Nat. Volatiles & Essent. Oils, 2022; 9(1): 165-175

NVEO
Natural Volatiles &
Essential Oils

Chronic Unpredictable Mild Stress Impacts On Chondrocyte Apoptosis And Long-Bone Growth In Adolescent Mice

Luh Ayu Asri Wijani[1] , Irwanto[1,*] , Reny I'thosom[2] , Alpha Fardah Athiyyah[1] , Purwo Sri Rejeki[3] , Risa Etika[1]

[1]Department of Child Health, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

[2]Department of Biomedical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

[3]Department of Physiology & Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

Chronic psychosocial stress is considered as a risk factor for somatic disorders, as its associated with alteration in the hypothalamic-pituitary-adrenal (HPA) axis caused increased of blood cortisol level and its ability to induce oxidative stress leading to apoptosis. Currently, there are no known reports on the underlying mechanism of the connection between skeletal and mental health. This study, therefore, aims to use models of depression to explore how long bone growth and chondrocyte apoptosis are affected by chronic unpredictable mild stress [1[. Seven-week-old male Mus musculus mice were exposed to chronic unpredictable mild stress (CUMS) for 7 weeks, whereas control mice were non exposed. At the endpoint, epiphyseal growth plate of femur was analyzed using histological and immunohistochemistry, the length of femur was measured using calipers. Compared with controls, exposure to CUMS resulted in approximately twice fold increase Caspase-3 expression presented chondrocyte apoptosis in femur epiphyseal growth plate of stressed mice. This a was associated with oxidative stress activating the intrinsic pathway leading to apoptosis chondrocytes in the growth plate. Furthermore, the length of femur was significantly decreased and the thickness of epiphyseal growth plate were not significantly reduced, suggesting endochondral ossification was interrupted during the long-bone growth process. This is related to protracted activation within the hypothalamic-pituitary-adrenal axis, and consequently, hypercortisolemia. In this stud we conclude that CUMS have negative impacts on bone, increased chondrocyte apoptosis in epiphyseal growth plate and disturb long bone growth in adolescent mice.

Keywords: Chronic stress; mental health; growth; chondrocyte apoptosis

Introduction

The COVID-19 pandemic Various factors affect the increased risk of skeletal health. Chronic psychosocial stress regarded as one of the greatest potential risk, although there's a strong association between chronic psychosocial stress and skeletal health but the mechanism underlying are still unclear. Also,

stress-related bone growth occurs due to multiple factors and is related to protracted HPA axis activation, which leads to the altered secretion of the Growth Hormone (GH). In the human body, the level of GH is increased by acute stress because the glucocorticoids on somatotrophs are directly stimulated. However, protracted HPA axis stimulation, as a response mechanism to chronic psychosocial stress, leads to the secretion of Corticotropin-Releasing Hormone (CRH) and, consequently, reduced GH levels [2]. This is in turn reduces the secretion of IGF-1 (Insulin-like Growth Factor), a member of the insulin/IGF/relax in protein family (nargrund 194) which plays a crucial purpose in long bone growth [2,3].

Exposure to stress can also cause increased ROS formation and decrease the body's ability to produce anti-reactive oxygen species (ROS), resulting in oxidative stress. Damage to the cell membrane will activate the intrinsic apoptotic pathway and cause cell death (apoptosis). The role of oxidative stress has been the focus of many studies. The human brain is particularly susceptible to oxidative damage [4], due to the high content of lipid substrates, as well as the high levels of oxygen requirement and free radical production. Previous studies have reported the relationship between chondrocyte apoptosis and oxidative stress. According to these studies, chondrocyte apoptosis is induced by excessive stress, through the p38 and p53 signaling pathways [5,6]. However, the recent study has demonstrated that chondrocyte apoptosis plays an important role in bone elongation [7].

The number of children and adolescents affected by mental health disorders has significantly increased [8]. Epidemiological studies focusing on the WHO World Mental Health surveys stated that the first onset of mental disorders usually occurs in childhood or adolescence [9]. The community surveys in United Stated (Merikange 2-8) reported at least one case of mental disorder in every 3 to 4 children and one case of severe emotional disturbance in every 10 children. The earliest age of onset distribution mental health disorders in children with median age 7-9 years for attention-deficit/hyperactivity disorder (ADHD), the phobia and separation anxiety disorder (SAD) with median age 7-14 years and 7-15 years for opposional-defiant disorder (ODD) [9]. Worldwide prevalence of mental disorders in children and adolescents is 13.4% (CI 95% 11.3-15.9) as follow: any anxiety disorder 6.5% (CI 95% 4.7-9.1), any depressive disorder 2.6% (CI 95% 1.7-3.9), mayor depressive disorder 1.3% (CI 95% 0.7-2.3), and ADHD 3.4% (CI 95% 2.6-4.5)[8].

Bone Elongation

Endochondral ossification is the process by which long bone cells, for instance, tibia and femur, derived from chondrocytes of the growth plate, undergo division and enlargement [7]. Meanwhile, linear growth or elongation of the cartilaginous growth plate is the major factor influencing the rate of bone growth [7]. In long bones, growth plates are found between the metaphysis and epiphysis, which border the hypertrophic zone, proliferative zone (PZ), and reserve zone (RZ). Several studies have suggested the RZ possesses stem-like cells which proliferate chondrocytes production. The total of these stem-like cells reduces with increasing age, consequently promoting the closure of the skeletal maturity-associated growth plate [10]. In the substitution of cartilage with bone, hypertrophic chondrocytes recruit osteogenic cells and stimulate vascular invasion. Osteoblasts are believed to have been differentiated from the cells of the bone marrow stroma [7]. However, based on recent studies regarding the mammalian growth plate, apoptosis does not occur in all hypertrophic chondrocytes, rather during development and remodeling, some hypertrophic chondrocytes are differentiated into

osteoblasts [11—13]. According to a report by Bahney on the use of cartilage grafts to promote bone regeneration, during bone repair, chondrocytes were directly differentiated into osteoblasts [13]. This chondrocytes-to-osteoblast trans differentiation findings is to be important role of bone elongation through endochondral ossification process [14—16].

This process is controlled by growth factors through a signaling pathway between IGF-I and GH [7]. IGF-l serves as the main regulator which controls bone elongation by facilitating hypertrophy and chondrocyte proliferation [17]. Recent study demonstrated IGF-l acts as both endocrine and paracrine growth regulator that stimulate local or systemic endochondral ossification [18]. In RZ of the growth plate, GH directly stimulates cell differentiation, while IGF-l mediates hypertrophic chondrocytes in PZ [7,19].

Chronic Unpredictable Mild Street

This is a common model for simulation chronic stress in humans by subjecting the test animal randomly to several minor-intensity stressors for a few weeks. Consequently, the test subject exhibits a chronic deviation from the normal behavioral patterns, for instance, apathy and anhedonia (inability to feel pleasure), and this altered behavior resembles the symptoms of major depressive disorder [1]. In this study, mice were exposed to a series of planned mild psychosocial stressors for 7 weeks (Table l) to induce behavioral, physical, and physiological alterations.

Table 1. Stressor schedule.

Time	Condition
Monday morning	Damp bedding (09.00-10.00) + Cage tilting (10.00-12.30)
Monday afternoon	Bathing (13.00-13.30) + Social stress (14.00-16.00)
Tuesday morning	No bedding (09.00-11.00) + Social stress (11.00-13.00)
Tuesday afternoon	Damp bedding (13.00-14.00) + Light/dark (14.00-16.00)
Wednesday morning	Cage tilting (09.00-11.30) + Bathing (11.30-12.00)
Wednesday afternoon	Damp bedding (12.00-13.00) + Light/dark (13.00-16.00)
Thursday morning	Social stress (09.00-11.00) + Bathing (11.30-12.00)
Thursday afternoon	Bedding changes (12.00-14.00) + Light/dark (14.00-16.00)
Friday morning	No bedding (09.00-10.00) + Damp bedding (10.00-12.00)
Friday afternoon	Cage tilting (12.00-14.00) + Light/dark (14.00-16.00)

Previous findings showed that cortisol-mediated increased formation of ROS/NOS that have been implicated in the psychopathological sequalae caused by chronic stress. Chronic stress induced imbalance oxidant and antioxidant, increased oxidative stress characterized by elevated MDA and decreased antioxidant defense system in mouse brain [20]. Several studies demonstrated that oxidative stress induces chondrocytes apoptosis [5,6]. Preclinical studies which mice were subjected to a series of mild and unpredictable physical and psychological stressors resulting depressive-like behavior revealed reduced bone mass (Liu and Liu 2017), and are associated with higher rate of fractures [21]. In contrast to another studies which mice were subjected to chronic subordinate colony housing (CSC) display anxiety-related behavior, revealed increased adrenal and decreased thymus weights. Micro-computed

tomography revealed significantly reduced tibia and femur length, increase growth plate thickness, suggesting disturbed endochondral ossification during long bone growth [3].

Given the differences response between the chronic unpredictable mild stress and the CSC models, we hypothesize that the chronic unpredictable mild stress does have impacts on bone elongation beside reduce bone mass and potential risk of fracture. Therefore, in the current study, we observed the impacts of chronic unpredictable mild stress on chondrocytes apoptosis in growth plate and long bone growth.

Materials and Methods

An experimental study was conducted from January to August 2021. Twenty-six male Mus musculus mice were randomly divided into two groups, control group and one intervention group. The control group (K0) was given normal diet for 60 days without exposure to stressors, and intervention group (K1) was given 7 days for adaptation continued with exposure to stressors randomly as CUMS method and then got terminated on day 61th. The following type of exposure consists of 7 different interventions, carried out once a day according to the schedule of administration in a week. A change of intervention was carried out, so that the mice did not adapt to the intervention given and exposure to stress could have a maximum impact. Each intervention in a day is carried out for approximately 7 hours with an estimated start at 08.00 in the morning until 15.00 in the afternoon.

The length of the femur was measured by calipers, the thickness and Caspase-3 expression of epiphyseal growth plate presented chondrocyte apoptosis were measured through histological examination, using Hematoxylin Eosin and Immunohistochemical staining. The data obtained were subjected to analysis using Windows 18.0 SPSS (Statistical Product and Service Solution) at a 95% confidence level, with a P-value of ≤ 0.05. Subsequently, the numerical data were presented as mean and Standard Deviation (SD) values, while the categorical data were presented as numbers and percentages. A man-Whitney Test and an Independent T-test were also performed to analyze the comparison between the intervention group (K1) and the control group (K0).

Results and discussion

The initial characteristic of body weight was measured, results the average of body weight in control group (K0) was 27.71 (±1.05) grams and intervention group (K1) were 27.73 (±10.91) grams with P >0.05 showed that there was no significant difference between K0 and K1. But after the intervention, the average body weight in K0 was 35.13 (±0.86) grams, while the average body weight in K1 was 32.60 (±0.56) grams. Paired Sample test in K0 and K1 obtained P value <0.05 shown that after intervention there was a significant difference between K0 compared to Kl.

The Effect of CUMS on Femur Length

This study as showed in Table 2. resulted the average of femur length Mus musculus mice in K0 was 2.831 (1 0.423) cm and in K1 was 2.469 cm (1 0.372). Concluded that the length of the femur in K0 were higher than K1, although they were given the proper nutrition during the intervention. Adequate nutrition is important variable that supports long bone elongation. There are strong correlation between undernutrition (racine 96,98) and stunting, as well as overnutrition and accelerated linear growth (racine 99-102). But in our study, the authors had given the proper nutrition for either K0 and K1 group. This

proved that the exposure of chronic unpredictable mild stress given by the authors caused a significant difference in the before and after intervention. This finding is accordance with previous studies, which stated that there are other factors besides nutrition that affect the growth of long bones. As mentioned, bone elongation is a complex process driven by multiple intrinsic and extrinsic variables, including: ethnicity, genetics, hormones, psychosocial, nutrition, chronic disease and other environmental factors [7 ,22].

Table 2. The effect of chronic stress exposure on long bone growth

Growth Plate	Group	Median	Min	Max	P
Femur Length (cm)	K0	3.0S	2.0	3.3	0.025
	K1	2.5	1.8	3.0	
Thickness (μm)	K0	331.449	142.27	534.74	0.191
	K1	268.283	132.86	607.18	

The mechanism stress-related bone growth is clearly multifactorial, and its associated with prolonged activation of the HPA axis. Various HPA axis responses occur due to chronic stress, and these responses fluctuate considerably. Certain studies proposed an association between increased HPA axis activity and chronic stress, however, other studies linked chronic stress to reduced HPA activity [23]. This study discovered reduced femur length was induced through chronic unpredictable mild stress-induced. This is probably due to the sustained hypercortisolemia caused by protracted HPA axis activation, which reduces GH circulation through secretion, or CRH (corticotropin-releasing hormone). Therefore, GH secretion is directly inhibited by high cortisol levels, leading to a further reduction in the IGF-l factor production within the liver, through the JAK/STAT pathway [2, 24]. IGF-l is a crucial mediator of bone growth, while GH directly and indirectly on target cells to increase tissue formation, and these two hormones play a key role in skeletal growth [25].

The role of IGF-I in growth has been demonstrated by studies of long bone growth in rats that are reduced in IGF-l deficiency in liver disease and acid-labile subunits [26]. It is suspected that GH has an independent pathway to IGF-l, because in mice that are deficient in IGF-I and GH will have reduced long bones than if the mice are deficient in IGF-l alone [27]. IGF-l is synthesized mainly in the liver, and to a lesser extent it is synthesized in bone, cartilage, and some other solid organs [24].

The term “psychological stress" refers to an unpleasant emotional experience that is followed by a behavioral, physiological or biochemical, physiological reaction [2]. These reactions can be either peripheral stress mechanisms or central stress mechanisms. However, the paraventricular nucleus (PVN) within the hypothalamus is generally believed to be the major regulator of stress responses [2]. The PVN comprises 2 neutron types: the first deals with the synthesis and release of oxytocin and arginine vasopressin (AVP), while the second type responsible for secrete corticotropin releasing hormone (CRH) and AVP [2]. PVN has broad functions in the hypothalamus and brainstem that play a role in autonomic control, such as metabolism, growth, reproduction, immune and other autonomic functions (such as gastro intestinal, renal, and cardiovascular functions [28]. The peripheral stress mechanism consists of the sympathetic-adrenal system (SAS) pathway, the HPA axis, and the HPG axis pathway [2,29]. In short-term stress, activation of the SAS pathway prepares the body to respond to the flight or fight mechanism and

after the stressor stops, the body return to a state of homeostasis. In long-term stress there's a prolonged stress response mechanism, resulting in pathological metabolism, changes in vascular function, tissue repair, changes in the immune system and nervous system, eventually chronic stress cause a reorganization of the HPA axis [4,29].

Preclinical studies by Foertsch et al in 2017, which examined the relationship of chronic psychosocial stress to long bone growth in adolescent rats, stated that chronic psychosocial stress had a negative impact on endochondral ossification of the growth plate and long bone growth. Mentioned that depressive disorders have a correlation with the incidence of osteoporosis and increased risk of fractures. But PTSD (Post Trauma Stress Disorder) has a different effect. Using a murine PTSD rat model obtained from micro computed tomography results showed a decrease in the length of the femur and tibia, an increase in the thickness of the epiphyseal growth plate and a decrease in mineral deposition, which indicated a disruption of endochondral ossification in long bone growth [3].

A study by Henneicke in 2017 stated that chronic stress exposure is a risk factor for decreased bone mass through the glucocorticoid signaling pathway Using a transgenic mouse model (glucocorticoid signaling knockout on osteoblasts and osteocytes) aged 8 weeks, it was found that on exposure to CMS (Chronic Mild Stress) there was an increase in serum glucocorticoid levels 3 times, a decrease in bone trabecular, and an increase I osteoclast activity. Uniquely, this occurred in male rats, while for female rats, chronic stress exposure did not cause skeletal effects [21].

The effect of CUMS on Epiphyseal Growth Plate Thickness

Our study found that the mean epiphyseal growth plate thickness in the K0 was 331.449 ± 137.831 µm and K1 was 268.283 ± 127.952 µm with $p>0.05$. It showed that CUMS gave no significant difference between K0 and K1 on the epiphyseal growth plate thickness of mice. However, overall, there was a decrease of average thickness epiphyseal growth plate in K1, which was ± 68,166 µm reduce compared to the K0. So, we are concluded that CUMS have negative impact to the thickness of the epiphyseal growth plate even though it was not significant. Reduced of thickness in the epiphyseal growth plate means that the growth plate will accelerate the fusion of epiphyseal growth plate. This can be explained that hypercortisolemia caused by CUMS affected production of IGF-I in a complex manner and disturb growth of long bones include reduce the thickness of the growth plate [2,24].

The growth plate is a narrow cartilage layer found between the metaphysis and epiphyseal, in the region where the long bones grow. The growth plate comprises 3 layers: the proliferation zone stem, the hypertrophy zone, and the cell layer or reserve zone. Endochondral ossification is the process by which long bones grow, beginning with cartilage formation and subsequently, remodeling into bone tissue. This process in initiated by the recruitment of chondrocytes within the stem cell zone, which triggers proliferation, then differentiation, mineralization, and apoptosis. In children and adolescents, the growth plate will experience maturation, there is a decrease in the thickness and width of the epiphyseal growth plate until it finally closes at the end of puberty [30]. In some cases, the fusion rate of the epiphyseal growth plate may be lengthened or shortened. Factors that influence the rate of fusion of the epiphyseal growth plate include nutrition and hormones [22,31].

A previous experimental study, with growth-restricted cystic fibrosis knockout cystic fibrosis transmembrane conductance regulator gene, showed a decrease in the length of the femur bone, a decrease thickness of the growth plate, and a decrease in serum IGF-I levels [32]. However, other

experimental studies suggested that psychological stress had negative impact on endochondral ossification such decreased the length of the femur, and increased the thickness of the epiphyseal growth plate [3]. Thus there are two different opinions in previous studies regarding the response of epiphyseal growth plate thickness to chronic psychological stress. This can be explained by another study, giving an early exposure estrogen to a rats, it was stated that epiphyseal growth plate fusion can occur in humans and rabbits, but in rats, growth plate fusion does not occur [30].

The Effect of CUMS on Chondrocytes Apoptosis

Our study as in Table 3 showed that there was a significant increased Caspase-3 expression in Kl compared to K0 group (P<0.001). Caspase-3 expression was found to be greater in K1, it proved that there was a significant effect of chronic unpredictable mild stress on chondrocyte apoptosis in the femur of mice. Caspase-3 is a marker of cell death or apoptosis, in this study its presented as chondrocytes apoptosis of the femur bone through the intrinsic pathway.

Table 3. The effect of CUMS on Caspase-3 expression

Group	Mean (%)	Title 3	P
K0	22.954	11.487	<0.001
K1	53.058	33.93	

Our study revealed that mice subjected to CUMS had increased chondrocytes apoptosis. This result is similar to earlier report that have also shown that oxidative stress induced chondrocytes apoptosis [5,6]. The primary neurohormonal involved in cortisol release with activation of oxidative, inflammatory signaling pathways in charge of stress-induced organ pathologies, is the HPA axis [20,33]. Cortisol is released in an attempt to sustain homeostasis in cells, however, the resulting hypercortisolemia is bound to cause organ damage, particularly for adrenal glands and the brain, which is particularly susceptible to oxidative damage [4]. Enlargement of the adrenal gland is a common indicator of chronic stress because excessive levels of cortisol have been reported to lead to adrenal hypertrophy [20,34]. Subsequently, psychopathological sequelae due to chronic stress are reportedly caused by cortisol-mediated increased formation of ROS/NOS [33,34]. According to several reports, psychological and physical stressors induce antioxidant depletion, consequently, leading to oxidative stress which activates the caspase pathway, inducing chondrocytes apoptosis [5,6].

Previous studies have suggested that chondrocytes apoptosis caused by oxidative stress is crucial in the progression of osteoarthritis [5,6], which is the most prevalent chronic joint disease indicated by progressive cartilage matrix degradation as a result of an imbalance in the activity of catabolic and anabolic chondrocytes [35]. Based on this study's findings, during endochondral ossification, hypertrophic chondrocytes have the capacity to differentiate and proliferate into osteogenic cells [36]. Generally, these chondrocytes undergo apoptosis and degeneration, with the last chondrocyte invaded by bone marrow derived osteoclasts/chondroclasts, as well as capillaries, while new bones are formed from newly arrived osteoblasts [16,36]. However, numerous studies have argued about the ability of mature chondrocytes to differentiate into bone cells [12,15,16]. During endochondral bone repair, chondrocytes are even believed to be direct precursors of osteoblasts [12], and it transdifferentiate by activating a stem cell-like state [11].

To the best of our knowledge, no studies has mentioned the correlation between chondrocyte apoptosis and bone elongation. Longitudinal bone growth occurs through endochondral ossification within cartilaginous growth plate at the ends of developing long bone, for instance, the tibia, humerus, and femur, which increase in length as the growth plate's chondrocytes expand and divide [7]. This process is regulated mainly by growth factor which is signaling between GH and IGF-1. Our study had showed that chronic unpredictable mild stress increased chondrocyte apoptosis by increasing Caspase-3 expression and reduced the length of the femur, although the underlying mechanism is still unclear.

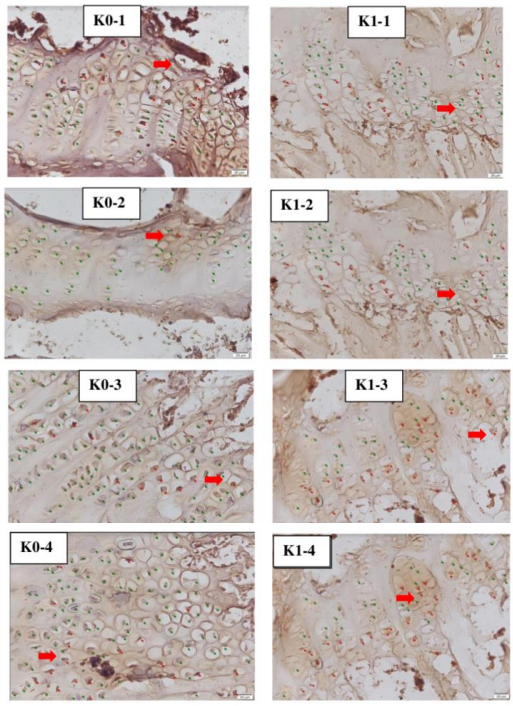

Figure 1. Chondrocyte apoptosis presented by Caspase-3 Expression with IHC. K0-1 to K0-4, Control Group. K1-1 to K1-4, Intervention Group with increased Caspase-3 Expression.

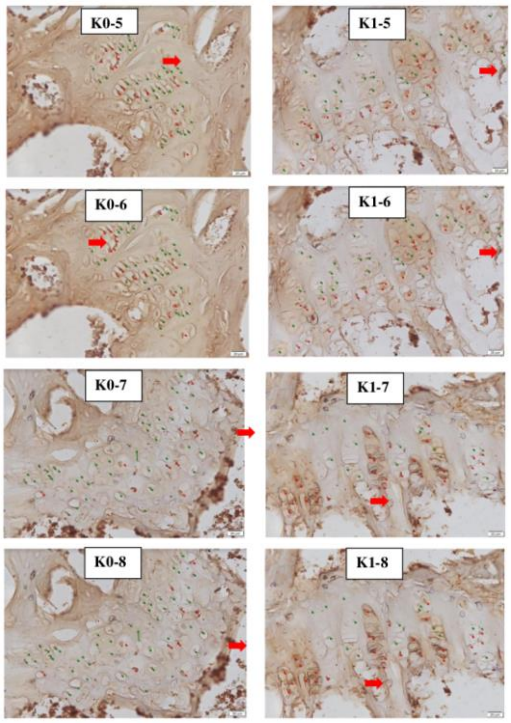

Figure 2. Chondrocyte apoptosis presented by Caspase-3 Expression with IHC. K0-5 to K0-8, Control Group. K1-5 to K1-8, Intervention Group with increased Caspase-3 Expression.

Conclusion

This study results in adolescent male mice subjected to a chronic unpredictable mild stress during long bone growth, have negative impact on the femur length but did not have a significant impact to the growth plate thickness. Increased Caspase-3 expression on the growth plate was associated with the apoptosis of chondrocyte that impacts bone elongation.

Availability of the Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon receipt of a reasonable request.

Acknowledgment

The authors are grateful to the technical staff of the Department of Pathology Anatomy, and to dr.Rimbun of the Department of Histology, University of Airlangga for rendering assistance during the histological studies.

Conflicts of interest

The author have no conflicts of interest to declare

References

1. Nollet, M. et al. (2013) Models of Depression: Unpredictable Chronic Mild Stress in Mice. Curr. Protoc. Pharmacol. 61, 5.65.1-5.65.17
2. Nargund, V.H. (2015) Effects of psychological stress on male fertility. Nat. Rev. Urol. 12, 373–382
3. Foertsch, S. et al. (2017) Chronic psychosocial stress disturbs long-bone growth in adolescent mice. Dis. Model. Mech. 10, 1399–1409
4. Krolow, R. et al. (2014) Oxidative Imbalance and Anxiety Disorders. Curr. Neuropharmacol. 12, 193–204
5. Li, D. et al. (2019) PI3K/Akt and caspase pathways mediate oxidative stress-induced chondrocyte apoptosis. Cell Stress Chaperones 24, 195–202
6. Sakata, S. et al. (2015) Oxidative stress-induced apoptosis and matrix loss of chondrocytes is inhibited by eicosapentaenoic acid: Oxidative Stress-Induced Apoptosis and Matrix Loss. J. Orthop. Res. 33, 359–365
7. Racine, H.L. and Serrat, M.A. (2020) The Actions of IGF-1 in the Growth Plate and Its Role in Postnatal Bone Elongation. Curr. Osteoporos. Rep. 18, 210–227
8. Polanczyk, G.V. et al. (2015) Annual Research Review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. J. Child Psychol. Psychiatry 56, 345–365
9. Kessler, R.C. et al. (2007) Age of onset of mental disorders: a review of recent literature: Curr. Opin. Psychiatry 20, 359–364
10. Raimann, A. et al. (2017) A journey through growth plates: tracking differences in morphology and regulation between the spine and the long bones in a pig model. Spine J. 17, 1674–1684
11. Hu, D.P. et al. (2017) Cartilage to bone transformation during fracture healing is coordinated by the invading vasculature and induction of the core pluripotency genes. Development 144, 221–234
12. Wong, S.A. et al. (2021) Chondrocyte-to-osteoblast transformation in mandibular fracture repair. J. Orthop. Res. 39, 1622–1632
13. Bahney, C.S. et al. (2014) Stem Cell-Derived Endochondral Cartilage Stimulates Bone Healing by Tissue Transformation: Cartilage Transformation Stimulate Bone Healing. J. Bone Miner. Res. 29, 1269–1282
14. Aghajanian, P. and Mohan, S. (2018) The art of building bone: emerging role of chondrocyte-to-osteoblast trans differentiation in endochondral ossification. Bone Res. 6, 19
15. Zhou, X. et al. (2014) Chondrocytes transdifferentiate into osteoblasts in endochondral bone during development, postnatal growth and fracture healing in mice. PLoS Genet. 10, e1004820–e1004820
16. Jing, Y. et al. (2020) Chondrogenesis Defines Future Skeletal Patterns Via Cell Trans differentiation from Chondrocytes to Bone Cells. Curr. Osteoporos. Rep. 18, 199–209
17. Lui, J.C. et al. (2014) Recent Research On The Growth Plate: Recent insights into the regulation of the growth plate. J. Mol. Endocrinol. 53, T1–T9
18. Tahimic, C.G.T. et al. (2013) Anabolic effects of IGF-1 signaling on the skeleton. Front. Endocrinol. 4,
19. Guntur, A.R. and Rosen, C.J. (2013) IGF-1 regulation of key signaling pathways in bone. Bone KEy Rep. 2,
20. Okoh, L. et al. (2020) d-Ribose–l-cysteine exhibits adaptogenic-like activity through inhibition of oxido-inflammatory responses and increased neuronal caspase-3 activity in mice exposed to unpredictable chronic mild stress. Mol. Biol. Rep. 47, 7709–7722

21. Henneicke, H. et al. (2017) Chronic Mild Stress Causes Bone Loss via an Osteoblast-Specific Glucocorticoid-Dependent Mechanism. Endocrinology 158, 1939–1950
22. Batubara, J.R. et al. (2018) Buku Ajar Endokrinologi Anak, Kedua. Badan Penerbit Ikatan Dokter Anak Indonesia.
23. Miller, G.E. et al. (2007) If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol. Bull. 133, 25–45
24. Rozario, K.S. et al. (2015) Gh and Igf-1 Physiology in Childhood.
25. Locatelli, V. and Bianchi, V.E. (2014) Effect of GH/IGF-1 on Bone Metabolism and Osteoporsosis. Int. J. Endocrinol. 2014, 1–25
26. Yakar, S. et al. (2002) Circulating levels of IGF-1 directly regulate bone growth and density. J. Clin. Invest. 110, 11
27. Lupu, F. et al. (2001) Roles of Growth Hormone and Insulin-like Growth Factor 1 in Mouse Postnatal Growth. Dev. Biol. 229, 141–162
28. Ferguson, A.V. et al. (2008) The paraventricular nucleus of the hypothalamus – a potential target for integrative treatment of autonomic dysfunction. Expert Opin. Ther. Targets 12, 717–727
29. Kumar, V. et al., eds. (2018) Robbins basic pathology, Tenth edition. Elsevier.
30. Emons, J. et al. (2011) Mechanisms of Growth Plate Maturation and Epiphyseal Fusion. Horm. Res. Paediatr. 75, 383–391
31. Shim, K.S. (2015) Pubertal growth and epiphyseal fusion. Ann. Pediatr. Endocrinol. Metab. 20, 8
32. Stalvey, M.S. et al. (2017) Reduced bone length, growth plate thickness, bone content, and IGF-I as a model for poor growth in the CFTR-deficient rat. PLOS ONE 12, e0188497
33. Panossian, A. (2017) Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals: Mechanisms of adaptogenic activity of botanicals. Ann. N. Y. Acad. Sci. 1401, 49–64
34. Panossian, A. et al. (2018) Novel molecular mechanisms for the adaptogenic effects of herbal extracts on isolated brain cells using systems biology. Phytomedicine 50, 257–284
35. Sekar, S. et al. (2017) Dietary Fats and Osteoarthritis: Insights, Evidences, and New Horizons: Dietary Fats & Osteoarthritis. J. Cell. Biochem. 118, 453–463
36 Enishi, T. et al. (2014) Hypertrophic Chondrocytes in the Rabbit Growth Plate Can Proliferate and Differentiate into Osteogenic Cells when Capillary Invasion Is Interposed by a Membrane Filter. PLoS ONE 9, e104638