Aus der Professur für Medizinische Soziologie und
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DISSERTATION

Extracellular Vesicles as the Potential Mediators of Psychosocial Stress Contribution to Osteoporosis: A narrative review

zur Erlangung des akademischen Grades

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Preface

Some of the results of this work have been published in the following scientific publications. (International Journal of Molecular Sciences, Multidisciplinary Digital Publishing Institute, Switzerland). Similarly, some of the results of this work have been included in encyclopedia.pub., as well as in the form of conference abstracts (International Society of Psychoneuroendocrinology 2021 annual conference):

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He, Y. (2021). Extracellular Vesicles in Osteoporosis. Retrieved from https://encyclopedia.pub/entry/10693.

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Abbreviations

AGEs	Advanced glycation end products
AR	Adrenergic receptor
BMMs	Bone marrow-derived macrophages
BMSC	Bone marrow mesenchymal stem cell
cAMP	Cyclic adenosine monophosphate
CREB	Cyclic adenosine monophosphate response element-binding protein
CUMS	Chronic unpredictable mild stress
ER	Endoplasmic reticulum
EVs	Extracellular vesicles
GPCR	G-protein coupled receptor
HSP	Heat-shock protein
IP_3	Inositol trisphosphate
miRNA, miR	microRNA
MSCs	Mesenchymal stem cells
MVBs	Multivesicular bodies
PIP_2	Phosphatidylinositol bisphosphate
PLC	Phospholipase c
RANK	Receptor activator of nuclear factor κ-B
RANKL	Receptor activator of nuclear factor κ-B ligand
ROS	Reactive oxygen species
Runx2	Runt-related transcription factor 2
SNS	Sympathetic nervous system

SOCS-3	Suppressor of cytokine signaling-3
TRIP-1	Transforming growth factor beta receptor II interacting protein-1
Ub	Ubiquitination
UTR	Untranslated region

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1 Summary (German)

Hintergrund: Kennzeichnend für Osteoporose sind eine verringerte Knochenmasse und die Zerstörung der Mikroarchitektur des Knochengewebes, wodurch sich das Risiko von Knochenbrüchen erhöht. Psychosozialer Stress und Osteoporose sind durch das sympathische Nervensystem, die Hypothalamus-Hypophysen-Nebennieren-Achse und andere endokrine Faktoren miteinander verbunden. Psychosozialer Stress hat eine Reihe von Auswirkungen auf den Organismus, und diese langfristige Erschöpfung auf zellulärer Ebene wird als mitochondriale allostatische Belastung angesehen, die eine mitochondriale Dysfunktion und oxidativen Stress beinhaltet. Extrazelluläre Vesikel (EVs) sind in den mitochondrialen allostatischen Belastungsprozess involviert und können in diesem Zusammenhang als Biomarker dienen. Als kritische Teilnehmer der Zell-zu-Zell-Kommunikation dienen EVs als Transportmittel für Nukleinsäuren und Proteine, verändern die phänotypischen und funktionellen Eigenschaften ihrer Zielzellen und fördern den Zell-zu-Zell-Kontakt. Daher spielen sie eine wichtige Rolle bei der Diagnose und Therapie vieler Krankheiten, wie z. B. Osteoporose.

Ziel: Diese Übersichtsarbeit soll die Eigenschaften von EVs und ihre Rolle in Hinblick auf den Zusammenhang zwischen psychosozialen Stress und Osteoporose zusammenfassen. Weiterhin wird untersucht, ob EVs in dem Zusammenhang eine potenzielle Mediatorenrolle zukommt.

Methoden Die Online-Datenbanken PubMed, Google Scholar und Science Direct wurden anhand thematischer Stichwörter durchsucht und die Verfügbarkeit aller ausgewählten Studien überprüft. Anschließend wurden die Ergebnisse der Artikel zusammengefasst und miteinander in Verbindung setzen.

Ergebnisse: Psychosozialer Stress führt zu einer Erhöhung der Transmitterkonzentrate wie Glukokortikoide und Katecholamine sowie einen erhöhten Glukosestoffwechsel, was jeweils Einfluss auf den Knochenumbau haben kann. Darüber hinaus führt psychosozialer Stress zu mitochondrialer allostatischer Last, einschließlich oxidativem Stress, was sich ebenfalls auf den Knochenumbau auswirken kann. Sowohl in vitro- als auch in vivo-Daten deuten darauf hin, dass EVs hierbei durch ihre Übertragung von bioaktiven Messengern eine relevante Mediatorenrolle einnehmen. Es ist anzunehmen, dass sie potenzielle Vermittler des Zusammenhangs von psychosozialem Stress und osteoporotischen Veränderungen sein können.

Schlussfolgerung: Entlang der eingeschlossenen Studien besteht ein Zusammenhang zwischen psychosozialem Stress, dem Knochenumbau und damit der Entstehung von Osteoporose. In der Genese des negativen Einflusses auf die Knochengesundheit scheint EVs durch ihre Aktivität als Messenger-Transporter eine relevante Mediatorenrolle zuzukommen. Dieses Wissen hat das Potenzial für zukünftige innovative Forschungskonzepte.

2 Summary (English)

Background: The characteristics of osteoporosis are decreased bone mass and destruction towards the microarchitecture of bone tissue, which raises the risk of fracture. Psychosocial stress and osteoporosis are linked by sympathetic nervous system, hypothalamic-pituitary-adrenal axis, and other endocrine factors. Psychosocial stress causes a series of effects on the organism, and this long-term depletion at the cellular level is considered to be mitochondrial allostatic load, including mitochondrial dysfunction and oxidative stress. Extracellular vesicles (EVs) are involved in the mitochondrial allostatic load process and may as biomarkers in this setting. As critical participants during cell-to-cell communications, EVs serve as transport vehicles for nucleic acid and proteins, alter the phenotypic and functional characteristics of their target cells, and promote cell-to-cell contact. And hence, they play a significant role in the diagnosis and therapy of many diseases, such as osteoporosis.

Aim: This narrative review attempts to outline the features of EVs, investigate their involvement in both psychosocial stress and osteoporosis, and analyze if EVs can be potential mediators between both.

Methods: The online database from PubMed, Google Scholar, and Science Direct were searched for keywords related to the main topic of this study, and the availability of all the selected studies was verified. Afterward, the findings from the articles were summarized and synthesized.

Results: Psychosocial stress affects bone remodeling through increased neurotransmitters such as glucocorticoids and catecholamines, as well as increased glucose metabolism. Furthermore, psychosocial stress leads to mitochondrial allostatic load, including oxidative stress, which may affect bone remodeling. *In vitro* and *in vivo* data suggest EVs might involve

in the link between psychosocial stress and bone remodeling through the transfer of bioactive substances and thus be a potential mediator of psychosocial stress leading to osteoporosis.

Conclusions: According to the included studies, psychosocial stress affects bone remodeling, leading to osteoporosis. By summarizing the specific properties of EVs and the function of EVs in both psychosocial stress and osteoporosis, respectively, it has been demonstrated that EVs are possible mediators of both, and have the prospects to be useful in innovative research areas.

3 Introduction

Stress occurs when individual senses or feels that homeostasis is at threat [1]. Homeostasis, in terms of physiological theory, is the self-regulatory process through which biological systems retain stability while responding to changing external situations [2]. The hypothalamic-pituitary-adrenal axis and autonomic nervous system play an essential role in regulating the response of body under threats [3]. Increased production of glucocorticoids, which typically motivate energy to help the body react against challenges, occurs when the hypothalamic-pituitary-adrenal axis is activated as a result of a threat to stress. And the activation of sympathetic nervous system can lead to the release of adrenaline from the adrenal medulla. These reactions mediate the necessary acute "fight or flight" response [4]. However, chronic stress that comes from repetitive negative life events responses may have harmful effects on the body [5], and is the risk factor for many diseases, in particular for psychiatric disorders, such as depression. Mitochondria, as a key constituent of cellular components, are involved in the process of chronic stress damage to the organism. The prolonged stress comes with glucocorticoid dysregulation, which increases glucose metabolism and reactive oxygen species [6]. These cumulative alterations result in mitochondrial DNA dysregulation, mitochondrial dysfunction, and increased cellular senescence [6]. Besides, the increased glucose metabolism generates advanced glycation end products, then increases cellular senescence [7,8]. These pathways may be the biological mechanisms by which stress affects the health of the organism.

In addition to these specific physiological reactions, one must also consider the allostatic load, or long-term consequences. Allostasis and allostatic load were used to introduce the conceptualization between organismal adaptability and adaptation to chronic and excessive stress. Allostasis is the process of maintaining physiological systems in the face of changing demand, which can also be accepted as altering biological "set points" in the body's homeostasis to promote survival, and adaptive or maladaptive allostasis may lead to the

maintenance of health or the development of disease [9]. Since it's difficult to readjust to the original homeostatic level due to chronic and excessive stress stimuli, the body system tries to set a new point in the face of chronic stress. And the difference between original and newly set points can be understood as the allostatic load [9], which is also known as "wear and tear on the body," refers to the accumulation of social, environmental, and psychological difficulties as a result of repetitive or chronic stress [10], this disrupts the integrity of multiple functional areas of the organism. The term allostatic overload, a severe state of allostatic load, refers to chronic exposure to stress responses that exceed an individual's coping ability [11]. The allostatic load and overload are considered harmful to health and may be associated with diseases [11], although research on the biomarkers and clinical criteria has yet to research a consensus. The concept of allostatic load was developed to explain the psychophysiological processes undergone by adaptation to stress cumulation and the effects on the body's self-regulatory system. But it's difficult to study in-depth the specific effects of chronic psychosocial stress on the organism with only theoretical support, and researchers have awarded this problem. As research has progressed, recent work has shown that at the cellular level, mitochondria and telomeres are considered to be essential links of allostatic load/overload affecting health [6]. Therefore, future studies focusing on mitochondria and telomeres can be more beneficial for researchers to conduct targeted experimental designs, thus helping shift the theoretical model of stress to experimental studies at the cellular level that better assess the cumulative burden of stress on body.

Over the past decades, with the increase in human life expectancy, organs and tissues are facing the challenges brought by aging. Many age-related degenerations of the body are gaining awareness. Among those degenerations, bone tissue, as an essential member, has attracted the attention of researchers. In healthy skeleton, bone tissue is constantly updated through a collaborative process to maintain the normal bone mass required by the body [12]. The main process of bone remodeling under normal physiological conditions refers to the

dynamic balance of bone resorption and bone formation, resulting in a stable reconstruction of bone tissue, thus maintaining the normal function of the bones [13].

Osteoporosis is the most frequent bone disease caused by physiological process imbalances [14]. During this process, bone mass decreases along with bone microarchitecture degrading, both of which lead to decreased bone strength, increased bone fragility, and a higher risk of fracture [15]. With the society of increased aging, osteoporosis and its most common complication, osteoporotic fracture, have brought huge economic losses and serious social problems to the international community [14]. Many negative behaviors are treated as risk factors for osteoporosis, such as smoking, alcohol consumption, and inadequate nutritional intake; notably, stress is also considered to be one of the risk factors for osteoporosis [16]. Persistent and repetitive stress suppresses osteoblast activity and increases bone resorption by osteoclast, possibly resulting in a long-term loss of bone mass [17], which links chronic stress to bone loss. Moreover, stress can stimulate physical and psychological responses and affect bone health by influencing behavioral factors in the body; for example, perceived psychological stress leading to changes in eating habits and lack of physical activity may be among the potential causes of low bone mass [18]. Decreases in bone mass are concerning since they may increase the risk of osteoporosis and fragility fractures.

Cell-to-cell interactions, however, bolster these processes are still a mystery to researchers. The stress-induced osteoporosis might be linked to EVs-mediated communication between cells [19], which has recently emerged as a significant regulator of cell-to-cell communication [20]. EVs are membrane vesicles secreted by many different types of cells that enter the extracellular space and participate in intercellular communication [21]. The study of EVs is an emerging field that has attracted the attention of researchers because of their involvement in intercellular communication, which is considered to have possible therapeutic and diagnostic utility [19]. It's important to consider the sterile inflammation process when researching the role of EVs involved in psychosocial stress. Sterile

inflammation, a critical feature of the psychological/physical stress response [22], is caused by a physical, chemical, or metabolically damaging stimulus instead of pathogenic bacteria [23]. Danger/damage-associated molecular patterns play a vital role during this process. By engaging with pattern recognition receptors, these patterns are released from injured or dying cells to activate the innate immune system [24]. Mitochondrial DNA, heat-shock proteins, S100 proteins, and high-mobility group box 1 are among the most significant molecular patterns linked with danger/damage [25–28]. By delivering immunomodulatory signals, circulating EVs maintain systemic immunological homeostasis and alter sterile inflammation induced by psychological stress [22]. Likewise, EVs impact osteoblasts and osteoclasts and, consequently, may have an effect on osteoporosis [29]. In particular, EVs are involved in the communication between osteoblasts and osteoclasts, as well as in the communication between these two and other cells [30,31]. There are many studies and reviews that separately consider the involvement of EVs during psychosocial stress process and the relationship between EVs and osteoporosis [29,32,33]. But few studies consider the role of EVs in psychosocial stress leading to osteoporosis. In order to analyze whether EVs operate as mediators of psychosocial stress and osteoporosis, a narrative literature review was carried out in this paper.

4 Material and Methods

A narrative review, rather than systematic review, was conducted when the review aims to have a deeper grasp of the subject matter. And combined with the consideration that the topic of this paper is difficult to carry out through systematic analysis, this paper is conducted as a narrative review. References were collected and searched in electronic databases based on the narrative literature review method to find out the role of EVs in both psychosocial stress and osteoporosis, and its potential function between these two by transporting biological cargo.

a. Databases:

PubMed, Google Scholar, and Science Direct

b. Inclusion criteria:

Type of studies: Research or review; languages: English; Time periods: 01/01/1990-31/05/2022. Only peer reviewed studies written in English were included.

c. Search strategy:

Database search was applied on the boolean operators “AND” and “OR” based on keywords identified in a literature review. The following keywords were used to identify the relevant literature: psychosocial stress; allostatic load; bone mineral density; bone remodeling; extracellular vesicles; exosome; microRNA; mitochondria; osteoblast; osteoclast; osteoporosis and so on. Moreover, these keywords or phrases were used in mixed combinations to get the maximum possible variation. And some key references were searched manually, and when necessary, the search was adjusted appropriately to give good depth. The contents of the references were manually screened to exclude those with no relevance to the topic.

d. Number of Articles:

Total studies included in the narrative review (n=175)

e. Review articles and document results:

Verify the availability of all the selected studies, then summarize and synthesize the findings from the found articles based on search strategy to focus on the following most relevant aspects: 1) The pathogenesis of osteoporosis. 2) The effect of psychosocial stress on osteoporosis. 3) Characterization of EVs and their association with stress and osteoporosis. 4) The possible role of EVs between psychosocial stress and osteoporosis.

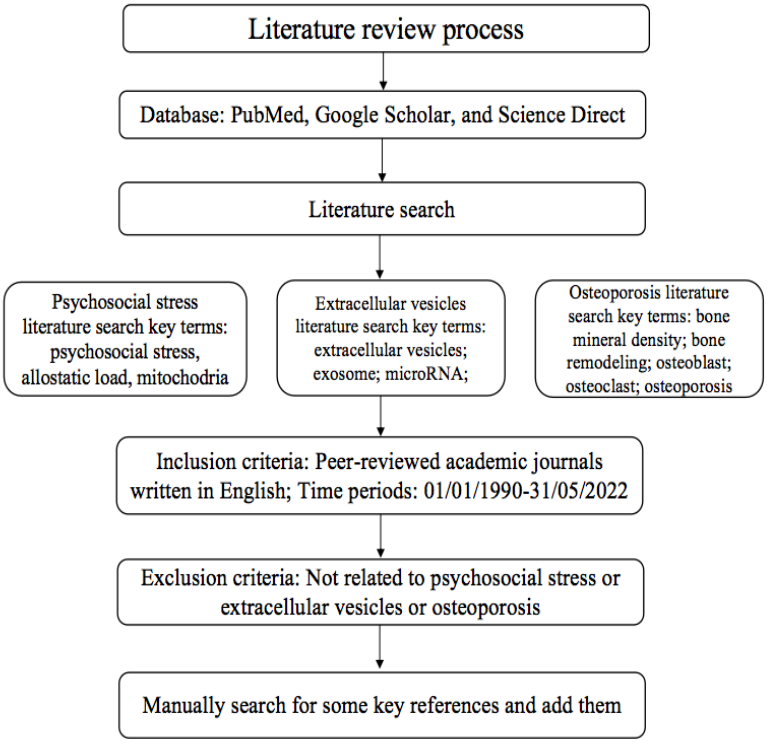

Figure 1 Narrative review process

5 Results

This narrative review included a total of 175 articles, and these papers were manually reviewed and categorized as: stress and related physiological changes, EVs, and osteoporosis. A portion of the articles cover the intersection of stress, EVs, and osteoporosis. In the results, osteoporosis and bone remodeling were introduced, followed by an analysis of the mechanisms by which psychosocial stress leads to osteoporosis. The characteristics of EVs were summarized, and the analysis of the possible role of EVs between psychosocial stress and osteoporosis. Some parts of this chapter (including pictures, tables) have been included in *International Journal of Molecular Sciences* (see also [34], p. 2–9) and *encyclopedia.pub* (From https://encyclopedia.pub/entry/10693) as individual research results in the form of thesis.

5.1 The Bone Remodeling and osteoporosis process

In order to prevent fractures, bone, one of the dynamic tissue, undergoes continuous reconstruction to supplement normal bone mass and thus maintain bone strength. As an essential physiological process in the development of the organism, pre-adult bone development allows bone mass to increase and reach its peak (typically between 25 and 35 years of age [35]); then, the balance of bone reconstruction in the adulthood phase can guarantee the normal bone mass level required by the organism. However, bone loss increases with age (most commonly in menopause), which may have a series of negative effects on the organism. The characteristics of bone remodeling process were summarized, based on the explanations given by He et al. 2021([34], p. 5–6).

The process of bone reconstruction is also known as bone remodeling, including bone formation and bone resorption. Bone remodeling process is associated with many cells, including osteoclasts, osteoblasts, osteocytes, and various immune cells, including T cells, B cells, and megakaryocytes [36]. In the bone-remodeling spaces, osteoclasts, osteoblasts, and osteocytes comprise the fundamental multicellular unit responsible for bone remodeling [37]. The bone remodeling process mainly involves bone resorption followed by bone formation to complement the cavity. And the remodeling cycle results in the complete filling of the resorption cavity with new bone [37,38]. Osteocytes, the most numerous cells in bone tissue, can perceive and respond to ambient mechanical stimuli and govern bone formation and resorption [39]. Therefore, osteocytes play a fundamental role in the bone remodeling process and maintenance of bone mass in the body. Runt-related transcription factor 2 (Runx2) and Osterix contribute significantly to osteoblast differentiation throughout the bone remodeling process [40,41], and osteoclast differentiation is primarily regulated by the receptor activator of nuclear factor κ-B ligand (RANKL)/receptor activator of nuclear factor κ-B(RANK)/osteoprotegerin pathway. Osteoblasts can generate RANKL, which can bind to RANK on the osteoclasts precursors, so stimulating their differentiation. Osteoblasts also release osteoprotegerin to compete with RANK to bind RANKL to tightly control osteoclastogenesis, hence limiting the osteoclasts' differentiation [42].

Bone remodeling is susceptible to various pathological conditions that lead to bone loss, and how to maintain a normal bone remodeling balance under various pathological conditions is still an urgent research problem to be solved.

5.2 The Effects of Psychosocial Stress on Bone Remodeling

Bone is a dynamic tissue in the life process of vertebrates. As an adaptive mechanism to environmental changes, in order to obtain and maintain the integrity of the bone structure and regulate the balance of minerals, bone needs to continuously remold. Many factors affect bone metabolism and increase the risk of fracture, such as thyroid disease, human immunodeficiency virus, type 2 diabetes mellitus, and some drugs [43–46]. The mechanisms of endocrine regulation of bone have been gradually revealed in recent years [47]; it has been found that psychosocial stress and stress-related endocrine regulatory processes may affect bone metabolism and impair bone health [48–50]. As stated above, chronic stress leads to cumulative dysregulation of multiple physiological systems, also known as allostatic load, and is treated as a biological pathway from psychosocial adversity to disease. Studies have shown an association between allostatic load and lower bone strength, as evidenced by lower spine bone mineral density and lower femoral neck strength values [51]. In terms of physiological and molecular mechanisms, many common pathways between psychosocial stress-induced allostatic load and the mechanisms of osteoporosis development have been revealed, such as glucocorticoids, blood glucose, catecholamines, reactive oxygen species, etc.

Glucocorticoids signaling is critical for many cellular processes. Still, glucocorticoids above physiological concentration are harmful to bone. Animal experiments demonstrated that higher glucocorticoid doses reduced bone formation, bone mass, and bone strength in mice [52]. In terms of mechanism, the released glucocorticoids during psychological stress and life adversity are harmful to the bone since they can inhibit osteoblastogenesis and the function of osteoblast, and promote osteoblast and osteocytes to apoptosis [53,54]. These are the main pathological mechanisms of glucocorticoids-induced osteoporosis. The glucocorticoids dysregulation caused by chronic stress leads to a similar bone damage effect, a crucial factor in chronic stress leading to osteoporosis. Besides, the dysregulation of glucocorticoids leads to increased glucose metabolism [6], which results in the production of advanced glycation

end products [7]. They can inhibit the proliferation of mesenchymal stem cells (MSCs), increase the number of apoptotic cells, and prevent the differentiation of MSCs into adipocytes, chondrocytes, and osteocytes [55]. The advanced glycation end products have also been shown to increase apoptosis in osteoblasts [56]. These effects lead to an imbalance in bone metabolism and may contribute to osteoporosis. Thus, increased glucose metabolism may lead to bone diseases.

Secreted catecholamines under chronic psychosocial stress may lead to osteoporosis. Adrenergic receptors (ARs) are a class of G protein-coupled receptors that mainly respond to the increased release of catecholamines (consisting of epinephrine and norepinephrine) as a result of sympathetic nervous system activation [57]. The potential mechanism that psychosocial stress leads to osteoporosis is the activation of β2-AR on osteoblasts and osteoclasts via catecholamine induction. The activated β2-AR pathway on the surface of osteoblasts downregulates bone formation through a series of pathways [58]. This is consistent with results from animal experiments that high bone mass in β2-AR gene deficient mice due to increased bone formation [59]. Notably, β2-AR agonist had an indirect stimulation on osteoclasts by increasing the expression of RANKL on osteoblasts, thus increasing osteoclast formation and bone resorption [60]. And sympathetic signaling directly regulates osteoclastogenesis through β2-AR expressed on osteoclasts via intracellular reactive oxygen species generation [61]. These researches demonstrate that altered catecholamines level due to chronic psychosocial stress may affect bone health.

Psychosocial stress generates neuroendocrine mediators that promote mitochondria structural and functional realignment, constituting mitochondrial allostatic load [62]. As an extension of the allostatic load model, the prolonged activation of allostatic mechanisms at the mitochondrial level (excessive mitochondrial fragmentation, reactive oxygen species production leading to mitochondrial DNA damage and respiratory insufficiency, and release of pro-inflammatory molecules) constitutes the mitochondrial allostatic load (as an extension

from [6], p. 304-307). The main cellular source of reactive oxygen species is mitochondria, and high glucose causes mitochondria to undergo excessive fragmentation, which causes reactive oxygen species overproduction [63]. Although the intermediate mechanisms may be different, elevated glucocorticoids can lead to the same result [64], and the increased reactive oxygen species production leads to oxidative stress. This is consistent with mitochondrial dysfunction in animal models of chronic stress [65]. Mitochondrial dysfunction under chronic stress causes oxidative damage, which affects bone health. Oxidative stress suppresses osteoblast' function by inhibiting mineralization in pre-osteoblastic cells and osteoblastic differentiation in bone marrow mesenchymal stem cells (BMSCs) [66]. Moreover, excess reactive oxygen species were also promoting bone resorption by osteoclasts [67]. They are involved in the dual regulation of osteoclasts and osteoblasts in the skeletal system, thereby reducing bone mass. Considering the damage caused by oxidative stress on bone remodeling, some antioxidants are now entering scientists' minds as therapeutic agents for osteoporosis, such as tocotrienols [68]. Stressed mitochondria during mitochondrial allostatic load increase the release of inflammatory signals, leading to chronic inflammation. Studies have shown that chronic psychological stress causes alterations in inflammatory cytokines such as elevated interleukin-6 and tumor necrosis factor-α [69], which are bone resorption stimulants and bone formation inhibitors [70]. Thus, the inflammatory response in the stress state may link it and osteoporosis, but the detailed mechanisms involved need further investigation.

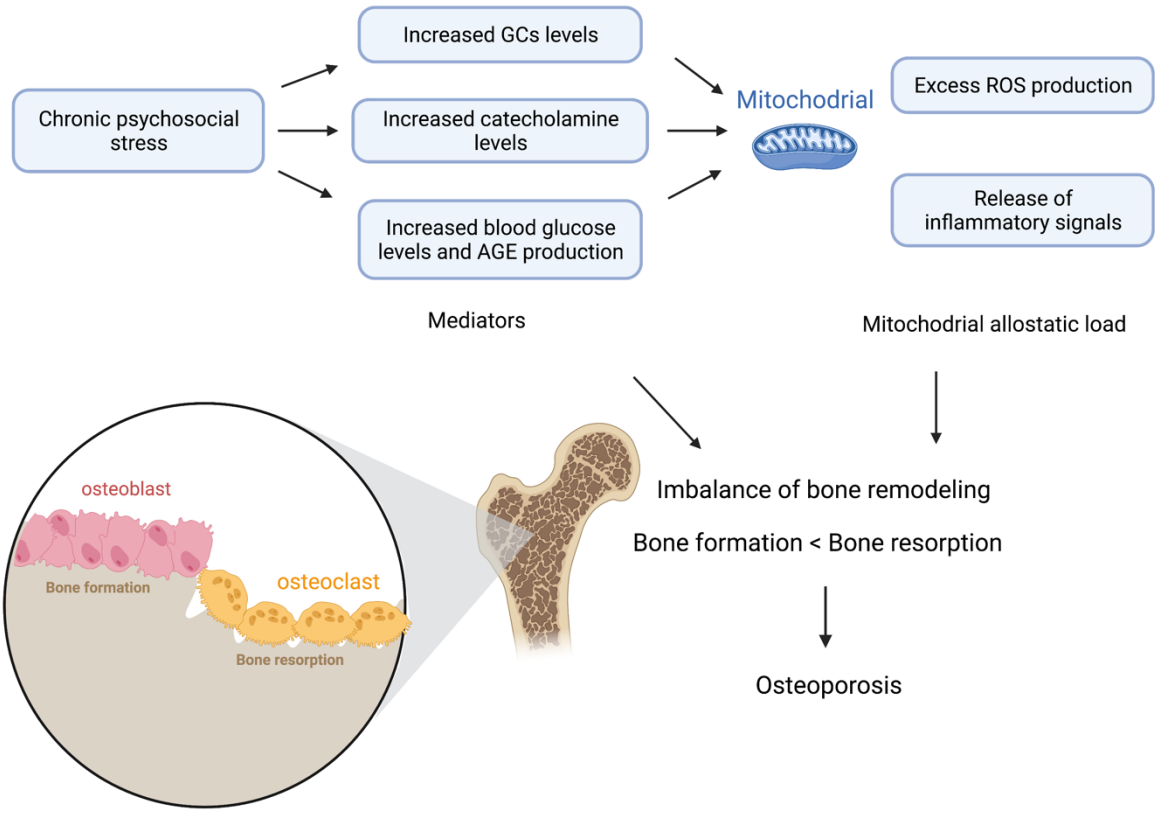

Figure 2 The effects of psychosocial stress on bone. Psychosocial stress generates neuroendocrine mediators that promote structural and functional realignment of mitochondria, forming allostatic mitochondrial load [62]. These altered neuroendocrine mediators include elevated glucocorticoids and catecholamine levels, blood glucose levels, and development of advanced glycation end products (AGEs), which have the potential to impact the bone remodel balance. In addition, these mediators influence the structure and function of mitochondria, forming the mitochondrial allostatic load, which consists of excessive reactive oxygen species (ROS) generation and the release of inflammatory signals. These are likewise osteoporosis risk factors.

5.3 The Characteristics and Roles of EVs

The definition and main categories of EVs were summarized, based on the explanations given by He et al. 2021([34], p. 2).

> EVs refer to vesicles with a lipid bilayer membrane structure that released by cells into the extracellular environment [21]. According to their subcellular origin and biogenesis, EVs divide into three main categories: small EVs (or exosomes), medium/large EVs (or microvesicles), and apoptotic bodies [71]. Exosomes are 40–200 nm diameter vesicles with uniform size, which are released from intracellular multivesicular bodies (MVBs) fused with the cytoplasmic membrane [72–74]. In contrast, microvesicles are non-uniform, 200–2000 nm diameter particles that are budding generated and expelled from the cytoplasmic membrane. Apoptotic cells undergo programmed cell death and release apoptotic bodies (800–5000 nm in diameter) that share certain properties with microvesicles [75].

Due to the overlap in size, lack of specific markers, and similar biological structural composition of these subtypes, the International Society for Extracellular Vesicles recommends using the term EVs to describe these groups unless their subcellular origin can be verified [71]. EVs carry multiple biomolecules, consisting of DNA, RNA, proteins, glycans, lipids, and metabolites [76,77]. They are regarded as communication vehicles for the transfer of substances, thus affecting the receptor cells, including function and composition. EVs can be derived from lots cells, such as MSCs [78], immune cells [79], tumor cells [80], platelets [81], and cardiomyocytes [82]. In addition, they are detectable in the majority of bodily fluids, including saliva, peripheral blood, urine, semen, and breast milk [83]. As a result, EVs are increasingly being treated as tools for diagnosing and treating diseases.

EVs, as transmitters, can affect receptor cells in a variety of biological ways, such as plasma membrane fusion and binding cell surface receptor [29,84]. Figure 3 illustrates the EV biogenesis, secretion, and impact on target cells. Since their genetic properties, nucleic acids transported by EVs may serve as potential biomarkers [85]. Table 1 summarizes the functions of EVs in human tissues. Moreover, gene therapy is currently a hot research topic in the

medical community. Still, its weakness is the delivery problem, as RNA molecules cannot reach the inside of target cells due to their easily degradable characteristic, hydrophilicity, and negative charge. The properties of EVs have attracted the attention of researchers, and their ability to transfer genetic material makes them widely promising for gene therapy. RNAs in mammalian cell-derived EVs include essentially all RNAs of the cell, messenger RNAs, microRNA (miRNA, miR), small nuclear RNA, small nucleolar RNA, ribosomal RNA, transfer RNA, etc. [86]. Since miRNAs have numerous properties suitable for research, most studies were focused on miRNA. MiRNAs are 17–24 nucleotide endogenous, non-coding RNAs, which post-transcriptionally silence target genes' expression by binding to the 3'-untranslated region (UTR) open reading frame region of target messenger RNAs (summarized from [87,88]), thus affecting a wide range of biological functions of the organism. Since miRNAs have the property of influencing gene expression, which is seen as a tool for disease diagnosis or treatment, and it is of great interest to further understand the function of miRNAs [89,90].

Due to the heterogeneity of the EVs family and the presence of sample contaminants, it poses a challenge for the purification, analysis, and clinical application of EVs. It is necessary to detail the storage, isolation, and analysis methods of EVs when explaining the experiments. Given the unique characteristics of EVs, many aspects need attention when studying circulating EVs in a specific way. First, during the collection and processing of the subject's blood, an overnight fast (including smoking, alcohol, coffee, etc.) should be maintained in the morning before blood collection, as well as avoiding strenuous physical activity the day prior to the blood collection to exclude the effects of potential factors on blood composition and the quantity, content, and purity of blood-derived EVs [91]. Furthermore, the choice of blood derivatives should be decided based on the purpose of downstream analysis after EVs isolation [91]. In general, plasma is more suitable for EVs studies than serum because blood coagulation releases EVs and affects experimental analysis results [92]. And it's better to remove platelets

as possible by a double spin to avoid platelets released EVs that interfere with the experiment results [93]. Common methods of isolating EVs are but not limited to ultracentrifugation, immunoaffinity, precipitation, microfluidics techniques, and size exclusion chromatography [71,94]. These methods may differ in recovery and specificity, thus requiring the researchers to determine possible isolation methods according to the purpose of the study.

Quantification and characterization of EVs is another essential process after EVs' isolation when EVs are used at the cellular level to study physiological processes of diseases. Although EVs can be effectively isolated using any of the methods listed above, to date, no single technique for quantification and characterization of EVs has been able to satisfy all EV characteristics in complicated clinical samples. Most current EVs-related studies use methods that complement each other to identify the EVs being studied. The common methods are but not limited to nanoparticle tracking analysis to quantify the size distribution of EVs and their quantity in a sample volume, transmission electron microscopy to determine the structure of EVs, and immunoblotting of specific proteins to determine the origin of EVs. Nanoparticle tracking analysis and transmission electron microscopy can be used to determine the physical characteristics of EVs by measuring the concentration of EVs in a sample and their size distribution, which can help infer the subtype of EVs [95]. Immunoblotting can be used to determine the biochemical characteristics of EVs by identifying specific proteins in EVs that can help identify pathophysiological markers of disease [96]. Of course, with the advancement of science and technology, a series of new methods for detecting EVs have emerged, such as electrochemical analysis and methods based on fluorescence techniques [95]. Although there are still many issues to be resolved during EV analysis, the supplementation of these advanced methods helps understand the functions associated with EVs properly and provides more opportunities for their use as biomarkers and potential therapeutic functions. And the most significant problem with following studies on EVs is the lack of standardization of EVs

isolation and characterization. Different EV processing techniques across studies lead to possible differences in reported results, which complicates the interactions between studies.

Another noteworthy experimental issue related to the study of EVs is how to determine the cellular origin of EVs. Since EVs act as participants in intercellular communication, the bioactive substances carried by these circulating EVs may act as biomarkers for specific diseases. A rapid and efficient method to determine the cellular origin of EVs is urgently needed. For example, many processes of tumor progression are driven by EVs, and identifying the tumor cells from which EVs originate can help monitor cancer progression, as well as provide more effective clinical treatments. Several emerging methods have emerged for determining the cellular origin of EVs. Such as intelligent probabilistic system [97] and EV-origin (the digital EVs quantification) [98]. These methods utilize the properties of EVs in combination with specific algorithms, thus helping to determine the origin of EVs. Therefore, as machine learning algorithms continue to progress, there is reason to believe that more effective methods for various research purposes in EVs will emerge in the future.

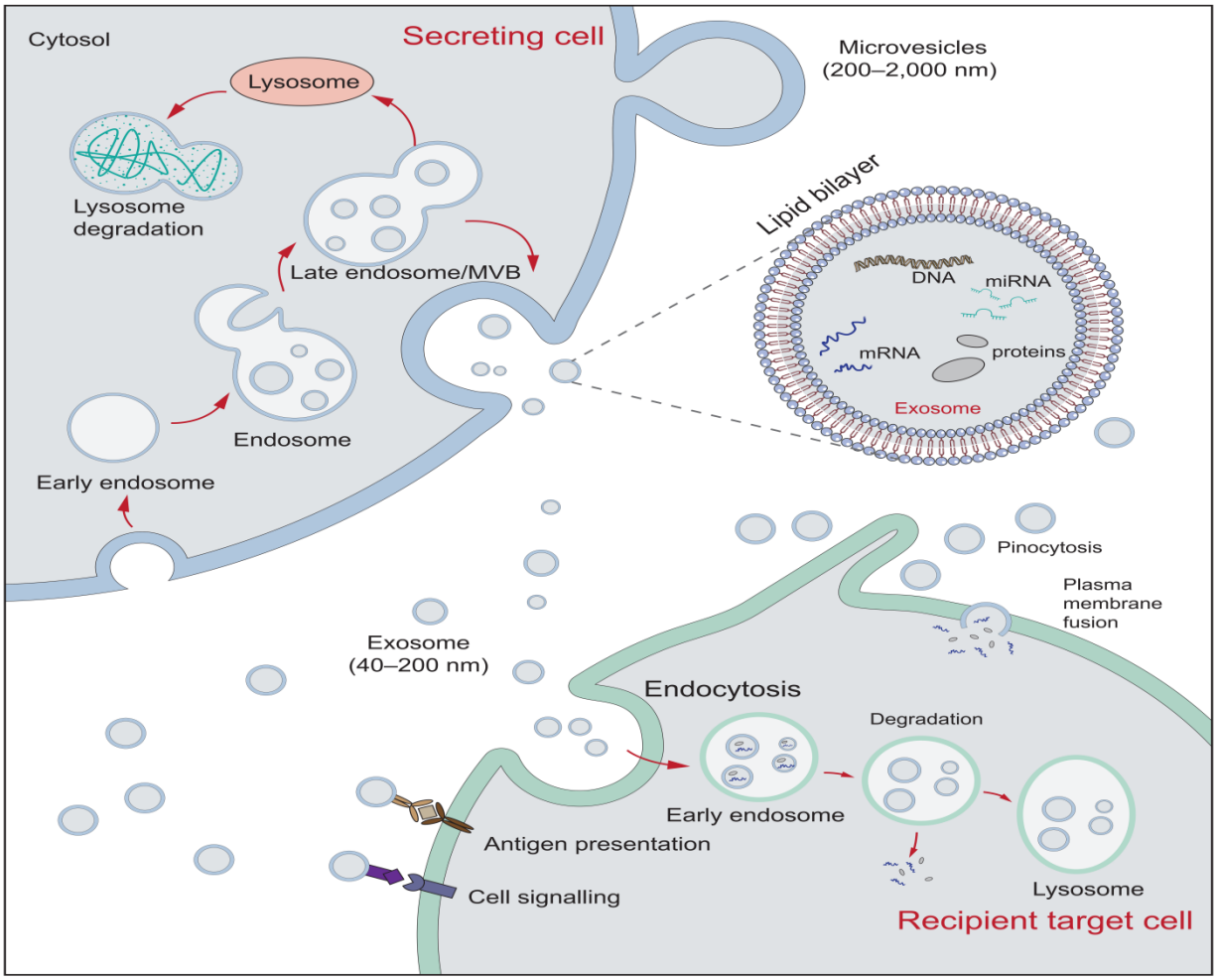

Figure 3 EV biogenesis, secretion, and impact on target cells in accordance with He et al. 2021 ([34], p. 3). Endocytosis of the cell membrane precedes the production of exosomes. The membrane of the endosome proliferates inward to generate vesicles, which then change into MVB. In addition to being secreted into exosomes (40–200 nm in diameter) by fusion with the plasma membrane, MVB can also be sent to lysosomes for degradation. And the process of membrane budding or exocytosis generates microvesicles (200–2000 nm in diameter). These EVs are able to connect with target cells by attaching to their receptors. In addition, target cells can ingest EVs by endocytosis, pinocytosis, and plasma membrane fusion [19], where EVs can release their contents to influence target cells or be eliminated by lysosomes.

Table 1 Role of EVs in human tissues, adapted from He et al. 2021 ([34], p. 3–4).

Tissue	Functions	Reference
Tumor	Biomarker Alters tumor microenvironment Regulates tumor immune response Involved in tumor angiogenesis	[83,84,99,100]
Bone	Biomarker Regulates osteogenic differentiation Affects osteoblast differentiation Regulates osteoblast and osteoclast function	[29,101–103]
Heart	Biomarker Promotes angiogenesis Cardioprotection and regeneration	[104,105]
Brain	Biomarker Influences inflammatory and regulatory pathways Neuroprotection	[106–108]
Kidney	Biomarker Involved in the development of renal fibrosis Contributing to kidney repair	[109]
Gastro-intestinal tract	Immunomodulation Response of anti-apoptotic, antioxidant stress Regulates the homeostasis of gut microbiota	[110,111]

5.4 The Role of EVs in the Stress Response

5.4.1 The Roles of EVs during Psychosocial Stress Process

Mitochondria is involved in the subcellular pathways that are influenced by metabolic and psychosocial stressors [32]. Psychosocial stress affects adaptive glucocorticoid signaling and glucose levels, leading to mitochondrial dysfunction, augmenting the formation of reactive oxygen species inside cells, causing oxidative stress, cellular damage, and systemic inflammation [6]. Furthermore, reactive oxygen species impact microRNAs' expression via epigenetic alteration and transcription factors [112] (see also [34], p. 4). The roles of cargos carried by EVs (miRNA and mitochondrial DNA) in the psychosocial stress process were summarized, based on the explanations given by He et al. 2021([34], p. 4).

> Numerous intercellular communication related miRNAs can be carried by EVs; it has been demonstrated that oxidative stress and inflammation related miRNAs are increased in plasma EVs isolated from human immunodeficiency virus-positive individuals receiving antiretroviral therapy, thus work as biomarkers of targetable pathways leading to disease pathogenesis [113]. Therefore, these altered miRNAs in EVs may treat as biomarkers of psychosocial stress. The damage to mitochondrial DNA is a component of mitochondrial allostatic load. Several human disorders, consisting of cancer, acute kidney injury, and Parkinson's disease, were related to altered mitochondrial DNA levels [114–116]. Consequently, the mitochondrial DNA level in bodily fluids and tissues may represent the mitochondrial function. Given that mitochondrial DNA is present in EVs and may interact with target cells via EVs' transport [33,117], somewhat like miRNA, the altered mitochondrial DNA in EVs may work as biomarkers for psychosocial stress process. In the stress response, mitochondria also cooperate with the endoplasmic reticulum [118]; hence, the endoplasmic reticulum may engage in the stress response process. Interestingly, the combination of physical/psychological and biological stress enhances endoplasmic reticulum stress [119]. And severe endoplasmic reticulum stress-mediated release of EV-associated danger/damage associated molecular patterns might involve in chronic inflammatory diseases [28]. Therefore, future research should focus on the role of endoplasmic reticulum in psychosocial stress.

5.4.2 Stress Modifies miRNAs in EVs to Regulate the Immune Response

The role of miRNAs contained in EVs between stress and immune response were summarized, based on the explanations given by He et al. 2021([34], p. 4–5).

> The immune functions may regulated by psychosocial stress, and the link between EVs and immune response has also been noted [120]. The function of circulating EVs as immune-regulatory signal transmitters is an interesting topic. Multiple stress models showed that miRNAs in EVs might affect immune response. For example, rats exposed to acute stressor (inescapable tail shock) resulted in altered miRNA expression in circulating plasma exosomes (decreased miR-142-5p and miR-203) [121]. These altered miRNAs in exosomes are potentially a critical component of the stress related immune response. The decreased miR-142-5p expression increases T cell function and promotes B cell hypersensitivity [122]. And miR-203 is capable of targeting the suppressor of cytokine signaling-3 (SOCS-3), a negative regulator of IL-6 and interferon-γ induced signaling pathways [123,124]; SOCS-3 also affects inflammatory responses by inhibiting IL-2 and IL-12 signaling [125,126]. The inhibition of SOCS-3 by miR-203 may result in a higher inflammatory response. In conclusion, stress suppresses miR-203 expression, leading to the activation of SOCS-3 and its suppression of pro-inflammatory cytokines. In addition to acute stress, chronic unpredictable mild stress (CUMS), a model of depression, can affect miRNA expression contained in serum EVs in rats (23 upregulated and 34 downregulated), with possible immunological consequences [127]. MiR-128-3p, which is upregulated after CUMS, stimulates gene expression of pro-inflammatory cytokines (Ccl5, Cx3cl1, and Cxcl7). Moreover, Shyamasundar et al. [128] demonstrate that miR-128-3p regulates inflammation in the normal rat kidney. Overexpression of miR-26a-5p (also upregulated after CUMS) attenuated the inflammatory response in mice with lipopolysaccharide-induced acute lung injury by decreasing total protein, neutrophil, and lymphocyte counts and expression levels of TNF-α, IL-1β, and IL-6 in bronchoalveolar lavage fluid [129]. And miR-455-5p was downregulated after CUMS, might selectively bind to SOCS-3 3'-UTR and decrease SOCS-3 production [130], thereby participating in the inflammatory response.

The miRNAs carried by EVs have also been found to have possible antidepressant symptoms. Li et al. [131] found that natural killer cells released EVs contain miR-27, which can relieve the symptoms of stress in the mice who were exposed to chronic mild stress. They also

demonstrated that miR-207 leads to a decrease in inflammatory factor release through cellular experiments, so that EVs-carried miRNAs may be involved in the regulation of inflammatory factors and influence stress symptoms. This provides a new method for depression treatment. Table 2 lists the effects of specific stressors on miRNAs in EVs.

Table 2 The miRNAs change in EVs under stressors, adapted from He et al. 2021 ([34], p.5).

Stressors	MiRNAs in EVs	Source of EVs	Change	References
Chronic unpredictable mild stress	miR-139-5p	Blood and brain from mice	↑	[132]
Chronic unpredictable mild stress	miR-126a-3p, miR-128-3p, miR-26a-5p, miR-191a-5p	Serum from rats	↑	[127]
Mechanical stress	miR-1246	Fibroblast	↑	[133]
Mechanical stress	miR-133a-3p, miR-203-3p	Fibroblast	↓	[133]
Chronic unpredictable mild stress	miR-455-3p, miR-187-5p, miR-206-3p, miR-455-5p	Serum from rats	↓	[127]
Inescapable tail shock	miR-142-5p, miR-203	Plasma from rats	↓	[121]

("↑" means upregulated; "↓" means downregulated).

5.5 The Role of EVs in Osteoporosis

The effects of EVs on osteoblasts, osteoclasts were summarized, based on the explanations given by He et al. 2021([34], p. 6–7).

5.5.1 EVs regulate the differentiation and activity of osteoclasts

As one of the contents delivered by EVs, microRNAs play a crucial function in bone homeostasis. For instance, miR-503-3p, abundantly expressed in EVs generated by osteoblasts, can suppress osteoclastogenesis by inactivating the RANK/RANKL signaling pathway [134,135]. Additionally, blood vessels are significant components in the process of bone repair and regeneration [136]. Song et al. [137] found that EVs derived from vascular endothelial cells target bone more effectively than EVs derived from osteoblasts or BMSCs and suppress the activity and differentiation of osteoclasts via miR-155. Thus, the miR-155-containing EVs may be a target against osteoporosis. Interestingly, tumor cells impact osteoclast by secreting EVs. Lung adenocarcinoma cells generated EVs showed upregulated miR-21 level, which enhanced osteoclastogenesis by targeting programmed cell death protein 4 [138]. Similarly, breast cancer cells secrete miR-20a-5p-containing EVs, promoting osteoclasts' proliferation and differentiation [139].

EVs can influence bone remodeling by controlling the differentiation and activity of osteoclasts directly. Huynh et al. [140] discovered that EVs generated from osteoclast precursors induce vitamin D-dependent osteoclasts formation. EVs from cultures enriched with osteoclasts, however, suppressed osteoclastogenesis. That means EVs from mature osteoclasts contain RANK, which might prevent the RANK activation on the osteoclast surface in a manner similar to that of osteoprotegerin. Besides, the RANK-containing EVs can deliver regulatory molecules to RANKL-expressing cells by RANK/RANKL interaction [140]. Moreover, osteoblasts generated EVs affect osteoclasts' function. The RANKL-containing EVs released by osteoblasts are transferred to the precursors of osteoclasts, increasing RANKL/RANK signal transduction and promoting osteoclasts formation [141]. To better understand EVs' function in osteoblast-osteoclast communication, scientists infused osteoblast-derived EVs with osteoclast-inhibiting medicines (zoledronate and dasatinib). They found that osteoblast EVs internalized and shuttled osteoclast-inhibiting drugs to inhibit osteoclasts' activity *in vivo* and *in vitro* [142], which paves the way for the using EVs

in the treatment of bone diseases. These findings demonstrate that EVs from various cell types may influence osteoclasts.

5.5.2 EVs Affect Osteoblasts and Osteogenic Function

Osteoblasts are the bone-forming cells of remodeling units and are essential for the growth and maintenance of the skeleton [143]. As stated before, osteoblasts released EVs regulate osteoclast activity. In turn, osteoclasts secreted EVs modulate osteoblast activity. Sun et al. [30] showed osteoclasts secrete miR-214-containing EVs that recognize osteoblasts via the interaction with ephrina2/ephrin type-A receptor 2. Furthermore, miR-214 inhibits bone formation via activating transcription factor 4 [144]. Osteoclasts generated EVs reside in both bone microenvironment and blood. Researchers discovered elevated miR-214 levels in the serum EVs of osteoporotic patients, suggesting that miR-214 in EVs may serve as a biomarker of bone loss [30]. Likewise, osteoclasts-derived miR-23a-5p-containing EVs suppress osteoblast function via Runx2 [145]. Therefore, the EV-mediated intercellular communication between osteoblasts and osteoclasts may work as a new path for bone remodeling research.

MSCs promote tissue regeneration and EVs generated by MSCs have gained considerable interest in bone remodeling field. EVs generated from BMSCs may influence osteoblast differentiation and osteogenic gene expression *in vitro*, hence enhancing osteogenic function [146]. MSCs generated EVs also promote osteogenic differentiation and mineralization during the latter phases of osteogenic differentiation. Moreover, the target prediction of differently expressed microRNAs in EVs indicates a high enrichment of osteogenic differentiation signaling pathways [147]. According to prior studies, several scientists have investigated the potential therapeutic uses of BMSCs. Specifically, Fang et al. [148] reported that BMSCs-derived EVs significantly reverse the decreased osteogenic differentiation of BMSCs in steroid-induced femoral head necrosis. These findings demonstrate the possible use of EVs generated from MSCs in bone regeneration treatment. Many research, as indicated in Table 3, support the involvement of EVs in bone remodeling.

Table 3 Summary of EVs associated with bone remodeling, adapted from He et al. 2021 ([34], p. 7).

Source	Bioactive Factors Containing	Target	Function	References
Osteoclasts	RANK	Osteoclasts	Inhibits osteoclast formation	[140]
Osteoclasts	miR-214	Osteoblasts	Inhibits the activity of osteoblasts through ephrina2/ephrin type-A receptor 2 interaction and targets activating transcription factor 4 to inhibit bone formation	[30,144]
Osteoclasts	miR-23a-5p	Osteoblasts	Inhibits the activity of osteoblasts by targeting Runx2	[145]
Osteoclasts	miR-214-3p	Osteoblasts	Inhibits osteoblastic bone formation	[149]
Osteoblasts	RANKL	Osteoclast precursors	Facilitates osteoclast formation by binding RANK on the osteoclast precursor surface	[141]
Osteoblasts	RANKL	Osteoclasts	Induces the apoptosis of osteoclasts	[142]
Preosteoblasts	TRIP-1	The extracellular matrix of bone	Promotes mineralization	[150]
BMSCs	miR-196a	Osteoblasts	Improves osteogenic function	[146]
BMSCs	miR-885-5p	BMSCs	Inhibits osteogenic differentiation by repressing Runx2	[151]
BMSCs	miR-151-5p	BMSCs	Promotes osteogenic differentiation	[152]
Endothelial cells	miR-155	Osteoclasts	Inhibits the activity and differentiation of osteoclasts	[137]
Endothelial cells	miR-31	MSCs	Inhibits osteogenic differentiation by repressing Frizzled-3	[153]

(BMSCs: Bone marrow mesenchymal stem cells; MSCs: Mesenchymal stem cells; RANK: Receptor activator of nuclear factor κ-B; RANKL: Receptor activator of nuclear factor κ-B ligand; TRIP-1: Transforming growth factor beta receptor II interacting protein-1; Runx2: Runt-related transcription factor 2).

5.6 EVs as Potential Mediators between Psychosocial Stress and Osteoporosis

Given the role that EVs play in psychosocial stress and osteoporosis, respectively, the focus here is on the role of EVs between these two. Psychosocial stress induces mitochondrial allostatic load, generating oxidative stress, a risk factor for osteoporosis. Interestingly, some EVs may be involved in the process of oxidative stress leading to osteoporosis. For example, *in vitro* experiment based on H_2O_2 induced oxidative stress treatment of BMSCs showed an increased abundance of miR-183-5p in BMSC-derived EVs, and miR-183-5p overexpression inhibits BMSC proliferation and osteogenic differentiation [154]. Since miR-183-5p also increases osteoclastogenesis [155], this possible effect on bone remodeling made miR-183-5p in EVs may act as a mediator between oxidative stress and osteoporosis; in other words, miR-183-5p in EVs from BMSC may link the psychosocial stress and osteoporosis. This is the first model we have summarized for the involvement of EVs in psychosocial stress contributing to osteoporosis, that is mitochondrial allostatic load model. The possible mechanisms are shown in Figure 4.

The impact of several stress models on miRNAs in EVs and the role of miRNAs in EVs during osteoporosis development were stated previously. And the effect of EV contained miRNAs between stress and osteoporosis were summarized, based on the explanations given by He et al. 2021 ([34], p. 7–8).

> Concerning the effect of acute stress on EVs, Beninson et al. [121] showed that rats exposed to acute stressors resulted in decreased miR-142-5p expression in plasma exosomes. Given that miR-142-5p enhances osteoblast activity and matrix mineralization [156], the miR-142-5p-containing exosomes might work as mediators between stress and osteoporosis. Since bone remodeling is characterized by a continuous process, emphasis was placed on explaining the potential influence of EVs' alterations on bone homeostasis under chronic psychosocial stress.
>
> Concerning the impact of the depression model on EVs, Fang et al. [127] reported that CUMS, which can lead to allostatic overload, alter the miRNA content of rat serum EVs. MiR-126a-3p and miR-128-3p, the most significantly upregulated miRNAs in

serum EVs of rats after CUMS, link to bone remodeling. MiR-126a-3p suppresses osteogenesis of human adipose-derived mesenchymal stem cells by suppressing Wingless and Int-1 activation [157], whose function is to maintain bone formation and suppress bone resorption [158]. Besides, miR-128-3p inhibits the osteogenic potential of MSCs [159]. Given miR-455-3p and miR-187-5p, the most significantly downregulated miRNA expression in serum EVs of rats after CUMS, were likewise associated with bone remodeling. MiR-455-3p protects osteoblasts from oxidative stress, an osteoporosis risk factor, hence promoting osteoblasts' growth [160]. Additionally, miR-187-5p promotes BMSCs differentiate to osteoblasts [161]. However, CUMS leads to a downregulated miR-23a-3p expression in EVs [127]. In turn, the inhibition of miR-23a-3p promotes osteoblast proliferation and differentiation [162], which contradicts the above findings. Nevertheless, several most significant altered miRNAs in mice's serum EVs after CUMS were involved in bone formation dysfunction and may contribute to osteoporosis. Table 5 lists the currently known EVs connected with stress and bone.

An important concern is whether circulating EVs changed by chronic psychosocial stress may target and impact bone tissue. However, until now there is no direct study exploring this issue. And the key to studying this issue is to clarify whether EVs in bone cells are associated with circulating EVs. Fortunately, a portion of research exists on this topic. The osteoblasts released EVs containing particular miRNAs circulating in the bloodstream and transferred their contained biological components to receptor cells [163]. In contrast, distant tissues influence bone tissue by secreting EVs. For instance, EVs derived from growth hormone-secreting pituitary adenoma can be internalized by osteoblasts, then stimulate osteoblast proliferation and bone formation [31]. This means EVs can serve as mediators between bone and other tissues. EVs are a promising option as a mediator of chronic psychosocial stress-related effects on bone [164], despite the fact that there is no direct evidence that circulating EVs modified by chronic psychosocial stress can target bone.

The second model, which is sympathetic nervous system model, was summarized for the involvement of EVs between psychosocial stress and osteoporosis, and figure 5 depicts the possible mechanism of EV participation in stress-induced osteoporosis, also known as sympathetic nervous system model via the α1-AR. In addition, a recent study focused on the role of β2-AR in the link between sympathetic nervous system and bone loss, thus is a good

complement to the sympathetic nervous system model. β2-AR signaling is activated by increased norepinephrine during sympathetic stress, which in turn stimulates the transcriptional response of miR-21. Osteoblastic miR-21 is then transferred through EVs to osteoclast progenitors, blocking programmed cell death 4 and boosting osteoclastogenesis. As a consequence, the homeostasis of bone remodeling is disturbed, resulting in bone loss [165]. This uncovered that sympathetic signaling drives miRNA transfer in EVs to regulate bone homeostasis, helping to provide new strategies to help treat psychosocial stress leaded bone loss. New content was added to the sympathetic nervous system model based on the results of this experiment as the third model, that is, sympathetic nervous system model via the β2-AR, shown in figure 6.

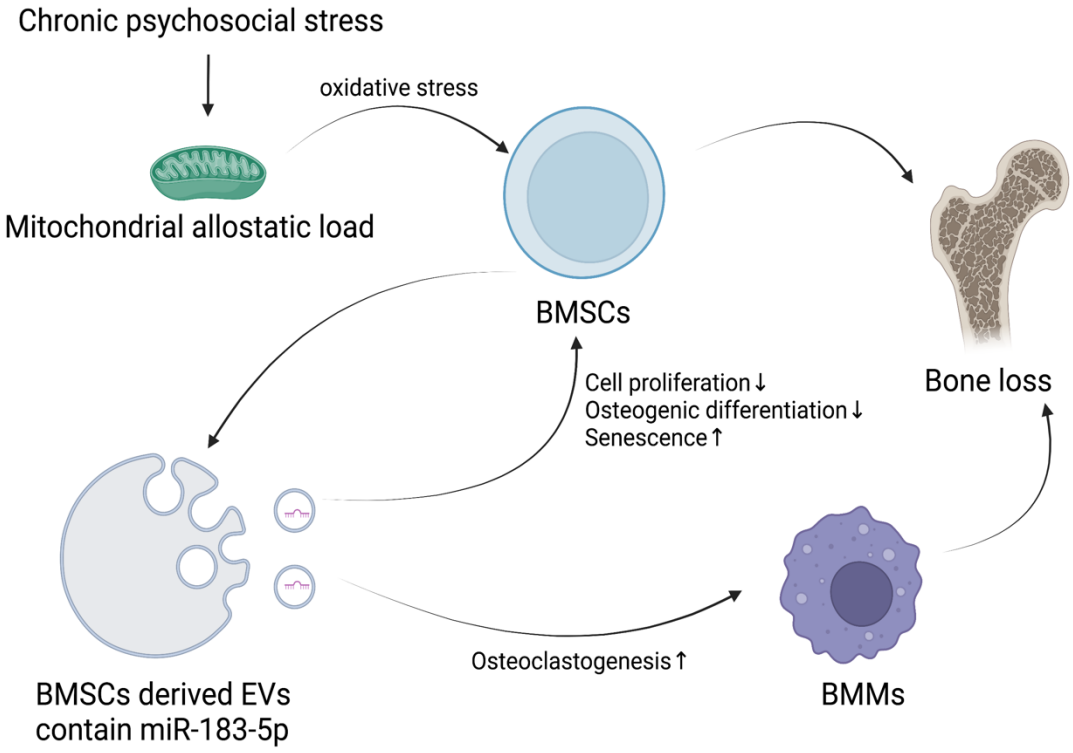

Figure 4 Mitochondrial allostatic load model. The potential mechanisms of EVs in psychosocial stress leaded osteoporosis via mitochondrial allostatic load. Chronic psychosocial stress induces mitochondrial allostatic load, along with excessive oxidative stress. Oxidative stress increases the quantity of miR-183-5p in BMSCs-derived EVs, and miR-183-5p inhibits cell proliferation, osteogenic differentiation, and accelerates cell senescence in bone marrow mesenchymal stem cells (BMSCs) [154]. miR-183-5p also increases osteoclastogenesis of bone

marrow-derived macrophages (BMMs) [155]. These effects on bone remodeling lead to bone loss and may contribute to osteoporosis development.

Table 4 The currently known EVs associated with psychosocial stress and bone, adapted from He et al. 2021 ([34], p. 8–9).

MiRNAs in EVs	Stress-Induced Change	The Effect of MiRNAs in EVs on Bone	References
miR-126a-3p	↑	Inhibits the osteogenesis of human adipose-derived mesenchymal stem cells	[127,157]
miR-128-3p	↑	Inhibits the osteogenic differentiation of MSCs	[127,159]
miR-26a-5p	↑	Inhibits the osteogenic differentiation of mouse adipose-derived mesenchymal stem cells	[127,166]
miR-139-5p	↑	Inhibits BMSC osteogenesis by targeting Wingless and Int-1/β-catenin signaling pathway	[132,167]
miR-455-3p	↓	Protection of osteoblasts from oxidative stress	[127,160]
miR-187-5p	↓	Promotes differentiation of BMSCs to osteoblasts	[127,161]
miR-1-3p	↓	Stimulates the osteogenesis of mouse MSCs and inhibits their adipogenesis	[127,168]
miR-23a-3p	↓	Inhibits the osteogenesis	[127,162]

("↑" means upregulated; "↓" means downregulated; BMSCs: Bone marrow mesenchymal stem cells; MSCs: Mesenchymal stem cells).

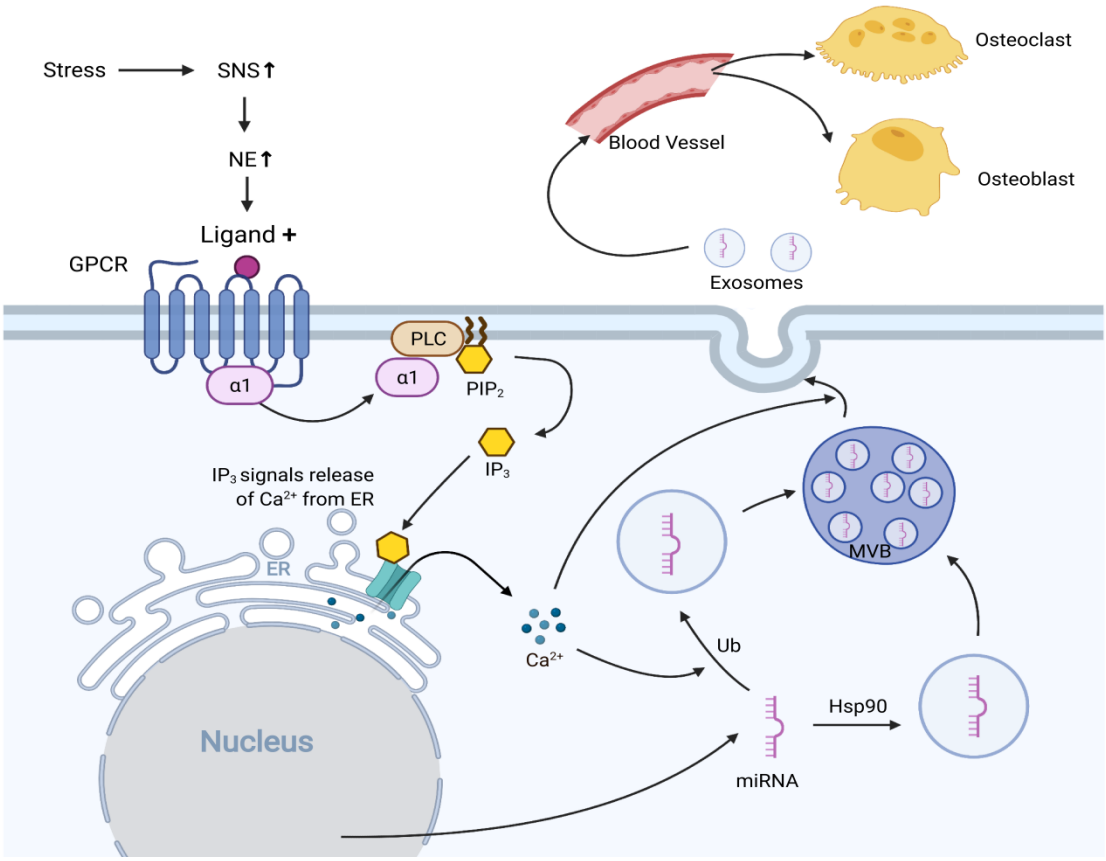

Figure 5 Sympathetic nervous system model (α1-AR) in accordance with He et al. 2021 ([34], p. 9). Through the sympathetic nervous system (SNS), EV may be involved in psychosocial stress-induced osteoporosis. Psychosocial stress causes the release of norepinephrine (NE) from sympathetic nerve terminals by stimulating the SNS; the released NE can bind to the α1-adrenergic receptor (AR), which is coupled to the G-protein coupled receptor (GPCR). GPCR dissociates upon receptor activation and stimulates phospholipase C (PLC), which catalyzes phosphatidylinositol bisphosphate (PIP_2) into inositol trisphosphate (IP_3). IP_3 interacts with the IP_3 receptor on the endoplasmic reticulum (ER), which causes an increase in cytosolic Calcium (Ca^{2+}) [169]. Ca^{2+} in the cytosol stimulates ubiquitination (Ub) and directs particular miRNAs to endosomes, whereas heat shock protein 90 (HSP90) directs other miRNAs to endosomes [170]. Then, endosomes are guided to multivesicular bodies (MVBs). Through the mediation of Ca^{2+}, the MVBs fuse with the plasma membrane of the cell, therefore releasing endosomes into the extracellular environment, where they are referred to as exosomes [121,164]. Osteoblasts and osteoclasts ingest exosomes, which then release the genetic information they carry, therefore altering their physiological activities and contributing to the development of osteoporosis.

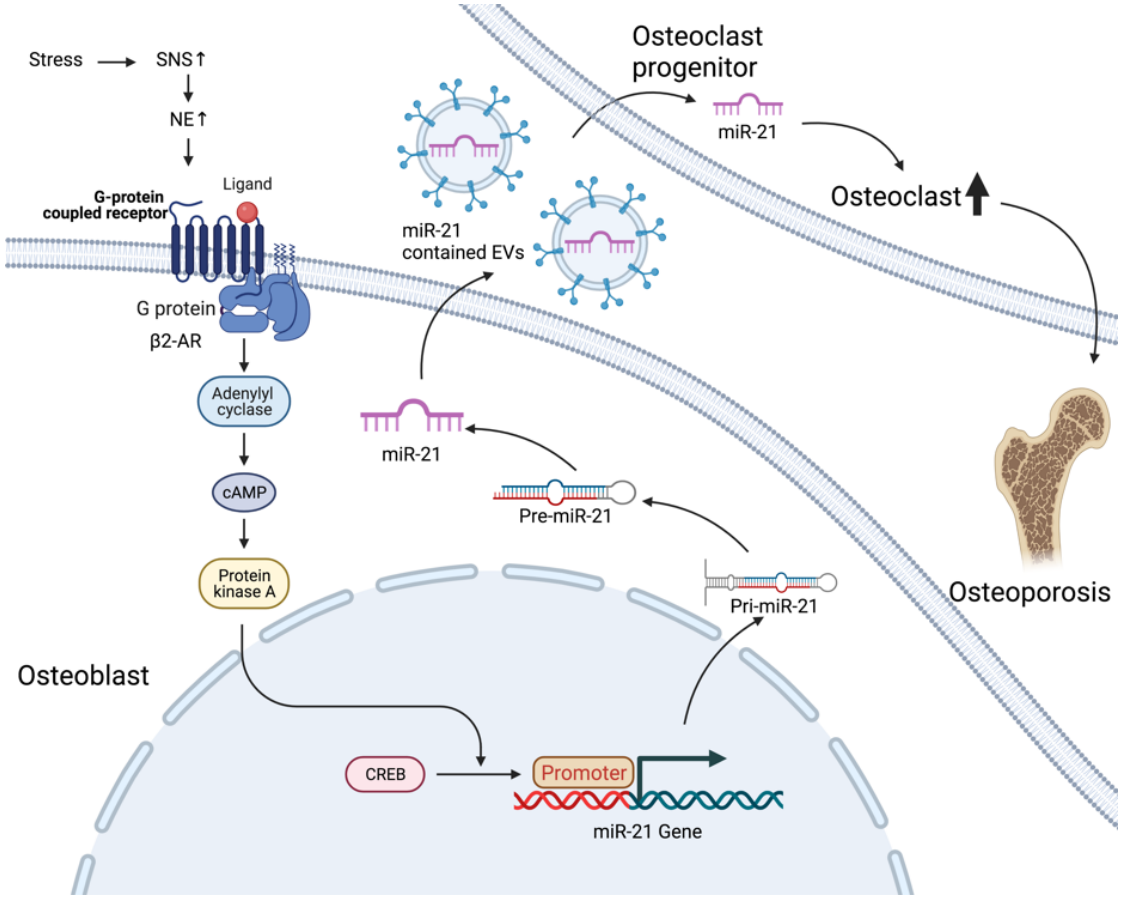

Figure 6 Sympathetic nervous system model (β2-AR), adapted from Hu et al. 2022 ([165], p. 7). Stress activates the sympathetic nervous system (SNS), releasing norepinephrine (NE) that binds β2-adrenergic receptor (AR) and triggers the transcriptional response of miR-21 through Cyclic adenosine monophosphate (cAMP)/Protein kinase A/cAMP response element-binding protein (CREB) signaling, where CREB binds to the promoter region of miR-21, and then mature miR-21 is loaded into EVs and transferred into osteoclast progenitors, where miR-21 is released and promotes osteoclastogenesis, ultimately leading to imbalanced bone remodeling and reduced bone mass, resulting in osteoporosis [165].

6 Discussion

Stress has both advantages and disadvantages for the organism. The positive effect is that acute stress mobilizes the organism's stress response to better cope with the stressor [171]. The negative effect is that prolonged, repeated, excessive stress leads to a series of strain harmful effects or cumulative burdens on body, also known as allostatic load [10], as mentioned above. The concept of allostatic load is an exact way to reveal the compliance and possible damage caused by chronic stress to the organism. With the accelerated pace of life nowadays, stressors from work and family are constantly affecting the health of the body. Stress and the diseases it causes are essential factors that affect the population's health and cannot be ignored in society. In the present study, contents are focused on the role of psychosocial stress contributing to osteoporosis. On the one hand, osteoporosis is the most common bone disease and imposes a substantial socioeconomic and population health burden [14]. On the other hand, there is a lack of previous literature that studies in-depth the cellular mechanism of psychosocial stress on the bone. In particular, combining the focus of research on EVs in recent years, this review takes the characteristics and roles of EVs into consideration and treats EVs as a possible mediator between psychosocial stress and osteoporosis, providing a new path for the research of both diseases. EVs have come to the attention of researchers in recent years due to their involvement in intercellular communication. Due to the characteristics of cell-to-cell transport of cargoes, the current research hotspots on EVs mainly explore their potential functions as targeted therapeutics and as biomarkers [104].

Since the introduction of EVs, their inherent qualities have garnered considerable interest. Compared to typical intercellular communication using soluble proteins, EVs offer several advantages. The outstanding advantage of EVs is their natural lipid and surface protein composition, which allows them to evade phagocytosis as well as control their safety [172]. As previously mentioned, EVs can carry nucleic acids, protecting them from the extracellular environment, thus making them stable in circulation and preventing degradation [77]. Besides,

EVs can transfer multiple miRNAs at once and target various mRNAs in a cell at the same time [86]. Based on this, EVs may have unique advantages in gene diagnostic therapy. Moreover. The release of EVs appears to be a relatively targeting process that may contribute to studying some disease processes [77].

The role played by EVs in intercellular communication makes them a critical aspect in the study of cellular communication in the organism, but EVs' characteristics also pose specific challenges to researchers. Different from previous studies of circulating biomarkers, the analysis of EVs often requires more complex experimental procedures [71], such as isolation, quantification, and characterization. In addition, there is no method yet for determining the cellular origin of EVs that is rapid and standardized, which is essential to studying the disease development process in which EVs are involved. The emergence of uniform criteria for determining the cellular origin of EVs in the future could greatly assist researchers in identifying the biological pathways in which EVs are involved and determining their role.

It is also unknown if EV-mediated transport of biological contents may change the function of target cells in a real physiological context. In contrast to laboratory studies in which high quantities of pure EVs may given to cells, there are numerous and complex factors that need to be considered in a real organism environment. In addition, the delivery efficiencies of EVs carrying biological cargo is not entirely understood. To achieve an agreement on the role of EVs and their potential clinical applications, such as biomarkers and therapies, in real situations, further research is required. This paper analyzed the involvement of EVs in the effects of psychosocial stress on body, the possible role of EVs in the osteoporosis process, and proposed three possible models for explaining the role of EVs in the process of psychosocial stress contributing to osteoporosis, mitochondrial allostatic load model and sympathetic nervous system model.

Numerous studies have shown that osteoporosis is linked to psychosocial stress [50,173]. However, EVs have not been experimentally investigated as potential mediators between psychosocial stress and osteoporosis yet. The small size and light weight of miRNAs, their ability to be efficiently transfected in cells, and the mechanism that miRNAs silence mRNAs make miRNAs a hot topic of research among EVs-carrying cargoes [174]. Considering the vulnerability of miRNAs in blood to the *in vivo* environment [22], the study of miRNAs enclosed in EVs may be superior to blood studies. The protective properties of EVs allow miRNAs preserved inside them to remain stable and less susceptible to degradation and can be transported over long distances in body fluids [77]. Therefore, EVs are good tools to help miRNAs involved in a variety of biological activities *in vivo*.

As demonstrated above (Result 5.4 and 5.6), stress-induced miRNA level change contained in EVs, and many altered miRNAs impact bone remodeling, which suggests miRNAs in EVs may link stress and osteoporosis. Consequently, this paper proposes a theoretical model that accepts the EVs transport mechanism. However, the role of EVs-mediate miRNAs modifications in real physiological settings is still unknown, and more research is needed to determine whether these modified miRNAs can build a bridge between psychosocial stress and bone metabolism. A doubt still exists when thinking about whether the progression of osteoporosis (reduced bone mineral density or other complications) is influenced by EV transmitted stress. In addition, the overall role that EVs play in bone remodeling has to be considered for the cargo they carry. Since it is important to consider in depth the effect of miRNA levels on osteoblasts/osteoclasts and also the effect of miRNAs on different stages of osteoblasts/osteoclasts. More experiments are needed to demonstrate the dominant effect of the specific miRNA on which cells (osteoblasts/osteoclasts), thus helping to determine the true role they play in bone remodeling. This is the shortcoming of the present review, as few previous research considered EVs' role between psychological stress and osteoporosis [165], only the possible role of EVs based on past studies or reviews was

summarized, and the proposed model may only “theoretically feasible.” Therefore, there are still many experimental and clinical studies to be done.

Future research should look into how psychosocial stress affects circulating (plasma or serum) EVs to find out if they play a role in bone homeostasis and contribute to the progression of osteoporosis, which helps researchers unravel the link between psychosocial stress and osteoporosis. The current literature on the connection between these two is mostly focused on the sympathetic nervous system and hypothalamic-pituitary-adrenal axis [173]; therefore, this review summarizes the potential effects of EVs linking stress and osteoporosis. This review also provides ideas for the next experiment step in our research group, and the differences in circulating EVs in the stress group compared with healthy controls are currently investigating, and as mentioned previously, the differences in miRNAs in EVs are a crucial focus, followed by a comparison of miRNAs that may affect bone health. Thereby got the initial idea of whether miRNAs in circulating EVs are potentially involved in the effects of psychosocial stress on bone. More cellular-level experiments will also be conducted in the future to determine the crosstalk effects of certain specific miRNAs in EVs between psychosocial stress and osteoporosis.

If it comes to the clinical application of EVs, there is still a long way to go in the future. First, despite the existence of several ongoing or completed clinical trials [175], EVs have not yet been used in the clinic. This is because of the relatively complex characteristics of EVs and the lack of sufficient knowledge about them. In addition, there are many factors that must be considered when it comes to the clinical application of EVs, such as the yield and purity of EVs, the exact function of the cargo carried by EVs, and how to control their immunotoxicity, etc. Researchers are still a long way from fully understanding all the properties of EVs.

7 Reference

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8 Affidavit

Ich versichere an Eides statt durch meine Unterschrift, dass ich die vorstehende Arbeit selbständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich oder annähernd wörtlich aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegebenen Literatur oder sonstiger Hilfsmittel bedient habe. Die Arbeit hat in dieser oder ähnlicher Form noch keiner anderen Prüfungsbehörde vorgelegen. Zudem erkläre ich mich damit einverstanden, dass meine Arbeit mit einer Plagiatssoftware geprüft wird.

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9 Curriculum Vitae

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10 Projects and third-party funding

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11 Publication list

He, Y.; Wuertz-Kozak, K.; Kuehl, L.K. & Wippert, P.-M. Extracellular Vesicles: Potential Mediators of Psychosocial Stress Contribution to Osteoporosis? *Int. J. Mol. Sci.* **2021**, *22*, 5846. https://doi.org/10.3390/ijms22115846. [IF 5.92]

He, Y. (2021). Extracellular Vesicles in Osteoporosis. Retrieved from https://encyclopedia.pub/entry/10693.

Puerto Valenica, L., He, Y. & Wippert, P.-M. (under review). Biomarkers on the effectiveness of non-pharmacological interventions in chronic back pain: A Systematic Review.

12 Conferences

He, Y.; Cazzanelli, P.; Wuertz-Kozak, K. & Wippert, P. -M. Might the cargo of extracellular vesicles constitute a biological link between psychosocial stress and osteoporosis? A narrative review. Psychoneuroendocrinology, **2021**, 131: 105480. https://doi.org/10.1016/j.psyneuen.2021.105480 [IF 4.91]

13 Acknowledgment

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