodilatation and increased permeability of blood vessels, and (2) emigration of phagocytic leukocytes (e.g. neutrophils) from the blood flow into the interstitial fluid (30). Vasodilatation causes an increase in blood flow to promote the supply of phagocytic leukocytes and plasma proteins to the site of tissue damage. An increased permeability of blood vessels promotes the migration of leukocytes and plasma proteins through the vessel walls. Leakage of plasma proteins causes a decrease in colloid osmotic pressure. This promotes fluid movement from the blood vessels towards the interstitial space, resulting into localised oedema (21, 30). Once fluid starts leaking from the vessels, the interstitial pressure increases, and swelling occurs, which is the typical macroscopic sign of inflammatory oedema (31, 32). If the oedema is prolonged and adds mechanical loads to an already distorted tissue site, by further increasing the interstitial pressure, this may escalate the cell distortion (27, 31, 32). Other typical signs of inflammation are redness, heat, and pain. Redness and heat are associated with enhanced blood flow. Pain can be caused by local distension due to oedema, or by injury or stimulation of nerve endings (21, 30).

In deep tissue injuries, the level of cell deformation has been considered the primary aetiological factor. This is because cell deformations exercise an immediate effect on the viability of the distorted tissues and will rapidly trigger an inflammatory response, and

potential secondary damage due to inflammatory oedema. Ischemia becomes an important aetiological factor if loading is not relieved for a prolonged period of several hours. Ischemia may also impact tissue viability earlier, as a tertiary damage pathway, following obstruction of the vasculature by inflammatory oedema (20, 32, 33). Ischemia-reperfusion injury is a related damage process occurring once blood flow restores after relief of a mechanical load (reperfusion) (16, 34). Lastly, impaired lymphatic drainage, caused by obstruction of the lymph vessels by external mechanical loading and potentially, by an evolving oedema, worsens the biochemical and biomechanical states of tissues. Due to a lack of clearance routes, metabolic waste products accumulate, and the volume of interstitial fluid (oedema) further increases (25, 26).

In vivo animal studies indicate that skeletal muscle tissue, which is affected in many deep tissue injuries, is more susceptible to deformation-related damage than skin (35, 36). Moreover, deep tissue injuries evolve faster and cause more extensive tissue damage than superficial pressure ulcers. As a consequence, once deep tissue injuries are detectable by visual skin assessment, serious injury has already occurred (17, 37).

Early detection of deformation-induced cell and tissue damage allows timely application of preventive measures before the damage becomes macroscopic, irreversible and clinically significant. As visual skin assessment is not suitable for early detection of deep tissue injuries, alternatives have been proposed, based on different medical technologies, such as high

frequency ultrasonography, biomarkers and quantification of subepidermal moisture (27, 38-40). For research purposes, various other imaging technologies (e.g. magnetic resonance imaging) and real-time computer modelling techniques are also applied. However, translation of each of these methods to medical devices which are clinically applicable, robust, adequately sensitive and specific, reliable and cost-effective in practice requires vast efforts and resources (39). Biomarkers are indicators of internal physiological processes (e.g. lactate levels indicating an anaerobe metabolism) and can be used to assess the integrity of soft tissues (22). Concerning pressure ulcers, two main physiological processes, described in histological studies, are inflammation and oedema (or interstitial fluid accumulation) (41-48). One study has demonstrated the potential of C-reactive protein (CRP) levels in blood samples as a chemical biomarker of inflammation associated with muscle damage in deep tissue injury (49). In contrast, biomarkers sampled from the skin surface (e.g. sweat lactate) were not able to reflect damage to the subcutaneous tissue and muscle tissue (39). A different type of a biomarker, a physical biomarker, is biocapacitance which is the change in the electrical capacitance of tissue as a result of accumulation of interstitial fluids. This is the biomarker of interest in subepidermal moisture measurements and is a direct indicator of the presence of micro-oedema in a tissue (32, 50, 51).

The complexity of the aetiology of pressure ulcers and the specific roles that inflammation and inflammatory oedema play in the aetiology points to the need for rigorous synthesis of the literature to extract the relevant information that is known thus far. This study therefore

aimed to provide an in-depth overview of the physiological processes of inflammation and oedema initiated by sustained mechanical loading in subcutaneous tissues.

METHODS

A scoping review was performed to provide an in-depth overview of the literature related to inflammation and oedema associated with pressure ulcers, including deep tissue injury. The review process was based on the methodological framework proposed by Arksey and O'Malley (52). This framework describes five subsequent stages: (1) identifying the research question; (2) identifying relevant studies; (3) study selection; (4) charting the data; and (5) collating, summarizing and reporting the results. Due to the explorative nature of the review, no methodological quality assessment of the eligible studies was performed. The review was performed in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines (53).

Research question and key concepts

Following research question was determined in advance: within published literature, what is known about the physiological processes of inflammation and oedema in subcutaneous tissues initiated by sustained mechanical loading? Inflammation was defined as the local response of living tissue to damage caused by sustained mechanical loading (54). Oedema was defined as an abnormal collection of interstitial or intracellular fluid, associated with tissue damage caused by sustained mechanical loading (28).

Identifying relevant studies

Relevant studies were searched for in four electronic databases from inception to 28 May 2018: MEDLINE (using the PubMed interface), EMBASE, Web of Science, and Scopus. Given the scoping nature of this review, a broad search strategy was designed. For this purpose, advice from a librarian technician was sought. Search terms were developed to capture three key processes: (1) tissue damage due to sustained mechanical loading (pressure or shear or a combination of both), (2) inflammation in subcutaneous tissues (muscle and subcutis), (3) localised oedema in skin or subcutaneous tissue. The search strategy was designed for PubMed, and converted for each subsequent database (Table 1). In addition, we reviewed the reference lists of included studies for further relevant literature (55).

Study selection

Eligibility criteria were established based on the research question and a pilot search in PubMed. Studies providing data of temporal and spatial mechanisms of inflammation and/or oedema due to pressure and shearing forces in deep tissue were considered for inclusion, if published in English, French, or Dutch. Studies investigating the molecular response to mechanical loading, or describing the development of an animal model were excluded. Both in vivo (animal and human) and in vitro (e.g. cell culture or tissue engineering) studies were considered, and no restrictions for inclusion were applied for study design, setting, participants, and year of publication.

Titles and abstracts of all retrieved records were screened for eligibility using the online application Rayyan (56). A second reviewer independently screened 15% of the records. A substantial Cohen's Kappa, as a measure of interrater agreement, of 0.68 was reached (57). Based on discussion of the discrepancies, a broader range of publications was retained (e.g. publications describing other physiological processes involved in pressure ulcer development), to decrease the possibility of missing any relevant publication. In a next step, the eligibility of the remaining studies was further evaluated, based on the full text. Each case of doubt was discussed with a third reviewer.

Charting the data

Prior to the search, a data charting form was designed to promote interpretation, comparison, and synthesis of the findings from the included studies (52). A pilot test allowed further refinement of the form, which included in its final version: name of the authors, the year of publication, title, study population, aim, study design, methods (intervention/comparison, assessment methods), and relevant findings.

Collating, summarising and reporting the findings

The key concepts of this review, inflammation and oedema, provided the main structure to collate, summarise, and report all relevant findings. Due to the heterogeneity of studies and the broad scope of this review, the findings were presented in a narrative way.

RESULTS

Literature search

The literature search, performed in May 2018, yielded a total of 18296 records. The full texts of 46 studies were retrieved to assess for eligibility. This assessment resulted in the exclusion of 35 studies which did not describe any mechanisms of inflammation and oedema in pressure ulcers. Screening of the reference lists of relevant studies yielded one additional study. Finally, 12 studies were included in this review (Figure 1). A chart table presenting an overview of the study characteristics was added as attachment to this review.

Most included studies (n = 8) used a quasi-experimental design (34, 48, 58-63). From the other studies, two used an observational design (40, 64), one combined an observational design with a quasi-experimental design (65), and one was a non-systematic review with an educational purpose (28). The quasi-experimental studies were mostly performed in an animal model (mouse, rat, pig). One study additionally applied human tissue cultures (59) and the combined study included human volunteers for the quasi-experimental part of the study (65). The observational studies were performed in hospitalised patients with pressure ulcers (40, 64), or residents of a long-term care facility at risk of developing a pressure ulcer (65).

The quasi-experimental studies and the combined study concerned load applications varying in amount (9 kPa-250 kPa, 100N, 1-10 kg) of mechanical loads. In most studies, mechanical

loads were applied for two hours (34, 48, 58-60, 62, 63). The development of inflammation and associated oedema were described using various methods: histology (48, 58-63), biochemistry (34, 59-61), high resolution ultrasonography (40, 64, 65), magnetic resonance imaging (48, 62, 63), or immunohistochemistry (59). To study the effect of ischemia-reperfusion, measurements were performed at time-frames varying between one day or less (34, 58-60, 62, 63) till 14 days (48). One study compared varying number of ischemia-reperfusion cycles (59).

Sample sizes from the quasi-experimental studies varied from n = 2-3 per study group (58), to n = 25 per study group (61). Histopathological assessments were performed in samples of n = 2-3 (58) per study group, to n = 8 per study group (34). The sample size for the observational studies varied between 9 (64) and 119 (65).

Funding for the individual studies was absent or not mentioned in seven studies (28, 40, 59, 61-65). Three studies were funded by independent scientific institutions or the local government (34, 48, 60). Some co-authors of the study of Quintavalle, Lyder (65) declared to be a shareholder, consultant, or employee of the company which delivered the ultrasound device.

Inflammation

Inflammation in pressure ulcers develops upon cell death caused by tissue deformation, ischemia, reperfusion, impaired lymphatic drainage, or any combination of these damage pathways. Tissue damage initiates the release of inflammatory mediators (cytokines). These mediators cause chemotaxis of inflammatory cells, nutrients, fluids, and clotting factors to the damaged area (28, 29). Typical signs of inflammation are infiltration of leukocytes (e.g. neutrophils, monocytes, macrophages), and oedema (28, 48, 58-63).

Time frame of the inflammatory response

According to the included studies, the inflammatory response evolves over time (Table 2). Seven studies provided time indications concerning the infiltration of inflammatory cells. Early/mild infiltration of neutrophils in muscle tissue was determined between zero and four hours after relief of the mechanical loads (34, 48, 60, 62). In contrast, the study of Houwing, Overgoor (60) reported extensive infiltration of neutrophils at two hours after relief of the mechanical loads. In the same study, the infiltration of neutrophils increased during the following days. Other studies reported extensive infiltration of neutrophils, monocytes, and lymphocytes on day 1 and/or day 3 (48, 58, 61-63). According to Nelissen, Traa (48), this inflammatory response is part of the pro-inflammatory phase. The same authors determined a decrease in the number of neutrophils and lymphocytes (to minimal/mild), and an increase in the number of macrophages on day 5. These changes were characteristic of the anti-inflammatory phase. At the same time, tissue remodelling was initiated. Histopathological

assessments performed on day 14 demonstrated a decrease in the number of neutrophils, lymphocytes, and macrophages, to normal/minimal levels.

One study provided data on the level of inflammatory markers over time (60). The researchers identified a statistically significant increase in plasma hydrogen peroxide two hours after relief of the mechanical loads, compared to no reperfusion ($p < 0.05$). In contrast, the level of reduced glutathione, measured in biopsies, was statistically significantly ($p < 0.05$) reduced immediately after relief of the mechanical loads compared to a control area not exposed to mechanical loading.

Three studies applied magnetic resonance imaging (MRI) to analyse the inflammatory response (48, 62, 63). Transverse relaxation time (T2)-weighted MRI demonstrated an increased signal intensity at the location of load application immediately after relief of the mechanical loads. In contrast, no increase in signal intensity was observed during load application (48, 62, 63). However, a higher signal intensity was not specific for the presence of inflammation. Other tissue changes associated with increased MRI signal intensity are: oedema, necrosis, and haemorrhage (48, 62). Stekelenburg, Oomens (62) and Stekelenburg, Oomens (63) demonstrated that the signal intensity remained elevated 20 hours after relief of the mechanical loads. Using multiparametric MRI, Nelissen, Traa (48) demonstrated that the effect of two hours application of a mechanical load, expressed as an increase in signal intensity, was the largest between day 3 and 5. This period corresponds to the pro-

inflammatory phase. On day 14, some signs of increased signal intensity were still visible, indicating that muscle tissue was not fully recovered. Although, differences in time course, extent and severity of tissue damage were observed between animals.

Effect of the duration and amount of mechanical loading on the inflammatory response

Three studies analysed the effect of the amount of mechanical loading on the inflammatory response (58, 61, 63). Bosboom, Bouten (58) compared muscle damage in rats, exposed to mechanical loads of 10 kPa for two hours (n = 2) or six hours (n = 3), 70 kPa for two hours (n = 3), or 250 kPa for two hours (n = 3). Muscle damage was defined as the infiltration of inflammatory cells and/or loss of cross-striation of muscle fibres. Histopathological assessment demonstrated muscle damage only in the group exposed to a mechanical load of 250 kPa for two hours.

Sari, Nagase (61) compared the effect of a mechanical load of 1 kg (4 hours) or 10 kg (4 hours), versus no mechanical load, on the development of deep tissue injury (n = 25/group). Histopathological assessment of the 1 kg group demonstrated minor infiltration of neutrophils and lymphocytes on day 1, with some muscle regeneration on day 3. In contrast, the assessment of the inflammatory response in the 10 kg group demonstrated extensive infiltration of neutrophils and lymphocytes on day 1, and more clear signs of (unspecified) inflammation on day 3.

Stekelenburg, Oomens (63) compared the effect of a mechanical load of 150 kPa for two hours (n = 7), versus 50 kPa for four hours (n = 3), on muscle damage development in rats. Analyses of magnetic resonance images (T2 weighted MRI), taken immediately after relief of the mechanical loads, demonstrated only an increase in signal intensity in the animals subjected to a 150 kPa load. This increase in signal intensity in the 150 kPa group may indicate the presence of inflammation, oedema, necrosis, or haemorrhage (48, 62).

Influence of the number of ischemia-reperfusion cycles and tissue type on the inflammatory response

Two studies analysed the influence of the number of ischemia-reperfusion cycles on the inflammatory response (34, 59). Gust, Hong (59) demonstrated that the infiltration of inflammatory cells in mouse adipose tissue increased with increased number of ischemia-reperfusion cycles (two or four cycles) and increased duration of load application (1.0, 1.5, 2.0 hours), compared to the control group of no ischemia-reperfusion ($n \geq 5$/group). More than 90% of the infiltrating inflammatory cells were neutrophils. The strongest increase [9.74, (SD 1.57)] was determined in the two hours ischemia/four cycles group. The authors did not report the reperfusion time. Statistically significant differences were determined between animals subjected to 1.0, 1.5, and 2.0 hours ischemia after four episodes of ischemia-reperfusion ($p < 0.05$). In contrast, in dermal tissue, a smaller increase [maximum value: 2.67 (SD 0.65)] in cellularity was determined, without any significant differences between groups. The authors additionally compared the expression of inflammatory markers in adipose and dermal tissue

of mice and human. In the adipose tissue of mice, a statistically significant ($p < 0.001$) increase in all inflammatory markers (monocyte chemoattractant protein-1, interleukin-6, heme oxygenase 1, interleukin-1β,) was identified. However, in dermal tissue of mice, only a statistically significant ($p < 0.001$) increase in the expression of monocyte chemoattractant protein-1 was identified. Similarly, in adipose tissue of human, the expression of migration inhibitory factor, interleukin-1β, and tumour necrosis factor was statistically significantly increased. In human dermal tissue, the results did not indicate any statistically significant increase in the expression of inflammatory markers.

Jiang, Tu (34) used a rat model to compare the extent of inflammation caused by three ischemia-reperfusion cycles (one to four hours reperfusion), versus two hours ischemia and no ischemia (n = 8/group). Histopathological assessment demonstrated mild infiltration of inflammatory cells in muscle tissue and dermal tissue in the ischemia group, and severe infiltration of inflammatory cells in muscle tissue in all ischemia-reperfusion groups. In addition, biochemical analyses were performed to assess the level of inflammatory mediators associated with oxidative stress [superoxide dismutase (SOD), malondialdehyde (MDA), endothelin-1 (ET-1), and nitric oxide (NO)]. The results demonstrated a statistically significant increase in inflammatory mediators (or decrease for SOD) ($p < 0.05$) in rats exposed to ischemia-reperfusion compared to rats only exposed to ischemia and rats not exposed to a mechanical load. These differences increased with reperfusion time and decreased slightly in the four-hour reperfusion group.

Oedema

Oedema is an abnormal collection of fluid, associated with an imbalance in fluid distribution. Two types of oedema can be distinguished: intracellular oedema, and interstitial oedema. Intracellular oedema is primarily a consequence of ischemia. Ischemia causes hypoxic cellular events, such as failure of the adenosine triphosphate (ATP) dependent sodium potassium pump in the cell membrane. Due to the disturbance of the sodium and potassium ionic gradient between intracellular and extracellular fluid, the intracellular osmotic pressure rises. The latter results in an increased influx of fluid into the cell and, as a consequence swelling of the cell (66). Interstitial oedema is caused by increased hydrostatic pressure, decreased colloid osmotic pressure, and impaired lymphatic drainage, which are all associated with activation of an inflammatory response (i.e. release of inflammatory mediators) (29, 67). From a histological perspective, the presence of interstitial oedema has been associated with widening of interstitial spaces (48, 60, 62).

Time frame of the development of oedema

Similar to the infiltration of inflammatory cells, the presence of oedema evolves over time (Table 3). Five studies provided time indications concerning the presence and extent of oedema (34, 48, 60-62).

Two studies reported increased MRI signals distal from the location of load application, which were initiated during load application (48, 62). At the location of the mechanical loading, MRI signals increased immediately after relief of the loads (48, 62, 63). Nelissen, Traa (48) associated these increased MRI signals with the presence of oedema, based on histopathological assessment of biopsies taken shortly (two hours) after relief of the load. However, increased MRI signals may also be associated with inflammation, haemorrhage, and necrosis (48, 62).

Three studies examined tissue samples using electron and/or light microscopy. Electron microscopy confirmed the presence of (microscopic) oedema at the location of mechanical loading immediately after relief of the loads (60). Likewise, light microscopy demonstrated the onset of oedema between one to two hours after relief of the loads (48, 60, 62).

In the study of Sari, Nagase (61), the mean muscle oedema index increased between day 1 and day 3. According to Nelissen, Traa (48), the presence of oedema was at the highest level between day 3 and day 5, corresponding to the pro-inflammatory phase. Subsequently, minimal/mild levels of oedema were described on day 5. Houwing, Overgoor (60) reported that the amount of oedema diminished after one week. In accordance, Nelissen, Traa (48) reported the absence of oedema on day 14.

Influence of the amount of mechanical loads applied on the development of oedema

One study analysed the influence of the amount of mechanical loads applied on the mean muscle oedema index in rats (n = 4-5/group) (61). The researchers demonstrated that the muscle oedema index was statistically significantly higher in rats subjected to a 10 kg load, than in rats subjected to a 1 kg load or no load. This result was observed on day 1 ($p < 0.05$ and $p < 0.01$), and on day 3 ($p < 0.001$ and $p < 0.001$) after relief of the loads. No statistically significant differences were identified between the 1 kg group and the control group. The 10 kg group additionally demonstrated widened interstitial spaces in muscle tissue on day 1 and 3.

Location of the development of oedema

Four studies reported on the location of oedema (34, 40, 64, 65). Of these, three studies used high resolution ultrasonography (HRUS) (40, 64, 65). On the HRUS images, a decrease in echogenicity corresponded with an increase in fluid content or oedema (68).

Aliano, Low (40) identified hypoechoic areas in the subcutis and deep muscle in 10/12 (83.3%) patients with superficial pressure ulcers (category I and II) and 8/8 (100%) patients with suspected deep tissue injury. Similarly, Yabunaka, Iizaka (64) reported the presence of fat oedema in 8/8 (100%) patients with superficial pressure ulcers, and 3/3 (100%) patients with deep pressure ulcers.

Quintavalle, Lyder (65) described the development of pressure ulcers through different patterns of oedema, in long-term-care facility residents at risk for developing pressure ulcers (n = 119). A first pattern concerned the presence of a hypoechoic area (oedema) directly below the intact epidermis, with no changes in deep tissue. This pattern corresponded with the pattern observed in healthy volunteers upon friction at the skin level. The second pattern concerned the presence of oedema in the deep subdermal area and corresponded to the pattern observed in healthy volunteers subjected to prolonged mechanical loading. The second pattern was further subdivided into three subgroups, based on the extent and location of the hypoechoic area: (1) pockets of oedema between bone and dermis, (2) oedema extending upward from the subcutaneous tissue into the dermis, (3) oedema extending upward through the dermis and pooling under the intact epidermis.

Jiang, Tu (34), compared the development of oedema in rats subjected to three cycles of ischemia-reperfusion (n = 32), versus ischemia only (n = 8). Histological assessment of rat skin and muscle tissue demonstrated oedematous muscles in rats subjected to three cycles of ischemia-reperfusion. In contrast, only mild tissue changes, including loosening of muscle, were described in rats subjected to ischemia only. No oedema was described in epidermis and dermis. No results on the development of oedema in the subcutis were provided.

DISCUSSION

This review aimed to provide, for the first time, an in-depth overview of the physiological processes of inflammation and oedema in the context of pressure ulcers and deep tissue injury. The findings confirm that inflammation and oedema are complex processes involved in the development of early stage deep tissue injury, and that these processes escalate over time. Some evidence, mainly based on animal studies, was found on the evolution of inflammation and oedema caused by mechanical loading, the role of neutrophils in the inflammatory process, and the type of tissue affected by inflammation and oedema. However, this evidence was difficult to compare between studies due to heterogeneity concerning study design, sample characteristics, amount and duration of the applied mechanical loads, time of follow-up, and the assessment methods that were used.

As indicated above, an important finding was that inflammation and oedema evolve and intensify over time. Once healing has been initiated and no supplementary mechanical load was applied, oedema and inflammation decrease to minimal/normal levels. The gradual increase of inflammation and oedema can be explained by the process of ischemia-reperfusion (69). Upon reperfusion, oxygen delivery increases, which has been associated with an increase in the amount of reactive oxygen metabolites (e.g. superoxide, hydrogen peroxide). Reactive oxygen metabolites are capable of altering the structure and function of many biomolecules, such as membrane lipids, structural proteins, enzymes, receptors, and nucleic acids (70, 71). This process triggers an inflammatory cascade, resulting in accumulation

of neutrophils, microvascular barrier disruption (e.g. causing interstitial oedema), alterations in plasma membrane permeability (e.g. causing cellular oedema), and cell apoptosis (70, 72). As a consequence, the effects of a single load application may increase over several days. Furthermore, one study indicated that a prolonged period of mechanical loading, and especially repeated ischemia-reperfusion cycles, reinforce the damage accumulation (59).

Neutrophils are the primary mediators of the inflammatory response (29, 70). In accordance, several studies included in this review reported neutrophil infiltration in muscle tissue (shortly) after relief of the mechanical loads (48, 60-63). Neutrophils are attracted by other inflammatory mediators, such as reactive oxygen metabolites. As described above, the amount of the latter increases during reperfusion. Reactive oxygen metabolites stimulate the activation of neutrophil adhesion molecules on the surface of endothelial cells and neutrophils. The consequent adhesion of neutrophils to the endothelial cell wall promotes diapedesis of neutrophils to the interstitial spaces (70, 71). On the one hand, neutrophils promote tissue repair through phagocytosis and activation of the immune response. Conversely, neutrophils may cause tissue damage through the release of proteolytic enzymes, and reactive oxygen metabolites (71, 73-75). Furthermore, activated neutrophils contribute to the development of interstitial oedema through different mechanisms. Firstly, adhesion of neutrophils to the endothelial cell wall (leukocyte plugging) reduces the diameter of the post capillary venules, which results in an increase in hydrostatic pressure. Secondly, the release of proteolytic enzymes may damage the endothelial basement membrane (70). As a

consequence of their involvement in tissue damage and oedema, neutrophils were considered to be major contributors to reperfusion damage (70, 71).

Histopathological findings suggested that the infiltration of inflammatory cells predominantly occurs in muscle tissue and the subcutis, as opposed to the dermis and epidermis (34, 59, 60). Muscles are the most metabolically active and the strongest vascularised tissues. Therefore, muscles are considered to be more sensitive to ischemia and hypoxia (36, 76). In this context, the critical ischemic time for muscles at normal temperature has been estimated around 4 hours, whereas in other tissues the critical ischemic time seems to be much longer: 8 hours for nerves, 13 hours for fat, 24 hours for skin, and 4 days for bone (77). As a consequence, early prevention of ischemia or reduction of ischemic time is of special importance in body sites which consists mainly of muscle tissue (e.g. lower limbs, heart) (78). Moreover, a finite element model indicated higher principal compressive stresses and strains in muscle layers compared to the skin and subcutis (79). As a consequence, muscle damage occurs prior to skin damage (36). Research suggested that adipose tissue has an important role in the inflammatory response through the production of adipocytokines. Adipocytokines are specific inflammatory mediators which have been associated with various conditions, such as obesity-related disorders and breast cancer (80, 81). In this context, one of the included studies demonstrated a statistically significant increase in the gene expression of inflammatory cytokines in subcutaneous adipocytes following ischemia-reperfusion (59). This finding has been confirmed by the in vitro study of Hong, Park (82), who additionally reported the

upregulation of cytoprotective and proapoptotic genes in matured adipocytes. According to Gust, Hong (59) matured adipocytes contribute to a major degree to the development of inflammation in tissues subjected to ischemia-reperfusion. It should be noted that, besides adipocytes, the subcutis consists of a loose layer of areolar connective tissue (collagen fibres, elastic fibres, and reticular fibres), various kind of cells (e.g. fibroblasts, macrophages, plasma cells, mast cells), and a semifluid ground substance. In accordance with these components, the subcutis has four important functions: (1) anchoring the dermis to the muscle fascia, (2) protecting the underlying muscles and bones, (3) supporting the passage of blood vessels, lymph vessels, and nerves between the skin and the muscles, and (4) thermoregulation (21). The influence of a mechanical loading has, to our knowledge, not been described for most of these functions. Although, shear forces may cause stretching and angulation of the blood vessels in the subcutaneous layer. This reduces the blood flow and promotes thrombosis, necrosis, and inflammation (83).

In patients with intact skin (at risk of developing pressure ulcers), superficial pressure ulcers (category I and II), or deep tissue injuries (category III and IV), oedema was identified in muscle tissue and the subcutaneous layer (40, 64, 65). Furthermore, Quintavalle, Lyder (65) described that oedema, initiating in the subcutaneous tissues, may extend through the dermis up to the level of the dermal/epidermal junction. Although, this study was performed in patients with intact skin (with, or without erythema) and no follow-up data were presented. Overall, the

results from the three studies cited above support the bottom-up approach of pressure ulcer development.

Several studies on pressure ulcers described that either a high mechanical loading for a short period, as a low mechanical loading for a long period can lead to the development of pressure ulcers (14, 20, 33, 84). Although, a certain threshold should be exceeded (85). These findings were confirmed by three studies included in our review. Signs of inflammation were observed if a mechanical loading of 150 or 250 kPa was applied for two hours, or a mechanical loading of 1 kg or 10 kg was applied for four hours (58, 63). No signs of inflammation were observed using a mechanical loading of 10 kPa, 50 kPa, or 70 kPa, even if a longer duration of loading was applied (4-6 hours) (58, 63). This was in contrast to the studies mentioned above. Possible explanations are differences in the durations of loading and methods used.

In contrast to the limited literature on inflammation and oedema in pressure ulcers, more extensive literature on these processes exists for other conditions affecting muscles, such as compartment syndrome, crush syndrome, vascular injury, and delayed onset muscle soreness (DOMS) (86). When comparing the main findings of our review with this literature, we identified some similar patterns. Both compartment syndrome, crush syndrome, and vascular injury were characterised by ischemia-reperfusion injury (86-88). Compartment syndrome and crush syndrome are to a major degree associated with elevated interstitial pressure, and more specifically intracompartmental pressure, as a consequence of oedema. Moreover, in all

four conditions, neutrophils are the first invading inflammatory cells, followed by macrophages, and promote further muscle damage (89-91). In case of extensive muscle damage, systemic conditions may occur, such as adult respiratory distress syndrome (ARDS), and multiple organ failure syndrome (MOF) (92).

Literature on acute injuries (e.g. burns, crush injuries) describes the presence of three characteristics wound zones: a central zone of necrosis, a peripheral zone of stasis, and a hyperaemic zone around (93, 94). However, our literature search revealed no studies reporting these zones in deep tissue injuries, which are characterised by rather chronic damage pathways. Although, the persistence of oedema or lymphatic stasis near deep tissue injuries may be an important area for future research, which may offer important cues to the management of deep tissue injuries.

Most of the quasi-experimental studies performing histopathological assessments, restricted these assessments to the level of muscle tissue (48, 58, 61, 62). However, insights into tissue changes at the level of the dermis and epidermis are important to identify early stage deep tissue injuries. Moreover, several body sites which are susceptible for the development of pressure ulcers, due to a bony prominence, are poorly covered by muscle tissue (e.g. sacrum, heels, elbows) (21). Therefore, we recommend researchers to compare tissue changes at skin level between animals/humans with or without deep tissue inflammation and oedema. This research will additionally enhance the body of knowledge on the pathophysiological processes

involved in the development of deep tissue injuries and may support the selection and use of appropriate biological markers for early detection of deep tissue injury.

Another limitation of the quasi-experimental studies was the restricted follow-up time, which mostly lasted no longer than a few hours to one day. However, two other studies suggested a gradual increase in the inflammatory response and the development of oedema over several days, until healing was initiated (48, 61). In addition, most analyses were based on a single mechanical loading of two hours . This is in contrast to clinical practice where patients are subjected to longer (e.g. 4 hours) and recurrent periods of mechanical loading. Research has indicated that, once a threshold has been exceeded, tissue damage increases with the amount of the mechanical loads and the loading time (95). Moreover, longer and recurrent periods of mechanical loading have been associated with a more intense inflammatory response (59, 89, 96). Additional knowledge is needed on the course of inflammation and oedema associated with recurrent mechanical loading, reflecting repositioning frequencies in clinical practice.

A few observational studies were performed in humans. However, most had a cross-sectional design and/or were restricted in sample size. According to Salcido, Popescu (97), similarities exist between the skin of mouse, rat, pig, and human. However, the microcirculation in human skin and muscle seems to be more complex. To be able to correlate early changes in deep tissues with pressure ulcer development, longitudinal studies are needed. Furthermore, sample sizes in future studies should be large enough to account for the known heterogeneity

in individual damage threshold (20, 97). According to the pressure ulcer conceptual framework of Coleman, Nixon (20), this damage threshold is affected by the balance between mechanical boundary conditions and individual susceptibility and tolerance. Both are influenced by individual risk factors, such as mobility, nutritional and perfusion status, skin status, and moisture.

An important gap in current knowledge which has been identified by the authors of this review, is that all the published studies where inflammation and oedema have been investigated did not consider abnormal inflammatory responses. Abnormal inflammatory responses can be associated with acute or chronic conditions (e.g. severe trauma, diabetes mellitus), use of pharmaceuticals (e.g. corticosteroids), undernutrition or overnutrition, and higher age, all of which are characterised by immunodeficiency (98, 99). Specifically, patients at risk for a pressure ulcer often suffer damage to their nervous system, either the central (e.g. spinal cord injury, brain trauma or stroke) or the peripheral (e.g. diabetic neuropathy). There is strong coupling between the functions of the inflammatory system and nervous system. Damage to the nervous system typically results in chronic inflammation or abnormal inflammation response, which affects the sensitivity to cytokine signalling, the extent of activation of the inflammatory response (including inflammatory oedema), and the time and rate of its cessation (31). There is clearly a need to study inflammation and oedema in animal models in which the nervous system has been damaged centrally or peripherally, in order to

understand the impact of such neural dysfunctions on the patterns of formation of inflammation and oedema caused by exposure to sustained mechanical loading.

CONCLUSION

Inflammation and oedema are complex processes involved in the development of early stage deep tissue injuries. Limited evidence, mainly based on animal studies, indicated that both processes initiate in the subcutaneous tissues, and increase in intensity during the first days after relief of the mechanical loads. Further research should focus on how early stage deep tissue inflammation and oedema manifested into unique tissue changes at the level of the dermis and epidermis, and how abnormal inflammatory responses manifest (e.g. when the nervous system is not functioning normally).

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INDIVIDUAL TABLES

Table 1. Electronic search strategy for PubMed (MEDLINE database)

Table 2. Evolution of the inflammatory response after mechanical loading

Table 3. Evolution of the development of oedema after mechanical loading

Chart table (attachment)

FIGURE CAPTION

Figure 1. PRISMA flow chart

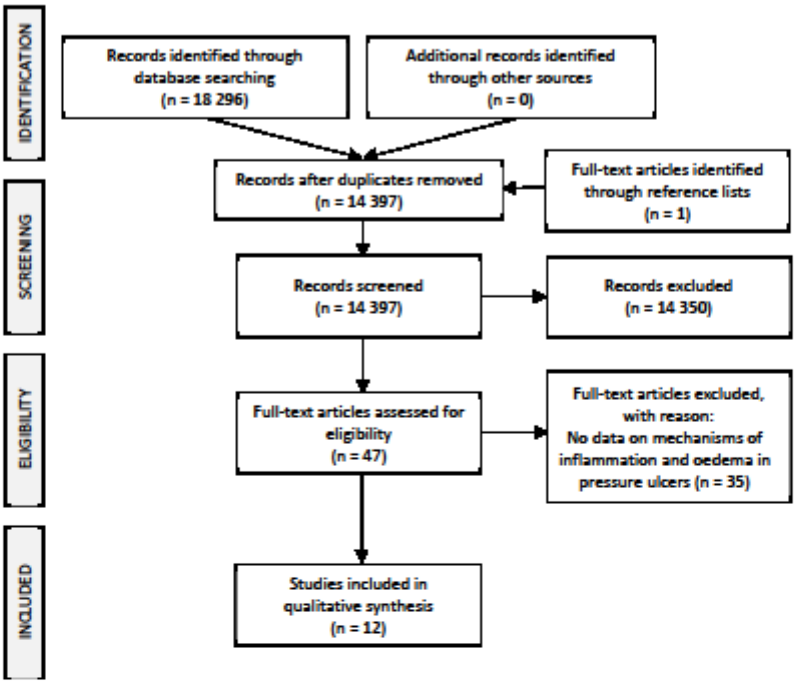

Figure 1. PRISMA flow chart

Table 1. Electronic search strategy for PubMed (MEDLINE database)

Search	Query
#1	((((pressure[MeSH Terms] OR pressure[Title/Abstract] OR shear[Title/Abstract] OR friction[MeSH Terms] OR friction [tiab]) AND ((pressure damage [tiab]) OR (mechanical stress [tiab]) OR (stress, mechanical [mesh]) OR damage [tiab] OR deformation [tiab] OR strain [tiab] OR (sprains and strains [mesh]) OR compressing [tiab] OR deforming [tiab] OR mechanical loading [tiab] OR (mechanical force [tiab]) OR mechanobiology [TIAB] OR (biomechanical phenomena [MeSH]))) OR ((pressure ulcer [mesh]) OR (pressure ulcer [tiab]) OR (decubit* [tiab]) OR (deep tissue injur* [tiab]) OR (pressure induced tissue damage [tiab]) OR (pressure injur* [tiab]) OR (pressure ulcers [tiab]) OR bedsore* [tiab] OR (pressure sore* [tiab]) OR (bed sore* [tiab]) OR (decubitus ulcer* [tiab]))))
#2	((inflammation [mesh] OR inflammation [tiab] OR (inflammatory response [tiab]) OR (subclinical inflammation [tiab]) OR (microinflammation [tiab]) OR (inflammatory marker [tiab]) OR (apoptosis [mesh]) OR (apoptosis [tiab]) OR (cell death [tiab]) AND necrosis [mesh] OR necrosis [tiab] OR (cell membrane permeability [mesh]) OR (cell membrane permeability [tiab]) OR (Immunity, Innate [mesh]) OR (innate immunity [tiab]) OR (sterile stimul* [tiab]) OR (toxic metabolite* [tiab]) OR (perfusion [tiab]) OR ischaemia [tiab] OR ischaemia [mesh] OR ischemia [tiab] OR reperfusion injury [mesh] OR reperfusion injury [tiab] OR reperfusion [mesh] OR reperfusion [tiab]) AND (muscle [tiab] OR fat [tiab] OR (adipose tissue, white [mesh]) OR (adipose tissue [tiab]) OR (subcutaneous tissue [mesh]) OR subcutan* [tiab] OR (soft tissue injuries [mesh]) OR (soft tissue [tiab]))))
#3	(edema [mesh] OR edema [tiab] OR oedema [tiab] OR (capillary permeability [mesh]) OR (transendothelial and transepithelial migration [mesh]) OR swelling [tiab] OR (subepidermal moisture [tiab]) OR (sub-epidermal moisture [tiab]) OR (interstitial fluid [tiab]) OR (interstitial fluid accumulation [tiab]) OR fluid [tiab] OR moisture [tiab] OR water [tiab] OR (extracellular fluid [mesh]) OR (extracellular fluid [tiab]) OR (capillary permeabilit* [tiab]) OR (microvascular permeabilit* [tiab]) OR (vascular permeabilit* [tiab]) OR permeability [mesh] OR permeability [tiab] OR (transepithelial and transendothelial migration [mesh]) OR (transendothelial migration [tiab]) OR (endothelial migration [tiab]) OR diapedesis [tiab] OR exudation [tiab] OR

	extravasation [tiab] OR free water [tiab] OR (bound water [tiab]) OR Vasodilation [TIAB] OR Vasodilatation [MeSH]) AND (Sub-epidermal [TIAB] OR Subepidermal [TIAB] OR Skin [MeSH] OR Skin [TIAB] OR (Subcutaneous Tissue [MeSH]) OR Subcutan* [TIAB] OR Epidermis [TIAB] OR Epidermis [MeSH] OR Dermis [TIAB] OR Dermis [MeSH]))
#4	(#1 AND #2) OR (#1 AND #3)

Table 2. Evolution of the inflammatory response after mechanical loading

Author, year **Animal, tissue**	**Amount of loads**	**Duration of loads**	**Inflammatory response after mechanical loading**							
			0 h	1 h	2 h	4 h	Day 1	Day 3	Day 5	Day 14
Bosboom et al., 2001[58] Rat, muscle	250 kPa	2 h					Infiltration of mononuclear cells			
Houwing et al., 2000[60] Pig, muscle and subcutis	100.0 N	2 h	No signs of inflammation Electron microscopy: neutrophil adherence Significant decrease in reduced glutathione	Early signs of inflammation (swelling and granulocyte infiltration)	Severe infiltration of granulocytes Significant increase in hydrogen peroxide ($p < 0.05$) No significant					

			($p < 0.05$) compared to control		change in reduced glutathione compared to control					
Jiang et al., 2011[34] Rat, muscle and dermis	9.3 kPa	2 h	Mild infiltration of inflammatory cells in dermis and muscle							
Nelissen et al., 2018[48] Rat, muscle	Not reported	2 h			In some cases early neutrophilic infiltration			Significant infiltration of neutrophils and lymphocytes	Decrease in neutrophils and lymphocytes, increase in macrophages	Neutrophils, lymphocytes and macrophages decreased to normal/minimal levels

Sari et al., 2010[61] Rat, muscle	1kg 10 kg	4 h					1 kg group: little infiltration of neutrophils and lymphocytes 10 kg group: extensive infiltration of neutrophils and lymphocytes	1 kg: muscle regeneration has started 10 kg group: more clear signs of inflammation		
Stekelenburg et al., 2006[62] Rat, muscle	1.1 N, 150 kPa	2 h				Early signs of inflammation: infiltration of polymorpho	Extensive infiltration of polymorpho nuclear leukocytes			

						nuclear leukocytes	and monocytes			
Stekelenburg et al., 2006[63] Rat, muscle	150 kPa	2 h					Extensive infiltration of polymorpho nuclear leukocytes and monocytes			

Table 3. Evolution of the development of oedema after mechanical loading

Author, year **Animal, tissue**	**Amount of loads**	**Duration of loads**	**Development of oedema after mechanical loading**								
			0 h	1 h	2 h	4 h	Day 1	Day 3	Day 5	Day 8	Day 14
Houwing et al., 2000[60] Pig, muscle	100.0 N	2 h	No oedema Electron microscopy: oedema	Early signs of oedema						Perivascular oedema diminishes after one week	
Jiang et al., 2011[34] Rat, muscle and dermis	9.3 kPa	2 h	No oedema								
Nelissen et al., 2018[48] Rat, muscle	Not reported	2 h			Mild oedema			Significant oedema	Minimal/mild oedema		No oedema
Sari et al., 2010[61] Rat, muscle	1kg 10 kg	4 h					1 kg group:	10 kg group:			

							small interstitial spaces 10 kg group: widened interstitial spaces, mean muscle oedema index significant higher than control and 1 kg	widened interstitial spaces, mean muscle oedema higher than day 1			

Stekelenburg et al., 2006[62] Rat, muscle	1.1 N (150 kPa)	2 h		Widened interstitial spaces		Widened interstitial spaces					

Chart table

Author, year	Title	Study sample/model	Aim	Design	Methods	Relevant findings
Aliano et al., 2014[40]	The correlation between ultrasound findings and clinical assessment of pressure-related ulcers: is the extent of injury greater than what is predicted?	• Hospitalised patients with pressure ulcer category I (n = 8), category II (n= 4), or suspected deep tissue injury (n = 8) • Young, healthy individual without pressure ulcer (n = 1)	To examine the hypothesis that deep tissue injury is present in either superficial pressure ulcers and suspected deep tissue injury	Prospective observational study	• High resolution ultrasonography (12 MHz)	• In all patients with pressure ulcers, regardless of the category, some kind of deep tissue injury was detected • Signs of deep tissue injury included: loss of dermoepidermal interface, and presence of hypoechoic lesions in the subcutaneous fat and deep muscle
Bosboom et al., 2001[58]	Quantification and localisation of damage in rat	• In vivo rat model (n = 11)	To develop an animal model that can be used to relate	Quasi-experimental study	• Intervention 1: mechanical loading: 10 kPa for 6 h (n = 2)	• Only the load application of 250 kPa caused muscle damage

	muscles after controlled loading: a new approach to study the aetiology of pressure sores		the controlled external loading to the location and amount of local muscle tissue damage		• Intervention 2: 10 kPa, 70 kPa and 250 kPa for 2 h (n = 3/group) • Control: non-loaded contralateral muscle • Interstitial fluid pressure measurements • Histology: 24 h after relief of loads	• Loss of cross-striation of muscle fibres • Infiltration of mononuclear cells into the damaged muscle tissue • The location of the damage was restricted to the diameter of the indentor
Edwards, 2006[28]	Tissue viability: understanding the mechanisms of injury and repair	N/A	To review the underlying pathophysiology of tissue viability	Review/educational paper	N/A	• Cellular oedema is caused by disturbance of ionic gradients due to deficient ATP-dependent sodium-potassium pumps in the cellular membrane. This increases the intracellular osmotic pressure.

						• Interstitial oedema is caused by increased hydrostatic pressure, decreased colloid osmotic pressure, obstruction of the lymphatic system, or stimulation of the inflammatory response • As part of the inflammatory response, mediators are released (e.g. cytokines). • Mediators attract nutrients, fluid, clotting factors, neutrophils, and macrophages. • Mediators cause a localised increase in capillary

						permeability and vasodilatation
Gust et al., 2017[59]	Adipose tissue drives response to ischemia-reperfusion injury in a murine pressure sore model	• In vivo mouse model (n ≥ 60) • In vitro human tissue culture (fibroblasts, adipocytes, adipose tissue explants (n = 12)	To evaluate the differential response of adipose tissue and dermal tissue to ischemia-reperfusion injury	• Quasi-experimental study	• Intervention: varying ischemia-reperfusion cycles (1-4) and duration of ischemia (1 h, 1.5 h, 2 h) (n ≥ 5/condition) • Control: no ischemia-reperfusion (n ≥ 5) • Histology • Immunohistochemistry: neutrophils • Biochemistry: RNA analysis for inflammatory markers (cytokines) • Computer-assisted image analysis: area of necrosis	• Increase of duration of ischemia and number of ischemia-reperfusion cycles is associated with increase of inflammatory infiltrate in adipose tissue ($p < 0.05$ in group of 2 h of ischemia – 4 cycles), but not in dermal tissue • Neutrophils concerned the majority of infiltrative cells (> 90%) • Inflammatory markers are significantly upregulated in adipose tissue following ischemia-reperfusion in

						murine ($p < 0.001$) and in human ($p < 0.05$) • Increase of duration of ischemia and number of ischemia-reperfusion cycles is associated with increase of the area of skin necrosis. After 4 cycles, the differences were statistically significant ($p < 0.05$) between each of the experimental groups
Houwing et al., 2000[60]	Pressure induced skin lesions in pigs: reperfusion injury and the effects of vitamin E	• In vivo pig model (n = 16)	To investigate the role of ischemia-reperfusion in pressure-induced tissue necrosis	Quasi-experimental study	• Intervention: standard pig food + vitamin E supplement, 14 days before experiment (n = 8) • Control: standard pig food (n = 8)	• Light microscopy of muscle: immediately after relief of loads: no damage visible; at 1 h: early signs of inflammation (swelling and granulocyte infiltration),

					• Mechanical loading: 100 N for 2 h • Time frame for biopsies: between 0 and 2 h and up to 330 h (14 days) after relief of loads • Histology • Biochemistry: inflammatory/oxidative stress markers (catalase, hydrogen peroxide, reduced glutathione)	anatomy of myocytes disappearing; at 2 h: severe infiltration of granulocytes, structure of myocytes completely disappeared, necrotic muscle • Electron microscopy of muscle: immediately after relief of loads: oedema, and adherence of neutrophils to the capillary endothelium • Granulocyte invasion increases further during the following days • Significant increase in plasma hydrogen peroxide ($p < 0.05$) at 2 hours of

						reperfusion versus no reperfusion • Significant decrease in reduced glutathione ($p < 0.05$) in tissue exposed to mechanical loading versus tissue not exposed to mechanical loading • Perivascular oedema diminishes after one week • After 2 weeks, some repair occurred, with formation of connective tissue • No damage to the epidermis was observed • Histological damage followed a pattern

						determined by arterial supply
Jiang et al., 2011[34]	Ischemia-reperfusion injury-induced histological changes affecting early stage pressure ulcer development in a rat model	• In vivo rat model (n = 48)	To create a pressure-induced injury animal model and explore the possible mechanisms and effects of ischemia-reperfusion injury	Quasi-experimental study	• Intervention 1: ischemia, 2 h (n = 8) • Intervention 2: 3 cycles of 2 h ischemia + 1-4 h reperfusion (4x n = 8) • Control: no ischemia or ischemia-reperfusion (n = 8) • Mechanical load: 70 mmHg (9.3 kPa) • Histology • Biochemistry: markers of oxidative stress/inflammatory mediators [superoxide dismutase (SOD),	• Ischemia group: mild injury, loosening of muscle tissue, and infiltration of inflammatory cells in muscle and dermis • Ischemia-reperfusion groups: severe injury, oedematous muscle fibres, infiltration of inflammatory cells in muscle after 3 cycles • Control group: no histological changes • Levels of inflammatory mediators were significant higher (or lower for SOD) (p

					malondialdehyde (MDA), endothelin-1 (ET-1), nitric oxide (NO)]	< 0.05) in the ischemia-reperfusion groups compared to the control group, and the ischemia group. These differences increased with increasing reperfusion time and decreased slightly in the 4 h reperfusion group, indicating recovery. No significant differences between ischemia group and control group
Nelissen et al., 2018[48]	An advanced magnetic resonance imaging perspective on the aetiology of deep tissue injury	• In vivo rat model (n = 59)	To investigate damage induction as well as remodelling in deep tissue injuries	Quasi-experimental study	• Intervention: mechanical loading for 2 h • Control: contralateral muscle (for histology)	• During mechanical loading: increased MRI signals starting distal from location of loading, and later both

					• Histology: day 0, 3 and 14 • Multiparametric MRI: before, during, immediately after relief of loads + during follow-up of 14 days	distally and proximally (associated with oedema) • Day 0, 2 h after relief of loads: mild oedema, in some cases early neutrophilic infiltration (first sign of inflammation) • Day 3: pro-inflammatory phase, moderate to severe degeneration, significant necrosis, oedema, haemorrhage, neutrophils, lymphocytes/plasma cells • Day 5: anti-inflammatory and remodelling phase, moderate to severe degeneration, reduced (minimal/mild) necrosis,

						oedema, haemorrhage, increased fibrosis, increase in the number of macrophages, decrease in neutrophils and lymphocytes • Day 14: neutrophils, macrophages, and lymphocytes decreased to normal/minimal levels; no necrosis, oedema, haemorrhage, in some cases mild fibrosis • MRI (transverse relaxation time): increased signal intensity immediately after relief of loads

						• The initial response to deformation occurred at some distance from the centre of indentation, affecting a relatively large area
Quintavalle et al., 2006[65]	Use of high-resolution, high-frequency diagnostic ultrasound to investigate the pathogenesis of pressure ulcer development	• Long-term-care facility residents at risk for developing pressure ulcers (n = 119) • Healthy volunteers at a medical centre (n = 15)	To explore and describe the pathogenesis of pressure ulcers	Prospective observational study + quasi-experimental study	• In healthy volunteers: preulcerative changes induced by friction (rubbing with a gauze pad), and mechanical loading (lying on a hard object for 1 h) • High resolution ultrasonography (20 MHz) • Comparison with documentation of clinical assessment	• Superficial pressure ulcers due to friction: oedema directly below intact epidermis, in some cases extending downward with some dermal oedema • Deep pressure ulcers: 3 patterns: (1) oedema between bone and dermal layer without dermal involvement, (2) oedema in the subdermal tissue with

						progression into the dermal layer, (3) oedema extending from the subdermal tissue via the dermis to the dermal/epidermal junction where it pools
Sari et al., 2010[61]	Hypoxia is involved in deep tissue injury formation in a rat model	• In vivo rat model (n = 75)	To examine the involvement of hypoxia in deep tissue injury	Quasi-experimental study	• Intervention 1: 1.0 kg load for 4 h (n = 25) • Intervention 2: 10.0 kg load for 4 h (n = 25) • Control: no mechanical loading (n = 25) • Histology: day 1 and 3	• Day 1: 1.0 kg group: muscle degeneration, very little necrosis, little infiltration of neutrophils and lymphocytes, small interstitial spaces; 10 kg group: marked increase in necrosis, extensive infiltration of neutrophils and lymphocytes, widened interstitial areas; control

					• Biochemistry: muscle oedema index on day 1 and 3 • Visual observation: healing time • Measurement of exudate and serum creatine phosphokinase (CPK) levels (marker of muscle injury): day 1 • Immunohistochemistry: HIF-1α (marker of ischemia): day 1 and 3	group: normal muscle tissue with small interstitial spaces • Day 3: some muscle regeneration in the 1 kg group, more clear signs of inflammation in the 10 kg group • Oedema: on day 1 and day 3, mean muscle oedema was significantly higher in the 10 kg group compared to other groups ($p < 0.05$ and $p < 0.001$).
Stekelenburg et al., 2006[62]	Compression induced deep tissue injury examined with magnetic	• In vivo rat model (n = 10)	To examine the early damage in muscle tissue after compressive loading	Quasi-experimental study	• Intervention: load application/indentation of 4,5 mm (1.1N, 150 kPa) for 2 h	• MRI during loading: some signal increase distal of loaded location

					• Biochemistry: muscle oedema index on day 1 and 3 • Visual observation: healing time • Measurement of exudate and serum creatine phosphokinase (CPK) levels (marker of muscle injury): day 1 • Immunohistochemistry: HIF-1α (marker of ischemia): day 1 and 3	group: normal muscle tissue with small interstitial spaces • Day 3: some muscle regeneration in the 1 kg group, more clear signs of inflammation in the 10 kg group • Oedema: on day 1 and day 3, mean muscle oedema was significantly higher in the 10 kg group compared to other groups ($p < 0.05$ and $p < 0.001$).
Stekelenburg et al., 2006[62]	Compression induced deep tissue injury examined with magnetic	• In vivo rat model (n = 10)	To examine the early damage in muscle tissue after compressive loading	Quasi-experimental study	• Intervention: load application/indentation of 4,5 mm (1.1N, 150 kPa) for 2 h	• MRI during loading: some signal increase distal of loaded location

						neutrophils, widened interstitial spaces adjacent to the necrotic region • At 20 h after relief of loads: extensive regions of necrosis; extensive infiltration of neutrophils and monocytes, revealing a pronounced inflammatory response, cellular debris occupied empty spaces
Stekelenburg et al., 2006[63]	A new MR-compatible loading device to study in vivo muscle damage development in rats due to compressive loading	• In vivo rat model (n = 10)	To examine type and location of muscle tissue damage, and damage progress after sustained compressive loading	Quasi-experimental study	• Intervention 1: load application/indentation of 4.5 mm (150 kPa) for 2 h (n = 7) • Intervention 2: load application/indentation of	• MRI: no signal increase during mechanical loading • MRI: increased signal intensity immediately after loading in the 150 kPa group which became significant ($p < 0.001$) after

					2.9 mm (50 kPa) for 4 h (n = 3) • Histology: at 20 h after relief of loads • MRI: immediately after relief of loads, up to 3 h + at 20 h	30 min and remained higher after 20 h • Partial damage (segmental necrosis) to the whole muscle • Damage (at 20 h) consisted of loss of cross-striation in parts of muscle fibres and infiltration of neutrophils and monocytes (extensive inflammatory reaction)
Yabunaka et al., 2009[64]	Can ultrasonographic evaluation of subcutaneous fat predict pressure ulceration	• Patients from a university hospital with at least one pressure ulcer on the great trochanter (n = 9)	To compare subcutaneous tissue in: • Ulcerated an nun-ulcerated skin • Patients with pressure ulcers in	Retrospective observational study	• High resolution ultrasonography (10 MHz)	• Heterogeneous hypoechoic area (hypothesised to be fat necrosis) in subcutaneous fat only present and irreversible in full-thickness pressure

			various stages in the progression towards healing • Superficial and full-thickness pressure ulcers			ulcers versus superficial pressure ulcers • Fat oedema present in superficial as well as full-thickness pressure ulcers • Fat oedema in superficial pressure ulcers was reversible (in deep pressure ulcers not examined)

N/A: not applicable; MRI: magnetic resonance imaging; ATP: adenosine triphosphate; RNA: ribonucleic acid; SOD: superoxide dismutase