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The osteogenic potential of physical activity

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Questa la scrivo in italiano!

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Introduction

This dissertation is the result of a three-year research work by Ph.D. student Alessandra Amato. During her doctoral program, Miss Amato investigated the effect of the mechanical stimulus from physical exercise on bone concerning biological age, the presence of comorbidities, and nutrition. Specifically, investigate the possible mechanisms of action through the study of Wolf's law and the hypotheses of miRNAs and GUTs. Muscles, bones, and tendons interact with each other like a functional syncytium. External factors such as exercise and nutrition translate into internal stimuli, and biochemical reactions, which are capable of modifying the muscular and skeletal structure. Connective tissue cells, that allow muscle strength transmission by complex pathways, are sensitive to mechanical stress and are subject to adaptation. Therefore, physical exercise is a stimulus that can influence tissue reorganization. Chapter one is the introduction to the research topic: It describes first the tissue and then how the workload acts on this by increasing the size and strength of tendons, ligaments, bones, and cartilage and the characteristics that the stimulus must have to be considered osteogenic. However, the mechanical stimulus given by scheduled physical activity interacts with various factors. Therefore, in the second part, the chapter deepens how three co-factors, by interacting with physical activity, influence the maintenance of bone tissue homeostasis. In particular, the effect of physical exercise on bone metabolism in the various stages of life, concerning nutrition and neurological diseases was described. The second chapter reports the main publications obtained by the Ph.D. student Amato starting from the practical implications concerned in the first chapter. The chapter opens with a review that analyzes the recent literature on the impact of diet and physical activity on bone health in children and adolescents and that laid the foundation of the second paper reported in the chapter, an original article that described the effects of different meals on bone metabolism of young gymnasts. To better understand the results found in the young gymnast's paper, Amato has analyzed in the third and fourth papers respectively a possible mechanism of action, namely that of the gut and the effect of specific training on bone metabolism in another stage of life age: adulthood. The last articles of the chapter, describe the effect of resistance training on bone health in a specific population: subjects with Parkinson's syndrome, along with an ongoing project involving subjects with multiple sclerosis, is Miss Amato's most recent work. Both neurological diseases have the loss of BMD in comorbidities with their symptoms. The third chapter shows the main activities carried out by Miss Amato during her Ph.D. years by describing her research areas of interest, the international mobility period, the skills acquired and the tools she used during her research work. The chapter closes with the abstract of all the published papers during the three years Ph.D. course. Finally, the Appendix reported the conferences that Miss Amato attended and the titles of the master dissertation of which she was co-tutor.

CHAPTER 1 – Bone tissue homeostasis and mechanical load: an essential relationship

1.1 Bone macro and microstructure

(Translated by (Anastasi, 2010; Farina, 2008; Tallitsch, 2012)
Bone is a connective tissue specialized in the support function, it is composed of the hard, mineralized extracellular matrix and the cellular component. The calcium-rich matrix forms a complex three-dimensional network, that encloses the same cells that produced it, within small gaps. The cellular part is the active part of the bone that allows it to change and adapt continuously according to different stimuli.

The cellular component consists of four different types of cells: Osteocytes, Osteoblasts, Osteoclasts (figure 1A), and Osteoprogenitor cells (figure 1B). The Osteoprogenitor cells give rise to other cell types, they are in large quantities in the endosteal and periosteal surfaces of the bone. The Osteoblasts synthesize the various tissue components participating in the formation of the osteoid and the regulation of its mineralization processes; in addition, to type I collagen, which is assembled into fibrils in the extracellular spaces and acts as a support for mineralization, osteoblasts produce some proteins, such as osteocalcin and osteonectin, which perform a support action in the deposition process of the calcified matrix. Osteoblasts are cells of mesenchymal origin. Osteoclasts are large cells, with a diameter ranging between 20 and 100 microns, equipped with many nuclei. They are mobile cells and specialize in the resorption of bone tissue. Thanks to the numerous microvilli, the osteoclasts attach themselves to a section of the bone matrix; they are generally housed in small gaps where they secrete proteolytic acids and enzymes, digesting both the supporting collagen and the inorganic matrix and solubilizing the minerals it contains. The resorption of bone tissue operated by osteoclasts plays an important role in the process of regeneration and remodeling of bone tissue, but not only. These cells are important for regulating serum calcium and phosphorus concentrations.
The secretion of the osteoblasts occurs according to a very precise orientation: initially it is polarized towards the pre-existing bone surface, but at regular intervals, it also turns in the other direction; in this way, the osteoblasts move away from each other, and remain imprisoned in the matrix during the mineralization phase, slowing down their metabolic activity and becoming an osteocyte. When the osteoblasts have exhausted their function they enter a state of quiescence and remain trapped in the calcified bone matrix. Taken together,

these will make up about 90% of the bone cell stock. It seems that the function of osteocytes is to participate in bone remodeling in response to various stimuli. Under the stimulus of calcitonin and parathyroid hormone, they also participate in the regulation of blood levels of calcium and phosphorus, controlling both the osteoclasts' and osteoblasts' activity. Osteoprogenitor cells, osteoblasts, and osteocytes are consecutive functional phases of the same cell type. On the other hand, Osteoclasts derive from precursors migrated into the bone tissue from the blood: the preosteoclasts; they differentiate from stem cells of the hematopoietic bone marrow.

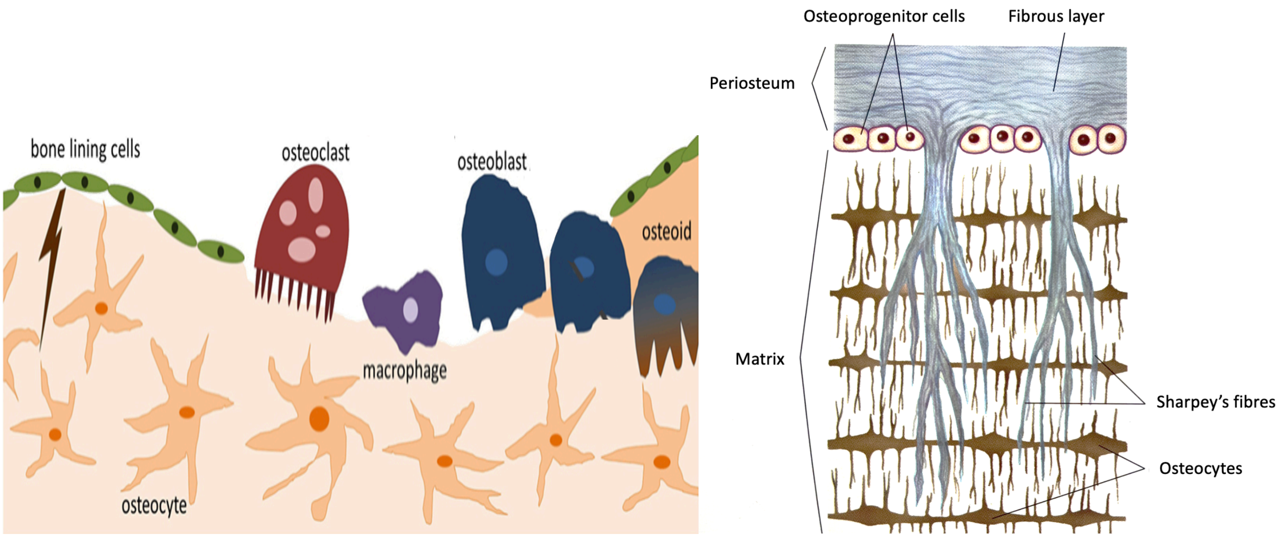


Figure 1. The bone cellular component. fig.1A: osteoclast, osteoblast, and osteocyte about their position in the bone tissue (Wittkowske, Reilly, Lacroix, & Perrault, 2016); Fig.1B: osteoprogenitor cells about their position in the bone tissue (modified by (Farina, 2008).

The Extracellular matrix (bone matrix or intracellular matrix), as a typical connective tissue, consists of an amorphous component, very small and essentially proteoglycan in nature, and an abundant fibrous component, consisting mainly of type I collagen fibers.

Within the bone tissue and the extracellular matrix itself, we can recognize organic (30-35%) and extra organic (65-70%) components. collagen fibers are responsible for bone flexibility, while the inorganic fraction is associated with their hardness.

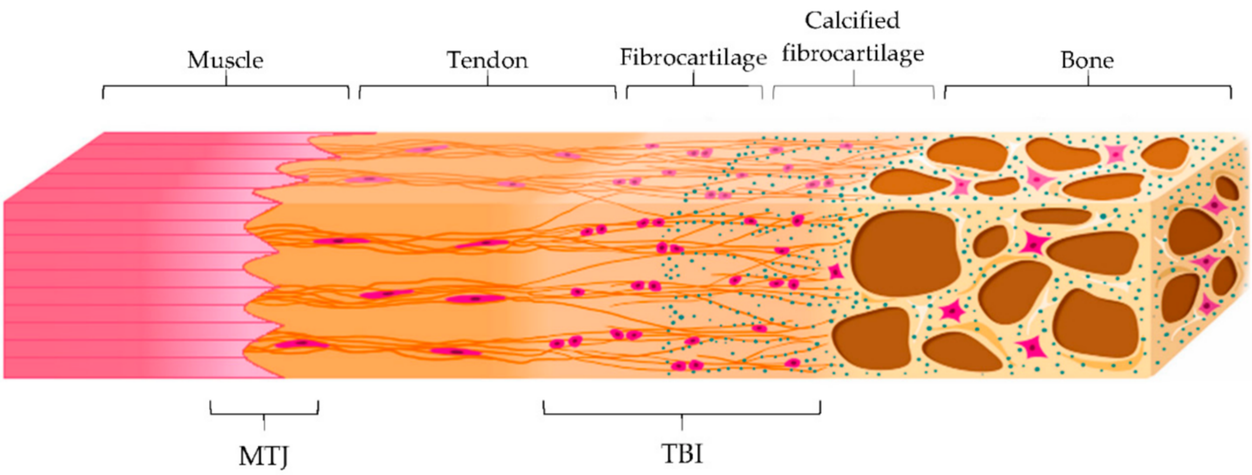
In addition to collagen, organic components of the matrix include proteoglycans, some non-collagenic proteins, cytokines, and growth factors. The most abundant element type I collagen is organized into fibers and acts as a support (matrix) for the sedimentation of salts during the mineralization process. The other protein components (osteocalcin, osteonectin, osteopontin) have the function of modulating this process of formation, mineralization, and adhesion between the cells and the osseous matrix.

Collagen fibers do not lay out randomly, but line up regularly, giving rise to an organic matrix known as an osteon. The collagen fibrils are arranged according to preferential lines of force, in such a way as to give the bone properties of resistance to mechanical stress. The osteon gives the bones considerable strength and compactness thanks to its lamellar structure. Collagen, like the other components of the organic matrix, is secreted by osteoblasts. Inorganic components of bone tissue, we recognize minerals such as calcium, phosphorus, fluorine, and magnesium, which give the bones the characteristic hardness. Calcium is found as calcium diphosphate, deposited in the form of crystals and anchored on fibrous collagen support; these crystals are arranged along the collagen fibers in an orderly manner. There are also other salts, such as calcium carbonate and traces of magnesium phosphate and calcium fluoride (also important in teeth). The presence of minerals gives the bones the characteristic of hardness. The outer surface of the bones is lined with a highly vascularized and richly innervated fibrous membrane: the periosteum; which, in addition to collagen fibers and fibroblasts, contains osteoprogenitor cells, and is firmly anchored to the bone with Sharpey's fibers (figure 1B). The periosteum is missing only in the attachment areas of large ligaments and tendons. Where the tendons enter the bones, their fibers intertwine with those of the tendons. The tendons are firmly anchored to the bone through the osteo-tendon junction, the anatomical point where the force of the contraction of the muscle fibers is transmitted to the bone. The tendon widens at the osteo-tendon junction. The peripheral fibers are fixed in the periosteum and the central ones penetrate the bone tissue giving rise to a fibro-cartilage structure that is subsequently mineralized in-depth, this organization allows better division of the stresses.

In the osteo-tendon junction there are 4 distinct areas:

• tendon
• fibrocartilage
• mineralized fibrocartilage
• bone

The fibrocartilage tissue interposed between tendon and bone absorbs insertional stresses. Tendon vascularization is guaranteed by capillaries present in the peritendinous sheets and the synovial sheaths. In addition, there are intrinsic systems located at the level of the myotendinous and osteotendinous junctions. Therefore, there is a pre-insertional area of relative hypo- vascularization which is the most common area of degeneration and tendon rupture. During the movement, the osteo-tendon junctions are subjected to intense stresses and are affected by the muscle tone during the phases of immobility.



Schematic representation of the myotendinous junction (MTJ) and the tendon-to-bone interface (TBI) with its different zones: tendon, fibrocartilage, calcified fibrocartilage, and bone. Modified from (Bianchi et al., 2021).

1.2 Connective tissue adaptation to mechanical load

The workload acting on the connective tissue results in an increase in the size and strength of tendons, ligaments, bones, and cartilage, but it is a moderate and time-consuming process compared to muscle. The bone and cartilage tissues have the capacity in acute to tolerate respectively 50-200 MPa and 5-40 MPa. MPa is the megapascal, a unit of measurement that evaluates pressure, stress, longitudinal elasticity, and tensile strength. A year of training can improve this capacity by 5-10 % but 4-8 weeks of inactivity decrease this by 30 % in mineralization and calcium content for the bone and water and proteoglycans for the cartilage (Schleip, 2015). The table below (Tab.1) compares the adaptations to mechanical stress anthe d inactivity of connective tissues and skeletal muscle.

Function	Capacity (acute)	Training (% increase)	Inactivity (% decrease 4–8 wks)
Connective tissue		Months-years	
Tendon	100 MPa	10	30 (+ stiff./cross-links)
Ligament	60–100 MPa	10	30 (+ stiffness/elastin)
Bone	50–200 MPa	5–10	30 (+ mineraliz./Ca++)
Cartilage	5–40 MPa	5–10	30 (+ water/proteogl)
Skeletal muscle		Months-years	
Strength		100	60
Muscle Mass		60	20–30

Tab,1. Connective tissue adaptation to exercise and inactivity, (Schleip, 2015).

Therefore, the connective tissue is highly dependent on normal daily load and weakens quickly when not subjected to mechanical load. Several studies show inactivity is related to the loss of strength and mechanical characteristics of the connective tissues. The absence of load causes a rapid alteration in the passive mechanical property, after 7/15 days, but not a rapid decrease in size. It was also found that a few days of inactivity causes a disorganization of the fibrils in the tendon structures and the lack of such mechanical stimuli, causes loss of bone mineral density (BMD) up to osteoporosis often found in bedridden subjects, (Blaber et al., 2014; Ohshima & Matsumoto, 2012; Sambandam et al., 2014). This stimulus is indispensable and cannot be replaced by other stimuli. It has been shown that following the prolonged absence of a mechanical stimulus, the connective tissues lose the expression of collagen receptors, mechanosensitive integrins (involved in the regulation of the cell cycle, the organization of the intracellular cytoskeleton, and the movement of new receptors on the cell membrane, allow rapid and flexible responses to events on the cell surface) and other proteins essential for tissue health. These adaptations do not appear to be unable to be compensated for by endogenous growth factors (e.g., TGF-beta, IGF 1, etc.), (Schleip, 2015). This underlines the importance of this stimulus for the proliferation of proteins that make up the structure of the matrix.

During mechanical loading, the connective tissue cells respond by modulating anabolism and catabolism through the protein synthesis of the central elements of the matrix (collagen type I), the proliferation and differentiation of osteoblasts, and the inhibition of synthesis and activity of osteoclasts (Rochefort & Benhamou, 2013; Thompson, Rubin, & Rubin, 2012; L. Zhao, Shim, Dodge, Robling, & Yokota, 2013). However, what is the mechanism by which the response of the cells that regulate bone metabolism is triggered following mechanical loading? One of the hypotheses under study is that the load causes an increase in the concentration and activation of microRNA (miRNA), an abundant class of short non-coding RNA, molecules composed of about 22 nucleotides (Luna, Li, Qiu, Epstein, & Gonzalez, 2011). MiRNAs are involved in multiple biological processes, including cell proliferation, differentiation, survival, and self-renewal of various cells including osteoblasts and bone marrow mesenchymal stem cells (BMSC) (Goff et al., 2008; Guo et al., 2015; Inose et al., 2009; Kook et al., 2009) multipotent stromal cells that can differentiate into a variety of cell types. Thus, mechanical loading directly affects miRNA concentrations which in turn regulate osteogenic and resorption factors.

1.2.1 History and key factors of remodeling knowledge

[from (H. M. Frost, 1990)]

The mechanical load must have specific characteristics to trigger the adaptation of the bone tissue, to be considered osteogenic, and ensure adequate bone mass also useful in the prevention of pathologies. Frost H.M., between the 80s and 90s, was one of the first to investigate the "Skeletal Structural Adaptations to Mechanical Usage" (H. M. Frost, 1990). We will briefly refer to his most important work which, clarifying the principles of Wolff's law, describes the mechanism of action that mechanical stimulus acts at the cellular level and modifies bone tissue. Frost describes the biochemical activities, which transform and adapt bone tissue as a result of the mechanical stimulus acting at the cellular level in the tissue. Generally, the remodeling at the multicellular unit (BMU)-based level occurs on some bone surfaces or "envelopes" and begins with a stimulus that triggers the activation of the turnover mechanism, resorption by osteoclasts, and formation by osteoblasts. This is a 3-4 month process. To be effective, this mechanical stimulus must create bone tension peaks between 50 and 100 microstrains. When the voltages remain below this range, the activation of the BMU is inhibited as well as when the stimulus exceeds this range. This is then referred to as the minimum effective strain (MES) to activate the remodeling process. However, activating the remodeling process has three key points: it does not respond in a detectable way to individual bone strains unless they damage the tissues; stimuli with peaks equal to or greater than the MES repeated over time are effective in stimulating remodeling, defined as "loading history"; stimuli with a lower intensity than the MES, even if repeated over time, are ineffective. BMU remodeling is affected by other factors besides mechanical use, including, hormones, sex, age, comorbidities, nutrition, microdamage, drugs, and toxins. Therefore, to maintain bone tissue healthy, the factors just mentioned must also be combined with the indispensable mechanical stimulus.

The specific purpose of this dissertation and the research work carried out in these three years of the Ph.D. program is to investigate how the mechanical stimulus interacts with three cofactors: nutrition, biological age, and comorbidities. Below are three introductory paragraphs to explain generically how the aforementioned cofactors interact with physical activity by influencing bone metabolism.

1.3 Bone metabolism in different stages of life

More than 50% of the bone mineral content (BMC) is formed during pre-adolescence. Therefore, BMD gains should be maximized during early life (Kambas et al., 2017). However, the tissue distribution is more important than the amount of BMC, this will affect the strength and endurance characteristics. Indeed, small changes in the amount of BMD (<10%), resulting from impact loading, are associated with large changes (> 60%) in bone structure and subsequent strength. The bone adapts to the load by increasing mass and reshaping itself to increase strength (Robling, Hinant, Burr, & Turner, 2002; Warden et al., 2005) affecting the surfaces of the endosteal or periosteal cortical bone or both surfaces at the same time. The predominant effect, in response to the increase in physical activity during puberty, in particular, is the thickening of the periosteal surface resulting in greater resistance to pressure load, fractures, or torsion (Warden & Fuchs, 2009). These adaptations of the periosteum to exercise persist over time, significantly reducing the risk of fractures in adulthood. In fact, during this phase, the density of the bone tissue decreases mainly from the endosteal surface

(Ahlborg, Johnell, Turner, Rannevik, & Karlsson, 2003; Seeman, 2003) (figures, 1), therefore reaching adulthood with a thick periosteum, built thanks to an adequate mechanical load during development, contributes to increasing resistance fracture in old age (Karlsson, 2007).

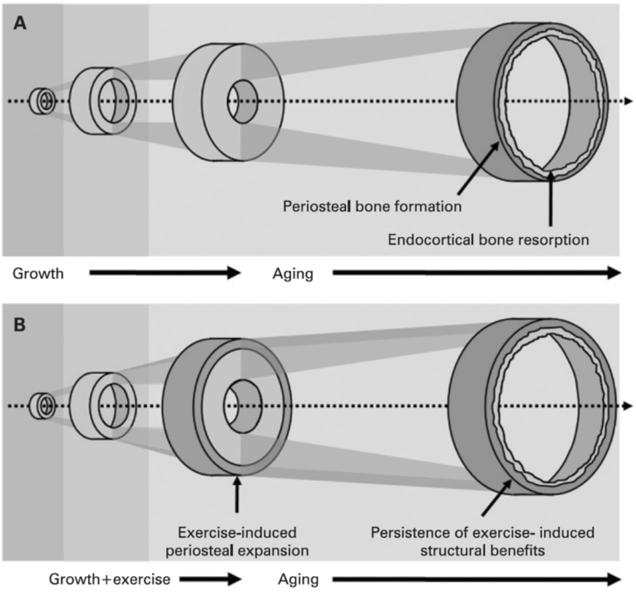


Figure 1 Bone structural changes associated with (A) aging and (B) exercise. (A) Bone loss during aging from endocortical surface. There is concomitant bone formation on the periosteal surface, which helps to maintain bone structure, but this is insufficient to maintain bone mass. (B) Exercise during growth facilitates periosteal bone formation, which optimizes bone structure. As bone loss during aging occurs from the inside out, the enhanced structure induced by exercise during growth has the potential to remain intact irrespective of age-related changes in bone mass, (Warden & Fuchs, 2009).

On the other hand, after maturity, the impact load can lead to an additional mass or reduced resorption on the endosseous surface which contributes less to the mechanical properties of the bone but whose characteristics are related to the health of the tissue, consequently, it is also important to carry out activities with an adequate mechanical loading in adulthood to contribute to bone tissue homeostasis (Bass et al., 2002; S. Kontulainen, Sievänen, Kannus, Pasanen, & Vuori, 2003; Martyn-St James & Carroll, 2009).

1.4 Bone metabolism in neurological diseases

Bone loss is often associated with other pathologies, especially neurodegenerative disorders characterized by motor impairment such as multiple sclerosis or Parkinson's disease (PD).

1.4.1 loss of BMD in Parkinson's disease: causes and remedies

The causes of bone mineral density decrease associated with PD are recently studied.

Parkinson's is a neurodegenerative disease in which dopaminergic neurons, in the midbrain substantia nigra, are lost causing an alteration in the brain circuitry controlling movement and consequently different motor dysfunctions such as walking, rigidity, tremors, bradykinesia (Vaidya, Dhamija, Guru, & Sharma, 2021) and "non-motor" dysfunctions as cognitive and mood-related problems (Aarsland et al., 2021). The cause is mainly attributed to genetics, since the 1990s genetic studies mapped more than 20 autosomal dominant and recessive genes involved in the pathogenesis of PD (Deng, Wang, & Jankovic, 2018; Lesage & Brice, 2009; Zimprich et al., 2004). However, there are several risk factors such as age, excess consumption of foods that can induce brain oxidative stress, sedentary lifestyle... Indeed, physical activity acts in the prevention of disease but also the management of symptoms. The brain and blood-brain barrier are damaged by a lack of physical activity that produces lactate and myokines essential for brain health (Adams, 2021) but not only: the first cause of the association between PD and bone disease is attributed to the reduction of physical activity. Motor alterations such as freezing of gait (FOG), for example, were associated with a lower BMD in a study comparing subjects with PD who present this symptom with others who do not (Choi, Cho, & Kim, 2021). Another recent study compares two physical impairments typical of PD: "The postural instability/ gait difficulty-dominant type" and "the tremor-dominant type". PD patients with postural instability or gait difficulty tended to have a higher prevalence of osteoporosis, compared to those with tremor-dominant type (Özcan, Acaröz Candan, & Gül, 2021). However, the loss of mechanical stimuli is not the only cause of bone loss, and in the most serious cases, of osteoporosis in PD patients. Indeed, such an association appears to be multifactorial. It's demonstrated a link between bone metabolism and the dopaminergic system. The homocysteine (Hcy), the main component of PD gold-standard drugs, has a toxic metabolite the effect of which seems to be associated with BMD decrease (Handa et al., 2019; Postuma & Lang, 2004; van Meurs et al., 2004). Furthermore, Deficiencies of 1α,25-dihydroxy vitamin D_3, and vitamin K, essential for bone remodeling, are commonly observed in PD patients (Handa et al., 2019). However, another possible mechanism of action of the association between PD and bone metabolism alteration could be genetic. Indeed, Parkinson's risk genes are expressed not only in the brain, but also in other tissues including bone cells (Xiong, Pan, Guo, Mei, & Xiong, 2021). These are called "PARK-designated genes", whose mutation is linked to Parkinson's disease and alteration of bone metabolism. Among those most studied, we mention some of them which play a key role in bone tissue homeostasis. PARK 2 and PARK 6 through β-catenin and autophagy signaling act on osteoblastic differentiation of BMSCs which results in accelerating bone repair; PARK 5 has an active role in osteogenesis by monitoring bone mineralization and on the other hand PARK, 7 has a negative regulation of osteoclastogenesis. Instead, PARKS 8 seems to act specifically on cortical bone increasing its thickness and decreasing the predicted risk of fracture. Finally, PARK 13 influences and activates the inflammatory responses during arthritis rheumatoid, often a symptom associated with Parkinson's disease. (Berwick et al., 2017; Cui et al., 2021; Deng et al., 2018; Kim et al., 2017; Lesage & Brice, 2009; Miyake et al., 2012; Xu et al., 2021; Zimprich et al., 2004). However, to date, there are no scientific publications that analyze the effects of structured physical exercise on the alterations of bone metabolism as a consequence of PD.

1.4.2 Loss of BMD in Multiple Sclerosis disease: causes and remedies

Multiple sclerosis (MS) is an autoimmune, inflammatory, and neurodegenerative disease that affects the central nervous system. MS is characterized by demyelination, axonal damage, and neurodegeneration. It is assumed that the causes are due to genetic and environmental factors (Rodríguez Murúa, Farez, & Quintana, 2022). The main motor impairments related to this pathology are balance, coordination, and strength, and MS is correlated with the diminution of bone mineral density too. Particularly, in some regions including the lumbar spine, femoral neck, and hip (Huang, Qi, Du, Chen, & Yan, 2015). However, the nature of this correlation is still uncertain, as in PD the lack of physical activity, due to the related motor alterations, seems to be the main one but not the only one. Also in MS, as in PD, there is a correlation between the therapy taken and bone loss. Corticosteroids often used to treat MS are related to decreased BMD (Kobza, Herman, Papaioannou, Lau, & Adachi, 2021). Glucocorticoids act mainly on the metabolism of three cells of the bone tissue: altering osteoblast differentiation (Hayashi et al., 2009), inducing osteocyte apoptosis (Weinstein, 2010), increasing osteoclast differentiation and maturation (Wijenayaka et al., 2011). Furthermore, Glucocorticoids act on renal excretion and intestinal calcium reabsorption too, altering calcium balance. Cleland et al studied some "Determinants" that influence bone metabolism in individuals with MS. This study showed that the amount of PA (evaluated with an accelerometer) is related to the loss of BMD but also that depression, symptomatic fatigue, and degree of disability (evaluated with EDSS), contributed independently from physical activity to BMD lost in MS. In the mechanisms of action hypothesized by Cleland, depression may increase activation of the sympathetic nervous system, stimulating bone resorption, and the independent association between disability and PA underlines that the disease process decreases BMD independent of its effect on physical activity or other variables, but that physical activity could help reduce this loss (Cleland et al., 2020). However, to date, studies that correlate the decrease in BMD to low PA evaluate the latter with questionnaires (Olsson, Oturai, Søndergaard, Sellebjerg, & Oturai, 2018) or with accelerometer (Cleland et al., 2020; Mojtahedi, Snook, Motl, & Evans, 2008) considering daily physical activity but there are no studies that plan to analyze the effect of structured physical activity such as resistance training, on bone metabolism.

1.5 Bone metabolism and nutrition

To understand the importance of physical activity and adequate nutrition association, we describe what happens when the balance between these two key elements for bone health is altered.

It is widely demonstrated that the low starting BMD (given by an inadequate acquisition during the growth phase), linked to inadequate caloric intake and physical exercise can predispose to the onset of bone diseases such as osteoporosis. The National Osteoporosis Foundation periodically renews the guidelines recommending specific physical activities in volume, intensity, and duration, suitable for the prevention of osteoporosis (and & Diseases; Foundation). While during adolescence, exercise, adequate in intensity and volume, can increase bone strength (V. P. Tan et al., 2014), in adulthood it helps reduce bone mass loss and density (Harding & Beck, 2017) and consequently avoid or slow down the onset of pathologies. However, inadequate exercise can alter bone metabolism, particularly in subjects most at risk of developing bone pathologies, such as women in menopause or amenorrhea (also caused by excessive and prolonged training over time associated with the caloric deficit). Indeed, some hormones, and in particular estrogen (female sex hormones) play a key role in regulating bone metabolism: They directly stimulate the synthesis activity of bone, there are estrogen receptors in osteoblasts; they stimulate the absorption of calcium by increasing the expression of receptors in the intestine; finally, they decrease osteoclastogenesis, the activity of osteoclasts and therefore bone resorption and increases the secretory reserve of endogenous calcitonin (Harding & Beck, 2017; Jin et al., 2021). Estrogen is produced in different tissues but synthesized in higher quantities by the ovarian follicles, this production decreases drastically during menopause. However, their concentration can also be affected by exercise (Cano Sokoloff et al., 2015): estrogen is also produced by adipose tissue (which becomes one of the main sources when the production of these hormones in the ovary ceases in menopause) and intense and prolonged exercise can lead to an excessive decrease in body fat, if not supported from an adequate caloric intake. This results in estrogen production decrease and consequently in all their activities. In the endocrine role of the adipose tissue, there is also the production of Leptin. Leptin regulates the sense of hunger at the hypothalamic level, but it also seems to have a role in bone metabolism, inducing the differentiation of osteoblasts, increasing the expression of alkaline phosphatase, osteocalcin, and type I procollagen, markers of osteoblastic maturation to an increase in the mineralization of the matrix. Additionally, leptin levels are higher in premenopausal women than in postmenopausal women. Estrogen is believed to have a stimulating effect on leptin but, conversely, in a feedback mechanism, leptin plays a role in modulating estrogen synthesis. (Cornish et al., 2002; Holloway et al., 2002; Salem, 2021).

Therefore, low levels of estrogen synthesis alter bone homeostasis by acting on several fronts indirectly and directly (Cano Sokoloff et al., 2015). Accordingly, inadequate training can alter hormone production, with a negative effect on bone health, mainly in women, an appropriate diet could help avoid or compensate for the effects of hormonal alterations (Amato, Baldassano, Cortis, Cooper, & Proia, 2018a). In addition to estrogen and leptin activity linked to adipose tissue, nutrition influences bone metabolism also through the GUTs hormones, the production of which is also linked to both physical activity and nutrition. The nutrients ingested stimulate their production and physical exercise could modulate their release (Broom, Batterham, King, & Stensel, 2009; King, Miyashita, Wasse, & Stensel, 2010; King, Wasse, Broom, & Stensel, 2010; King, Wasse, & Stensel, 2011; Martins, Morgan, Bloom, & Robertson, 2007). Once produced, intestinal peptides work by decreasing bone resorption (S. P. Schiellerup et al., 2019a). However, little is known about the mechanism of action that would increase GUT production following exercise and there are very few studies that link the effect of the production of these hormones, following various structured physical activities, with bone metabolism. This was one of the starting points of our research work.

CHAPTER 2 – From the literature review to practical implications: the effects of physical activity and co-factors interaction on bone metabolism

Chapter two describes the practical implications of the relationship between physical activity and co-factors such as nutrition, age, and comorbidities on bone metabolism, through the main publications of Alessandra Amato's Ph.D. program. The research work starts from a published review that includes the study of the recent literature guidelines on the role that each micro and macronutrient has on bone metabolism, and on the characteristics (volume, intensity, frequency, duration) that physical exercise must have to be an adequate osteogenic stimulus in a specific population: children and adolescents. Because, as described in the previous chapter, it is a key life stage for building adequate bone health. We will then describe an original article published that we experienced in the same age group, taking into consideration in the review, the effect of two types of pre-workout meals on bone resorption. Therefore, we have considered adulthood by investigating one of the possible mechanisms of action in the diet-PA-bone relationship: the GUTs hormone production (paragraph 2.3). Furthermore, we showed the effects on bone metabolism of another specific high-impact activity in this population (paragraph 2.4). Finally, we described, in the last two original published papers in the chapter, the study of the effect of a structured PA on two pathologies presenting osteoporosis between the signs respectively Parkinson's and Multiple sclerosis disease, which results are not present in recent literature.

2.1 The impact of diet and physical activity on bone health in children and adolescents

[from Proia, Amato et al. (2021)]

Introduction

Bone tissue is a real organ in constant change, with both locomotive and supportive functions. It can be stiff but also flexible. These characteristics depend on the bone's material composition. The bone grows in length with endochondral ossification processes and size with the modeling process of periosteal and endosteal surfaces (Bonewald, 2019). In particular, formation and resorption allow for increasing bone mass and changing tissue density. The cartilaginous epiphyseal plate is the space where bone grows in length as a result of chondrocyte proliferation and maturation replacing the cartilage tissue with bone construct. To allow the complete ossification process, osteoclasts and osteoblasts are directed, by blood vessels, into the new cartilage tissue (SA. Kontulainen, Hughes, Macdonald, & Johnston, 2007). This process runs constantly till the early twenties until the growth plate cartilage is replaced completely by new bone (Ballock & O'Keefe, 2003). When the bone stops growing in length it keeps increasing in cross-section to adapt to different mechanical loads and to compensate for bone loss. Osteoclasts and osteoblasts perform different tasks during the process of bone construction, the former are responsible to shape the long bone's outer surface by building on circumferential lamellae. Osteoclasts are responsible for endosteal bone surface resorption and overall bone remodeling. The process of bone shaping and remodeling is sex and age-dependent (Gómez-Bruton et al., 2020; Moon et al., 2015) leading to the bone with an optimal size, shape, and architecture to withstand the normal physiological loads imposed on it. Failure to gain a sufficiently strong skeleton during growth may predispose to bone fragility later in life. Thus, what are the key factors that may positively influence skeletal health in children and adolescents to maximize peak bone mass? Lifestyle, including diet and adequate physical activity, are external factors of bone mass development that during growth and also during adulthood can help the building of a strong adult skeleton (Amato, Proia, et al., 2021; A. Iannaccone, A. Fusco, S. J. Jaime, et al., 2020). Most of the literature that exists for children and adolescents about the effects of diet and physical activity on bone health, is translated from studies conducted in adults. Thus, so far relatively few studies have investigated the association between macronutrients or physical activity and bone mass depending on whether they are children or adolescents. Therefore, there are still many unanswered questions about what type of diet and physical activity may positively influence skeletal development. This review focuses on bone requirements in terms of nutrients and physical activity during childhood and adolescence. It explores the contemporary scientific literature that analyzes the impact of diet together with the typology and timing of physical activity that could be more appropriate depending on whether they are children or adolescents to assure optimal skeleton formation. A description of the role of PTH and gut hormones (GIP, GLP-1, GLP-2) as potential candidates in this interaction to promote bone health is also presented.

The influence of macronutrients and micronutrients on bone health

Nutrition is an essential process for the healthy growth and development of the skeleton. Diets are mainly composed of macronutrients (protein, fat, and carbohydrates) and also of micronutrients like dietary calcium, phosphorus, and vitamin D. Together they are essential factors in promoting bone health and preventing bone loss. In the following paragraph, we will focus on studies that have investigated the influences of macronutrients and micronutrients on bone to assure optimal skeleton formation.

Proteins

Protein consumption exerts a beneficial effect on bone health in adults, while very little is known about the short or long-term effects of protein supplementation on bone turnover and bone development in children and adolescents. As far as we know, the dietary allowances (RDA) in healthy children and adolescents are strictly derived by adults study (Dwyer, 2003; Schroeder & Sonneville, 2016) in which the recommended protein intake varies from 1.03 g/kg for all individuals to 0.97g/kg per day for children aged 18-24 months. In 2012 The EFSA Panel gave a scientific opinion on setting the Dietary reference value (DRVs) for protein, which also takes into account protein quality. The daily amount of protein is calculated according to age and sex and set the Population Reference Intake (PRIs) for adults goes from 0.66 g/kg body weight per day to 0.8 g/kg body weight per day. In older adults, the PRIs go from 0.8 g/kg body weight per day to 1.0 g/kg body weight per day concerning low energy requirement of sedentary elderly or more energy-dense protein for physically active groups. In infants and children, the EFSA Panel suggested a PRI from 1.65 to 1.1 g/kg body weight per day for the first year of life between 1.15 to 0.9 g/kg body weight per day from 2 to 5 years old children and from 5 to 18 years old the PRIs goes from 1.1 to 0.8 g/kg body weight per day (Agostoni, 2012). Moreover, not much data are present for children above or under average weight and young athletes and this might cause insufficient coverage in protein and energy requirements along children's growth (Pimpin, Jebb, Johnson, Wardle, & Ambrosini, 2016) to assure an optimal skeleton formation (Thierry et al., 2016).

Usually, in the adult athletes population protein RDA is defined as 1.2 and 1.7 g/kg/day (Gardner, Hartle, Garrett, Offringa, & Wasserman, 2019) but recent study affirm that protein intake in young boys before pubertal maturation should increase up to 2 g/kg/day protein intake to obtain higher bone acquisition as the positive effect of physical activity because the positive effect on skeleton health is due to protein intake instead than calcium intake (Chevalley, Bonjour, van, Ferrari, & Rizzoli, 2014; Thierry et al., 2016). Thus more research to clarify the point is necessary. Another open question is whether recommended protein intakes should be increased based on the source of protein. Many conflicting studies have tried to examine the beneficial or detrimental role of protein intake on bone health, based on the source of protein (animal vs vegetable) and the amount of protein ingested (high vs low quantities) (Heaney & Layman, 2008). Namely, the sulfur content may vary in sulfur-containing amino acids present in the protein source (animal or vegetable proteins). Therefore, greater production of sulfuric acid could induce low- or mild-grade metabolic acidosis that, in turn, may hurt bone remodeling by enhancing bone resorption (Bowen, Baird, Syrette, Noakes, & Baghurst, 2012). However, when a balanced diet with adequate intake of calcium, vitamin D, fruits, vegetables, and protein from animal sources is ingested, this does not exert a detrimental effect on bone and improves bone health (Cao & Nielsen, 2010) On the contrary, in pubertal girls when calcium intakes were less than 675 mg/day, the high-protein intake, especially from animal sources, hurt bone mass accrual deposition (Zhang et al., 2010). It is quite clear, from the literature, that sulfur amino acids play critical roles in metabolism and overall health maintenance. Animal-derived foods are a good source of sulfur-containing amino acids (Nimni, Han, & Cordoba, 2007). Besides their role in protein synthesis, methionine and cysteine are precursors of important molecules (Bauchart-Thevret, Stoll, & Burrin, 2009). The most popular food for children and adolescents that are a good source of dietary sulfur-containing amino acids are chicken and beef but also white eggs mainly present in products like an omelet, frittata, crepes, mayonnaise, biscuits, and cakes. So, the positive effects of protein on bone are exerted only if the energy requirements are satisfied by the carbohydrates and fats, otherwise, proteins are catabolized to sustain the energy demands. This is an essential adequate intake of fat and carbohydrates to maintain bone health (J. P. Bonjour, 2011; Dolan & Sale, 2019).

The Protein needs of adult athletes are higher than those of non-athletes (Phillips & Van, 2011; "Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine," 2000). Thus, we don't know if the quantity of acid formed, such as sulfate may hurt the young skeleton. Thus, further studies about the impact of the source of protein origin (animal or vegetable) on the bone of child athletes or highly active children would be of interest. Moreover, this concept is closely linked to food habits. There is some evidence that suggests substantial differences in the consumption of food containing vegetables or animal proteins based on age. Children ranging from 2 to 13 years old (in both sexes) are used to consuming more red meat, poultry or fish daily. Adolescents, from 14 to 16-year-old, especially girls have a marked decrease in animal protein consumption. However, the average intake the meat was generally higher for males (100 g/day) than for females (80 g/day) (J.-P. Bonjour, 2005)

Furthermore, it is important to underline that proteins may affect bones at various levels: (1) they represent the major component of the bone matrix, (2) they impact affect calcium excretion and absorption, and *(S. M. Shirreffs, Casa, Carter, & International Association of Athletics)* on serum concentrations of insulin-like growth factor (IGF-1) (J.-P. Bonjour, 2005). Therefore, adequate protein intake may be crucial for young athletes, for example, those involved in non-weight bearing-weight-bearing activities, like swimming who seem to be at increased risk for suboptimal peak bone mass development (Gomez-Bruton et al., 2016). Western diet often accounts for being responsible for osteoporosis or bone fracture due to the high protein content associated with hypercalciuria (Dolan & Sale, 2019). Thus, as protein intake increases, there is an increase in urinary calcium excretion, with most subjects developing negative calcium balance as well as an increased risk of fracture (Lorincz, Manske, & Zernicke, 2009). Therefore, the intake of protein must be adequate to fully realize the benefit of each nutrient on bone. In fact, for example, proteins can modulate the IGF-1 levels that, in turn, would impact both skeletal muscle and bone, reducing fracture risk and increasing the speed of recovery following a bone injury (Bikle et al., 2015). In addition, there is evidence of a positive bone turnover response after protein intake shortly after intense exercise in adolescents. A Study reveals a significant reduction in a marker of bone resorption (CTX) after protein intake based on whey protein beverage following intense physical activity such as swimming. Thus, protein intake, after exercise, is important for the increase of bone mass during childhood, in particular, if the subjects are active, or athletes (Theocharidis et al., 2020). On the other hand, if protein intake is low (0.7-0.8 g/kg) the amount of parathyroid hormone (PTH) levels will increase in the blood while moderate (1.0-1.5 g/kg) is associated with normal calcium metabolism, without altering bone homeostasis. After all, any lifestyle strategy that can promote bone buildup in children, without affecting whole-body homeostasis, is beneficial because it will drive toward higher peak bone mass and improvement in bone mineral density. Future research should focus on the long-term benefits of protein intake on blood markers of bone turnover in association with exercise, especially in children and adolescents practicing low and high-impact physical activity.

Fat and Carbohydrates

Many studies have explored the effects of fat and carbohydrate intake on bone health in children by focusing on calcium absorption rather than its direct effect on bone mineral density (BMD), bone mineral content (BMC), and bone remodeling. Thus, we will first focus on it to briefly make the point on what is known and the importance of the impact on bone growth in children. Studies have been carried out to understand whether o, a not fat or carbohydrate hindered calcium absorption in the intestine. Until now calcium absorption has been very studied concerning its impact on bone metabolism. As regards calcium absorption and fat, most of the studies were carried out on animal models fed with diets that contained a variable percentage of saturated fats (SF) (5%, 14%, 28%, and 45%). The results have shown poor calcium absorption probably due to the formation of non-digestible calcium and saturated fatty acids complexes in the intestine. In particular, a reduction in calcium absorption started when the diet administered contained up to 28% of fat, while there was a dramatic decrease in intestinal absorption of calcium when it reached 45% of fat. As a consequence, high-fat diet consumption in animals and humans is associated with a reduction of BMD and bone strength (Tian & Yu, 2017). Adverse microstructure changes occur in the cancellous bone compartment. Corwin and collaborators based on data from the Third National Health and Nutrition Examination Survey (NHANES III) conducted a study on 14850 subjects, confirming a negative association between saturated fat intake and BMD in both men and women (Corwin, Hartman, Maczuga, & Graubard, 2006).

Regarding the impact of carbohydrate intake on calcium absorption, numerous studies investigated the role of monosaccharides (particular glucose) and disaccharides (particular sucrose) showing an effect on the renal metabolism at the level of the distal tubule region of the nephron, therefore, influencing the reabsorption of calcium (Blaine, Chonchol, & Levi, 2015; DeFronzo, Cooke, Andres, Faloona, & Davis, 1975; Langsetmo et al., 2016; Lorincz et al., 2009). In particular, there will be a large increase in renal excretion following glucose ingestion. For example, Ericsson et al in human studies pointed out an exaggerated loss of calcium through the urine following the intake of a solution of glucose. The lactic acid formed by the osteoclast following the increase in glucose intake could induce the dissolution of calcium and magnesium from the bone surfaces, with a consequent increase in urine calcium excretion concerning the normal range. Other studies on both human and animal models showed that the insulin spike triggered by the ingestion of a high amount of glucose was directly proportional to the urinary levels of calcium excretion (Ericsson, Angmar-Månsson, & Flores, 1990). These data suggest that more attention should be paid to children's diets. The question is why the reduction of calcium absorption induced by fat and sugar could affect the growth of healthy bones in children. Because as they grow there is a decrease in the consumption of milk and a concomitant increase in the consumption of unhealthy food rich in saturated fat and soft drinks often attributed to gaining independence in choosing what to drink. In particular, data from the Continuing Survey of Food Intakes report (CSFII 1994-1996) suggest that with aging there is a reduction in milk intake with a ratio of about 30 ml and a concomitant increase of around 126 ml in sweetened drinks. This is associated with an increase in caloric intake, of approximately 30 kcal, and a concomitant reduction in calcium consumption of 34 mg for every 30 ml of milk displaced (Bowen et al., 2012). In young children, both athletes and not, the increased intake of soft drinks, or the consumption of food rich in saturated fat could hurt bone health and performance due to the impact of these on calcium absorption. This is the reason why it may be important to suggest using unsweetened drinks (water, milk) or orange juice, or sports drinks but only with small amounts of carbohydrates (<2%) and moderate amounts of sodium (S.M. Shirreffs, 2009) and fortified with calcium to obtain rehydration and reduce or prevent loss of BMD. Furthermore, following a healthy diet rich in unsaturated fat is also suggested. For example, animal studies have shown that polyunsaturated fatty acids (PUFAs) such as omega-3s have been shown to reduce bone resorption and increase bone formation (Watkins, Li, Lippman, & Feng, 2003). The positive effect of a diet high in PUFA was confirmed in humans. The subjects involved in the study were assigned to three different groups of intakes consisting of 8-13% of SFA, 12-13% of monounsaturated fatty acids (MUFA), and 9-17% of PUFA for a period of 6 weeks. There was no change in levels of bone-specific alkaline phosphatase (BSAP), selected as a marker of bone formation, across the three diets while there was a reduction of bone resorption following the PUFA-enriched diet (Griel et al., 2007). The results indicated that dietary PUFA may have a protective effect on bone metabolism. Studies are necessary to see the impact of unsaturated fat on bone remodeling in children. Carbohydrates are present in fruit and vegetables and influence the absorption of calcium and therefore might influence bone growth. For example, chicory and artichokes have a high content of non-digestible carbohydrates (they are not digested by mammalian enzymes) called fructans such as inulin, which increases the absorption of calcium (Cummings, Macfarlane, & Englyst, 2001; Shoaib et al., 2016). A study conducted on 9-13 years old boys and girls, for a total of twelve months, with 8 g/day supplementations of inulin-type fructan showed that calcium absorption, BMC, and BMD were significantly higher in the inulin-type fructan-supplemented group than in the placebo (supplemented with maltodextrin) control group (Abrams et al., 2005). As far as the mechanism of action is concerned, it was suggested that these types of molecules, that are not digested, will reach the colon where they will be fermented producing organic acids capable of reducing the pH by increasing the solubility and availability of calcium (Coxam, 2007). The daily intake of carbohydrates should not go down 50-55% of the diet. Adam-Perrot et al confirmed that consumption of a low-CHO diet leads to an increase in urinary calcium loss and a decrease in markers for bone formation. Moreover, in adults low-CHO diets lead to increased consumption of animal protein generating an acidosis that promotes calcium mobilization from bone, finally leading to an increase in urinary calcium (Adam-Perrot, Clifton, & Brouns, 2006). The lack of a consistent definition for "low carbohydrate diets" complicates efforts to compare the results of the studies already published in this field. While it is known in the adult population that when we refer to a "very low carbohydrate diet" we are talking about less than 70 grams/day based on the proportion of energy intake, a diet containing 200 grams of carbohydrate might be classified as moderately low for a 2.000 calorie intake, moderate carbohydrate for 1.500 calories and high-carbohydrate for 1.200 calories (Wylie-Rosett, Aebersold, Conlon, Isasi, & Ostrovsky, 2013).

In conclusion, there is a need for studies about the effects of carbohydrates and fat on bone metabolism in children to see how their impact bone growth. Future studies should focus on both the short and long-term benefits of carbohydrate and unsaturated fat consumption/supplementation on bone remodeling and BMD in children and adolescents, by looking also to those children participating in intensive training.

Micronutrients

Vitamin D and Calcium are known to play key roles in bone health. Optimal calcium intake is estimated to be 400 mg/day from birth to 6 months, 600 mg/day in infants (6 to 12 months), 800 mg/day in young children (1-5 years) and 800-1,200 mg/day for older children (6-10 years), 1,200-1,500 mg/day for adolescents and young adults (11-24 years) ("Optimal calcium intake," 1994; Zemel, 2017). Milk and milk products such as 125gr of yogurt or 50 grams of cheese, allow the intake of about 300 mg of calcium. However, alternative calcium sources are orange juice, some vegetables such as cabbage family or large leafy vegetables, spinach, legumes, and some cereals that contribute to the daily calcium intake by about 10%. However, vegetables having high calcium content, have reduced calcium bioavailability due to the high concentration of oxalates (Golden, Abrams, & COMMITTEE, 2014). In general, reducing the intake of dairy products below the recommended daily doses can hurt the bone not only due to the lack of calcium but also of other micronutrients such as phosphorus, potassium magnesium, or vitamins (B2 and B12, A, D) (Dror & Allen, 2014).

The effects of calcium alone and together with vitamin D supplementation on the bone health of children have been investigated in different trials using also twins children. Johnston and collaborators showed that in prepubertal twins, supplemented with 1612 mg of calcium daily for three years, there was an increase of BMD concerning the twin used as a control and supplemented with 908 mg of calcium despite the same intake of all nutrients and the equivalent level of physical activity (Johnston et al., 1992). A double-blind randomized control trial investigated whether there was a differential response to calcium supplementation in elite prepubertal gymnasts and school children controls. It was found that 1250 mg daily of calcium supplementation, with a low level of physical activity, had only a small positive change in tibia trabecular volumetric BMD in the control group. Moreover, it was found that there was no beneficial effect of additional calcium in prepubertal gymnasts who already consume their recommended nutrient intake of calcium (Ward, Roberts, Adams, Lanham-New, & Mughal, 2007). In a randomized trial, adolescent girls, aged 12 years, were enrolled and supplemented with daily calcium, 800 mg of calcium carbonate, and 400 IU of vitamin D for twelve months. Daily calcium and vitamin D supplementation promoted greater trabecular BMC and volumetric BMD acquisition in these preadolescent girls (Moyer-Mileur, Xie, Ball, & Pratt, 2003). Accordingly, Greene and collaborators confirmed that 800 mg of calcium and 400 UI of vitamin D supplementation, every day for 6 months, increased trabecular density, strength strain index, and increased tibial cortical area in female identical twins, aged 9 to 13 years (Greene & Naughton, 2011). However, a meta-analysis study showed that increased dietary calcium/dairy products, with and without vitamin D, significantly increased total body and lumbar spine BMC in children with low baseline calcium intakes (Huncharek, Muscat, & Kupelnick, 2008). So, it seems that calcium and vitamin D supplementation above the recommended nutrient intake has a modest influence on one, especially in active pre-pubertal children. On the other hand, calcium supplementation seems to be beneficial for the child population with low daily intake.

Children aged between 3 and 17 years old seem to take more than 50% of the recommended daily dose of calcium through dairy products as confirmed by studies carried out in France (2005-2007) and USA (Dror & Allen, 2014). The latter pointed out that children aged between 2 and 18 years old take about 950 mg/ day through dairy products (especially milk and cheese), which represents the main source of (Iuliano & Hill, 2019). The optimal quantity of phosphorus to take is in a ratio of 2:1 to calcium (in favor of the latter) since it could have the opposite effect. Sodium may also affect urinary calcium excretion, as both sodium and calcium compete for reabsorption in the renal tubules. It is estimated that for every 2300 mg of sodium excreted, approximately 50 mg of calcium is lost in women (Nordin, Need, Morris, & Horowitz, 1993). The influence of short-term calcium supplementation in adult athletes during exercise on bone remodeling has been investigated. Short-term supplementation with calcium, 60 minutes before physical activity, did not appear to affect bone resorption (V. Sherk et al., 2017), but this could be due to the quantity of supplementation or by the time of consumption before the trial. In fact, aA calcium supplement of 1000 mg, taken 30 minutes before exercise did not affect bone resorption in competitive adult male cyclists while 1350 mg of calcium taken 90 minutes before the exercise reduced bone resorption in competitive adult female cyclists (E. Haakonssen et al., 2015). Regards, vitamin D, the general recommended daily dose is 200 IU/day for children. Specifically, the Recommended Dietary Allowance (RDA) for children up to 1 year is 400 IU/day which increases up to 600 IU for children aged 1 year or older. Also, in this case, upper limits are set beyond which it could have a detrimental effect; specifically for children up to 6 months 1000 IU, from 6 to 12 months of age 1500 IU, from 1 to 3 years of age 2500 IU, from 4 to 8 years of age 3000 IU and from 9 and 18 years 4000 IU (Golden et al., 2014). However, it should be kept in mind that in addition to calcium intake, other factors affect bone health such as hereditary and environmental factors.

Dietary composition

Bone health could be more influenced by dietary long- or short-term changes rather than specific nutrients although there are insufficient studies to prove it.

The HELENA study failed to show, in Spanish adolescents, the association between Mediterranean Diet and bone mineral content (Julián et al., 2018) as well as Monjardino et al. (Monjardino, Lucas, Ramos, & Barros, 2014) found no association between forearm BMD and different dietary patterns. The work of Shin et al. observed that adolescents in the highest tertile with a dietary pattern rich in cereal and milk score significantly reduced chance of having low BMD compared to those in the lowest tertile (Shin, Hong, Kang, & Joung, 2013). The association between dietary patterns, physical activity BMC and BMD need to be further explored especially in children. About this issue, Muñoz-Hernandez et al showed that moderate to vigorous physical activity and less time for sedentary behavior seems to improve bone health in overweight or obese children with related poor adherence to the Mediterranean dietary pattern (Muñoz-Hernandez et al., 2018).

The review of Mariotti et collaborators, indicate that classic vegetarian diets provide more than adequate protein and amino acids concerning the dietary reference values, furthermore, children who are consuming sufficient energy to cover their necessities for growth should automatically reach sufficient protein intake and protein varies from vegetarian diets (Mariotti & Gardner, 2019). Taking into account that extreme dietary position a vegan diet or vegan who consumed only uncooked and unprocessed plant-based food might show lacking in micronutrients concentration, as Calcium, Vegans require calcium-fortified foods that in combination and variety may help meet their daily calcium needs (Brown, 2018). However, in adults, as well as in children, it is not clear enough the mechanism behind bone health and dietary intake therefore further studies are needed to clarify this complex mechanism.

The influence of physical activity on bone health

Regular physical activity during growth seems to be one of the most important factors influencing peak bone mass. According to the International Osteoporosis Foundation (IOF) about 22% of men and 46% of women aged 50 years, will experience osteoporotic fractures during the remainder of their lives (Hernlund et al., 2013a). Thus, what should be done to optimize peak bone mass is to maximize the increase in BMD during the first 25 years through appropriate physical activity, and to minimize the decrease in BMD after 40 years due to endocrine changes related to aging by regular physical activity. This easy strategy would reduce the occurrence of fractures later in life. However, so far, an exercise program for children and adolescents that will optimize peak bone mass in detail has not yet been defined.

The World Health Organization (WHO) has suggested that physical activity confers benefits for bone health in children and adolescents (Bull et al., 2020). Evidence comes from randomized small trials which have some limits like confounding factors inherent to the cross-sectional studies (e.g. type of exercise training, duration of the intervention, group of intervention) that make it difficult to figure out the osteogenic effects of physical activities. Many systematic reviews focus on bone strength in children and adolescents of both sexes about physical activity (V. Tan et al., 2014). Some of them reported changes in bone structure rather than bone mass linked to enhanced bone strength.

In general, moderate to vigorous physical activity was linked to positive bone outcomes especially in males although discrepancy in the methodology assessment made it difficult to establish the amount and type of physical activity that might lead to favorable bone outcomes and therefore that might exert the osteogenic action (Bland, Heatherington-Rauth, Howe, Going, & Bea, 2020). What is recognized to exert an osteogenic action are interventions that must include high-intensity exercise with enough ground reaction forces, to significantly increase bone mineralization and prevent osteoporosis and fragility fracture later in life. It was shown that high-impact jumping activities with ground reaction forces (GRFs) from at least 3.5 and 8.8 × BW (10 minutes for 2 to 3 times per week) are effective in increasing BMD and/or BMC in children and adolescents underling that the most important factor is the intensity and not the duration of the stimulus (Nguyen, 2018). Usually, the intensity of an osteogenic exercise was expressed as GRFs, the combination of the magnitude of force and speed by which it is applied (Economos et al., 2020; Nguyen, 2018). It was demonstrated that activities with the most osteogenic potential have GRFs greater than 3.5 times BW (per leg) with peak force occurring in less than 0.1 seconds (KB. Gunter, Almstedt, & Janz, 2012). Worthy of note is also a recent article about a project that assessed whether early elementary school children participating in 20 min of vigorous activity 3 to 5 days per week with 5 minutes of umping component (ground reaction forces between 4/ 7 times body weight) would improve bone quality and muscular strength (Economos et al., 2020). The study confirmed the effectiveness of the program. Thus, a higher intensity level of physical activity achieves positive effects on BMC, BMD, and accretion (Heidemann et al., 2013). The importance of vigorous exercises as a favorable predictor of bone strength was further confirmed. In particular, 1 h per day of moderate-to-vigorous physical activity was able to improve bone strength in six-year-old subjects (Consortia et al., 2020). Also, participation in unstructured weight-bearing physical activity had strong and consistent positive effects on bone development (KB. Gunter et al., 2012; V. Poitras et al., 2016) further confirms that the high level of physical activity intensity is associated with higher modification in bone parameters during childhood (Cardadeiro, Baptista, Ornelas, Janz, & Sardinha, 2012; Hasselstrøm et al., 2007). An evaluation study conducted from middle childhood to middle adolescence in more than 300 boys and girls in a 10-year longitudinal study, confirmed that high participation in moderate to vigorous physical activity during childhood led to bone strength benefits in late puberty and that improving bone BMC is necessary vigorous physical activity interventions (Janz et al., 2014). Thus, vigorous physical activity was associated with BMC at skeletal sites from childhood to adolescence, and the effect was not modified by maturity or age (Janz et al., 2014). Both engaging and maintaining high levels of vigorous physical activity participation during puberty in boys are associated with greater gains in bone mass and density (Marin-Puyalto et al., 2018). This is because at the age of 11-13 years old the growing bones of adolescents are more sensitive to mechanical loading than adult bones. Regarding the time spent on physical activity to achieve an osteogenic response, it was suggested that in adolescents about thirty minutes per day of vigorous physical activity have shown benefits for the femoral neck BMD(Gracia-Marco et al., 2011) (87) while 3 h per week could be enough to elicit an increase in bone mass (Vicente-Rodr??guez, 2006). Bone development is influenced by volume and intensity. Male adolescents (11-13 years) who participated in vigorous physical activity were positively correlated with increased whole body and lumbar spine BMC than sedentary, light, and moderate physical activity. Furthermore, the best benefits of BMC appear when 15 consecutive minutes of vigorous activity (like running) for those who did it from 5 to 15 minutes and who did it for less than 5 minutes. Therefore, with the same intensity of the stimulus (vigorous activity) those who carried out a longer duration showed better BMD than the rest of the subjects (Marin-Puyalto et al., 2019). However, it is important to note that not all activities have the same osteogenic effect on bone mass in children or adolescents. Such as in boys, long-term soccer participation, starting at a prepubertal age (Tanner stage 1-2) results in the greater acquisition of bone mass and a lower accumulation of body fat (Vicente-Rodriguez et al., 2004). The bone acquisition is higher also in adolescent male footballers compared to swimmers and cyclists (Gómez-Bruton et al., 2017; Vlachopoulos et al., 2017). This suggests that weight-bearing activities should be incorporated into the training of swimmers to develop a stronger skeleton during adolescence. This would allow for optimizing peak bone mass and reducing low bone status later in life. In addition, ground reaction forces during impact exercises, especially during pubertal growth, play an important role in maximizing bone mineral gain (KB. Gunter et al., 2012). Among these activities, gymnastics is particularly osteogenic for bone development in children because it is a high-impact activity and involves the subject at an early age during growth (Tournis et al., 2010). However, intense athletic activity in growing and maturing gymnasts often associated with inadequate dietary intake leads to relatively low-fat mass (Maïmoun, Georgopoulos, & Sultan, 2014) and consequently possible alteration of endocrine function (Malina et al., 2013). However, two major systemic reviews showed that prepubertal gymnasts have higher BMD and BMC than age-matched untrained controls. Therefore, gymnastics activities seem to be the one of most effective exercises for improving bone mineral gain in growing and maturing children (Burt, Greene, Ducher, & Naughton, 2013; Jürimäe, Gruodyte-Raciene, & Baxter-Jones, 2018). Thus, the positive effect on bone accumulation seems to outweigh the possible negative influence of other characteristics. What is important to underline is that the optimal timing of a physical activity intervention differs depending on the stage of maturity of the pediatric population. BMC increases linearly, with no sex differences until the onset of the pubertal growth spurt (Molgaard, Thomsen, Prentice, Cole, & Michaelsen, 1997; Weaver et al., 2016). Therefore, there are no differences in BMC and BMD between 6-year-old boys and girls (Moon et al., 2015) or younger subjects (3-5 years old) (Gómez-Bruton et al., 2020). However, some differences can be appreciated from the puberty phase onward at the tibial diaphysis the cortical bone increases in size more for boys than for girls (SA. Kontulainen, Macdonald, Khan, & McKay, 2005) therefore the BMD varies considerably for the same chronological age among boys and girls. The peak of growth rate occurred earlier but is smaller in females than in males and the female growth curve flattens before and with a lower peak than min en (Dunsworth, 2020). The mMalea longer prepubertal period of growth because their pubertal growth spurt occurs one/two years later than in girls (Cameron, Tanner, & Whitehouse, 1982; Seeman, 2001; Tanner, 1990). However, this can cause a transient period of increased porosity at the cortex during periods of rapid growth. Particularly boys demonstrate greater porosity than girls in this period (Nishiyama et al., 2012). Bone thickness remains relatively stable until late puberty, as endosteal apposition is unable to keep pace with the rapid periosteal resorption that dominates the process of metaphyseal investing during periods of rapid longitudinal growth (Gabel, Macdonald, & McKay, 2017). The lag of bone thickness growth may contribute to increased bone fragility and it could be a direct result of increased calcium demands, resulting in higher rates of intracortical bone turnover and increased porosity due to incomplete consolidation of bone (Parfitt, 1994). Looking inside the sexual difference in BMC during adulthood, Baxter-Jones and collaborators analyzed 30 years later, in a prospective study, 82 female and 72 male children (age range 8 to 15 years old) were both more and less physically active (Baxter-Jones, Kontulainen, Faulkner, & Bailey, 2008). Participants returned for follow-up at age 23 to 30 years (2002–2006) and the cohorts were divided into active, average, and inactive groups. When compared to the inactive group, active females and males had greater adjusted BMC. In young adulthood, the male and female adolescent active groups were still more active than their peers and had still greater adjusted BMC. It was concluded that the skeletal benefits of physical activity in adolescents are maintained into young adulthood. In conclusion, physical activity with certain characteristics seems to be better than others to improve bone mass. It should be dynamic (Gardinier, Mohamed, & Kohn, 2015) and vigorous (Cardadeiro et al., 2012; Consortia et al., 2020; Hasselstrøm et al., 2007; Janz et al., 2014; Marin-Puyalto et al., 2018) with impact and load (KB. Gunter et al., 2012) to have a strong and consistent positive effect on bone development. However, further research to better clarify the modulatory role of the different activities on bone health in children and adolescents are necessary to improve and maintain bone health.

Potential explanatory mechanism: PTH and gut peptides

Bone metabolism is influenced by hormones, such as the parathyroid hormone (PTH), which is essential for the maintenance of calcium homeostasis, but also by hormones produced by other peripheral districts like the gastrointestinal tract (Figure 1). What is known about the influences of these factors on the bone and how these are affected by nutrients and physical activity?

The parathyroid hormone (PTH) has multiple effects on the skeleton. PTH stimulates osteocytes, osteoblasts, and their precursors. The physiological function of PTH is to maintain extracellular fluid calcium concentration and to prevent hypocalcemia. Therefore, with a feedback mechanism, PTH production is closely regulated by serum calcium concentration: PTH secretion increases whenever

calcium concentration falls below normal and the hormone is accountable for the difference in blood calcium concentration directly influencing the bone rather than kidney metabolism.
The hormone can prevent hypocalcemia at the cost of progressive bone destruction and loss of bone mineral (Silva & Bilezikian, 2015).

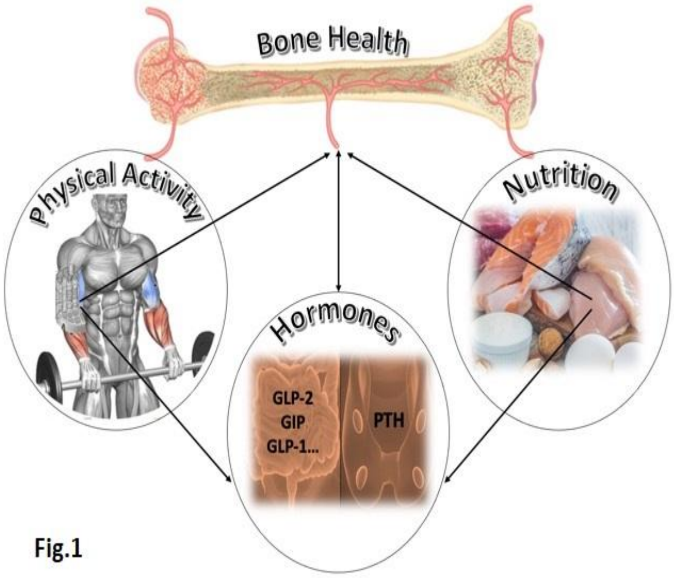


Figure 1. Potential key factors of bone homeostasis and remodeling: Bone is modulated by nutrition and by physical activity. Nutrition influences the release of hormones like gut peptides (GLP-1, GLP-2, GIP) and modulates the secretion of PTH. Both in turn influence bone remodeling. Physical activity, adequate in volume and intensity, could impact bone homeostasis by influencing hormones released by peripheral organs. Figure 1 is our own creation.

Despite the role of PTH on bone homeostasis and remodeling, there are few studies about changes in PTH secretion and actions in healthy young subjects. These studies are not recent and did not focus on the effects of macronutrients and physical activity on PTH secretion. One of these studies shows the opposite variations of PTH and 25 hydroxyvitamin D in a group of forty-two children living in south Argentina (Oliveri, Ladizesky, Mautalen, Alonso, & Martinez, 1993). The association between a high level of PTH, low levels of vitamin D, and reduced bone mass was confirmed in a letter study conducted in pubertal and prepubertal Finnish girls (Cheng et al., 2003). The secretion of PTH is modulated by vitamin D (Guillemant, Cabrol, Allemandou, Peres, & Guillemant, 1995) suggesting that micronutrients could affect PTH secretion. Thus, also macronutrients could modulate PTH secretion in adolescence. For example, lower PTH concentrations and beneficial effects on bone size were observed in early pubertal children that have high fruit and vegetable intakes (Tylavsky et al., 2004). This strongly suggests that nutrient supplementation influences PTH release in children and consequently bone homeostasis.

Physical exercise influences PTH release and increases PTH production suggesting a possible key role in bone formation and adaptation to its mechanical features (Lombardi, Ziemann, Banfi, & Corbetta, 2020). However, research into the effects of exercise on PTH expression and secretion is still limited and the study was conducted in adults. However, what was observed is that the increase in systemic PTH levels seems to depend on the type, intensity, and duration of exercise (Maïmoun & Sultan, 2009; J. Scott et al., 2011). It was demonstrated that bone adaptation during exercise is not only a function of the dynamic loading but also PTH release and that PTH signaling contributes differently at structural and tissue-levels (Gardinier et al., 2015). During exercise, there is an increase in calcium demand by the active muscle, but this increase is at the expense of bone mineralization at the periosteum (H. Frost & Schönau, 2000) In fact, calcium supplementation during exercise may reduce bone resorption markers in adult female cyclists (E. Haakonssen et al., 2015) and in general lessen the increase in PTH (V. Sherk et al., 2017). In postmenopausal women, the time of calcium supplementation also seems to be important for the PTH release (K. Shea et al., 2014). The excess of calcium during exercise may impair systemic PTH release with a feedback mechanism (E. Haakonssen et al., 2015). Further research is necessary to determine the effects of macronutrients and exercise alone and together on PTH secretion in children and adolescents to determine how this influences bone homeostasis and skeletal adaptations during growth. Gastrointestinal hormones have recently been seen to influence bone metabolism. The relationship between hormones secreted by the gut and bone is a recent study and opens the way to new interesting fields of research.

Bone and gut are strictly connected and gut hormones respond to food intake triggering bone resorption. Bone resorption is increased during the night compared to the day, and diurnal suppression is eliminated by fasting, confirming the role of gastrointestinal hormones in controlling bone homeostasis (S. P. Schiellerup et al., 2019b)

Among the gut hormones Gastric Inhibitory Peptide (GIP), glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2) are known to be involved in the regulation of bone turnover. These hormones have been extensively studied for their effects on glucose and lipid metabolism (S. Baldassano, Accardi, & Vasto, 2017a; S. Baldassano, Amato, & Mulè, 2016; S. Baldassano et al., 2020b; S. Baldassano et al., 2019b; Chon et al., 2017; Y. Song et al., 2019) and are of interest in the light of the interplay between bone and glucose metabolism that seems to be compromised in diabetes (Cipriani et al., 2020). The exact mechanism of action of these peptides on bone is unclear. The GLP-1 and GLP-2 receptors are expressed in the immature human osteoblast cell lines MG-63 and TE-85 (E. Pacheco-Pantoja, L. Ranganath, J. Gallagher, P. Wilson, & W. Fraser, 2011) but GLP-2 has not been identified in human osteoclasts, or any other bone-related cell type despite the impact of GLP-2 on osteoclast activity. GLP-2 is co-secreted with GLP-1 by L cells in the small and large intestines and was initially studied for its ability to stimulate mucosal growth and nutrient absorption in the intestine (S. Baldassano & Amato, 2014a). GLP-2 treatment is associated with the reduction of serum and urinary markers of bone resorption in postmenopausal women, while bone formation appears not to be affected by (J. Clowes et al., 2002; D. Henriksen et al., 2003). The GIP receptor is expressed in both osteoblast and osteoclast-derived cell lines and increases the expression of type 1 collagen. It maintains osteoblast homeostasis with an anabolic effect on bone. It also has an inhibitory effect on the bone resorption activity of PTH (Jouret et al., 2013).

This information suggests that gut hormones could impact bone health and affect bone quality. Thus, more studies are required to investigate the effects of diet and physical activity on the secretion of gut peptides in children and the modulatory role, if any, in assure an optimal skeleton formation

Conclusion

Several studies have investigated in adulthood the best strategies to maintain good lean mass and bone mass by modification of diet and physical activity (Dawson-Hughes, Harris, Rasmussen, Song, & Dallal, 2004; Gómez-Cabello, Ara, González-Agüero, Casajús, & Vicente-Rodríguez, 2012; Messina, Amato, D'Amico, Baldassano, & Proia, 2019; Senderovich & Kosmopoulos, 2018; R. Zhao, Zhang, & Zhang, 2017). Most of the adult studies have focused on osteoporosis risk subjects, post-menopausal women, and have analyzed the effect of minerals (mainly Calcium), vitamin supplementation (mainly vitamin D), and nutrients, especially protein supplementation (Daly et al., 2014). There are few studies on childhood and there are many questions to answer. The skeleton is not an inert structure playing a supporting role for muscles and a protective role for inner organs like the brain. It is able not only to regulate its physiology but to influence energy metabolism by continuum interplay with the peripheral organs like the adipose tissue-derived hormones, the gastrointestinal tract-derived peptides, and insulin, and by also producing bone-derived peptides like osteocalcin and lipocalin-2. Nutrition and physical activity may influence the release of these factors in children. How this could impact bone health in children and adolescents is not completely known. Thus, studies that will address questions about the complex interplay between bone, gut, white and brown adipose tissue, nutrition, and physical activity are required to provide near future new insight into this fascinating topic of metabolic endocrinology.

2.2 Analysis of body perception, pre-workout meal habits, and bone resorption in children gymnasts.

[From Amato, Proia et al. (2021)]

Introduction

Preadolescence represents a period of growth and the development of the body and per- sonal identity. These changes, which lead to adolescence, especially if rapid, can affect brain health such as self-esteem, body image perception, and bone homeostasis. Physical activity helps to increase body self-acceptance (Lubans et al., 2016) and bone growth in children (Bjarnason et al., 2002). However, aesthetic sports, such as artistic gymnastics, are considered risk factors for the development of distorted body image, eating disorders, and osteoporosis(Mora & Gilsanz, 2003; Salbach, Klinkowski, Pfeiffer, Lehmkuhl, & Korte, 2007). Athletes' appearance is judged in addition to their performance, and the physical appearance of gymnasts could be the result of a combination of intense physical activity and undernutrition, which could lead to a lean, almost anorexic physique(Klinkowski, Korte, Pfeiffer, Lehmkuhl, & Salbach-Andrae, 2008). The problem is that more than fifty percent of total bone mineral content is obtained during preadolescence, which represents the time to achieve an optimal bone mass (Lloyd et al., 1992). Excessive energy expenditure and inadequate nutrition in children with intensive training programs may affect body self-acceptance (Biddle, Ciaccioni, Thomas, & Vergeer, 2019), modify growth during puberty (Roemmich, Richmond, & Rogol, 2001), promote bone injuries and predispose children to osteoporosis in older age (Hart, Meehan, Bae, d'Hemecourt, & Stracciolini, 2018). The latter is because osteopenia and osteoporosis during adulthood, seem to be related to the degree of bone mineralization during childhood and adolescence (Santos, Elliott-Sale, & Sale, 2017). Adequate physical activity (Alice Iannaccone et al., 2020) and dietary habits with the supply of health-promoting substances are the basis of a healthy diet (Sara Baldassano et al., 2018; S. Baldassano, Accardi, & Vasto, 2017b). Therefore, correct dietary habits could be useful to maintain body weight and consequently improve body self-acceptance. How- ever, weight-category sports and aesthetic sports, such as gymnastics or dancing, are associated with a greater risk of developing disordered eating (Bratland-Sanda & Sundgot-Borgen, 2013; Lewiecki et al., 2008; Sundgot-Borgen et al., 2013). A positive body image seems to be linked with more favorable eating attitudes and behaviors (Tylka & Wood-Barcalow, 2015). Men usually have greater body appreciation compared to women (He, Sun, Zickgraf, Lin, & Fan, 2020; Swami, Weis, Barron, & Furnham, 2018). However, especially in weight-sensitive sports, in which body weight has a high impact on performance (Francisco, Alarcão, & Narciso, 2012), understanding the influence of a positive body image in the prevention of disordered eating in depth is a central issue. The few studies conducted on athletes have reported a more favorable body image of athletes compared to nonathletes ("Comparison of body image between athletes and nonathletes: a meta-analytic review," 2001; Soulliard, Kauffman, Fitterman-Harris, Perry, & Ross, 2019; Voelker, Petrie, Huang, & Chandran, 2019), and this seems also to improve confidence in sports and the state of flow in physical activity, leading to more successful sports performance (Soulliard et al., 2019). Moreover, less attention has been paid to the associations between a positive body image and physical activity in adolescents (Andrew, Tiggemann, & Clark, 2016). In general, what is known is that participation in physical activity seems to be associated with a more favorable body image (Bassett-Gunter, McEwan, & Kamarhie, 2017; Sabiston, Pila, Vani, & Thogersen-Ntoumani, 2019). Sportive adolescents have greater body image perception compared to nonexercisers (Goodwin, Haycraft, & Meyer, 2016; Jankauskiene & Baceviciene, 2019). It has also recently been shown that participants of leisure and competitive sports reported greater body appreciation, self-esteem, and lower body dissatisfaction compared to nonparticipants (Cox, Ullrich-French, Tylka, & McMahon, 2019; Jankauskiene, Baceviciene, & Trinkuniene, 2020; Neumark-Sztainer, MacLehose, Watts, Pacanowski, & Eisenberg, 2018). Self-esteem is considered a multidimensional concept that includes self-esteem domains such as school, work, social and emotional spheres, and family (Harter, 1999; Rotatori, 1994). There is a close and reciprocal relationship between self-esteem and body image observed starting from primary school children (Donnellan, Trzesniewski, Conger, & Conger, 2007) that is also confirmed in adults (Birkeland, Melkevik, Holsen, & Wold, 2012; Olchowska-Kotala, 2018). Thereafter, a positive body image is associated with greater self-esteem and proactive coping (Avalos, Tylka, & Wood-Barcalow, 2005). The self-evaluations of physical appearance contribute in a significant way to global self-esteem. Therefore, positive perceptions of body acceptance can be generalized to global self-esteem improvements (van de Grift, Cohen-Kettenis, de Vries, & Kreukels, 2018). Thus, it is possible to evaluate the self-esteem of subjects through their body image perception (Rotatori, 1994). Children need to have an adequate pre-workout meal because nutrient intake induces acute suppression of bone resorption within two hours following the meal (Clowes, Khosla, & Eastell, 2005). The international society for clinical densitometry suggests the use of bone turnover markers to monitor acute and chronic bone adaptation in children (Lewiecki et al., 2008). The C-terminal telopeptide region of collagen type 1 (CTX) is a very sensitive marker of bone resorption (de la Piedra et al., 1999). Unfortunately, data on these biomarkers are very limited in healthy preadolescent children. In particular, information on their change in response to variation in nutrient composition, following physical activity, in preadolescents, is limited. Therefore, the objective of the study was to investigate the association between artistic gymnastics and a more favorable body image of young gymnasts compared to sedentary peers and if a high-carbohydrate (HCM) or high-protein (HPM) preworkout meal can attenuate bone resorption in preadolescent rhythmic gymnasts. Moreover, the variables inherently related to body image, such as preworkout eating habits (type of meal consumed, the frequency of consumption, and the timing of consumption), were investigated. Based on the previous finding, it was supposed that gymnasts would be associated with more favorable body image perception. They would also have attenuation in bone resorption related to the macronutrient composition of the preworkout meal.

Materials and Methods

Study Population, Participant Characteristics, and Meals

Twenty-eight prepubertal girls participated in the study. The children's inclusion in the study was based upon the following criteria: (1) taking part in artistic gymnastics at a precompetitive level for at least four years and 12 h/week; (2) individuals were at the premenarcheal period; (3) individuals were Caucasian females aged 9–12 years old; (4) individuals were clinically healthy; (5) individuals were not receiving medication known to affect bone metabolism; (6) individuals had no history of immobilizing surgery or fractures; (7) individuals were on a maturity level of 1 or 2 on the tanner scale. The participants were not involved in other sports or recreational activities (e.g., dancing, biking) organized by the local community at the time of the study. To verify that they were clinically healthy, the subjects provided a health certificate for competitive sport activity. The certificate is released by their physician following a medical examination, urine test (urinalyses), electrocardiogram at rest and under stress, and spirograph (diagnostic test under Italian law to be able to practice competitive sports activities (Ministerial Decree 18/02/1982)). Candidates for the study were excluded if they had a chronic illness and/or if they were taking medications, vitamins, or mineral supplements. The parents/guardians of forty-two girls were approached initially, and thirty-eight consented to participate in the study. Ten girls were excluded because they did not meet the inclusion criteria. As a control group (C), for the analysis of body perception, we used a sample of 28 schoolgirls (average age = 10.62 0.7 years) attending schools in the same region (Palermo districts). The inclusion criteria for the control group were: (1) being female; (2) being within the same age range as gymnasts; (3) having completed all the questions measurements; (4) not practicing any sport. The exclusion criteria were the same as above. The gymnast group was divided into two groups, with 14 girls assigned to the high-protein meal (HPM; 300 kcal, 55% carbohydrates, 31% protein, 13% fat) group and 14 assigned to the high-carbohydrate meal (HCM; 300 kcal, 88% carbohydrates, 9% protein, 3% fat) group. The HPM consisted of bread, baked ham, and orange juice, and the HCM consisted of cereals and orange juice. Participants were provided with a 500 mL bottle of water to consume ad libitum. In this way, the amount of water introduced by the subjects was fixed. The energy and nutrient content of the meals are described in Table 1. The gymnast (both the HPM and HCM groups) and control groups are presented in Table 2.

Table 1. Energy and nutrient content of the meals.

		Carbohydrates (g)	Proteins (g)	Fats (g)	Kcal
HPM	Bread (45 g)	16.5	1.8	0	118
	Baked ham (79 g)	0.3	15.8	7.2	133
	Orange juice (180 mL)	16.3	1.3	0.45	79
	Total	33	19	7.7	330
		Carbohydrates (g)	**Proteins (g)**	**Fats (g)**	**Kcal**
HCM	Cereals (60 g)	49	5.5	1.4	242
	Orange juice (200 mL)	18.2	1.4	0.5	88
	Total	67.2	6.9	1.9	330

High-protein meal (HPM); high-carbohydrate meal (HCM); grams (g).

Table 2. Characteristics of the gymnast (both HPM and HCM) and control groups.

High-protein meal (HPM); high-carbohydrate meal (HCM); standard deviation (SD). Values are means ± SD. *n* = 14 in each group.

Group	Age (Years)		Weight (kg)		Height (cm)		BMI (kg/m^2)		Fat Mass (%)		Free Fat Mass (%)		Bone Mineral Density (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HPM group	10.2	1.2	35. 0	7.2	144	0.1	16.5	1.9	16.4	2.3	83.5	2.3	9.3	0.1
HCM group	10.7	1.0	38.5	9.6	148	0.1	17.2	2.8	17.1	3.0	82.8	3.0	9.4	0.1
Control group	10.62	0.7	39.1	3.6	148.5	1.6	17.9	1.6	-	-	-	-	-	-

The groups did not differ in age, weight, height, BMI, body composition, or training characteristics. Specifically, the years of training were 5.4 ± 1.7 for the HPM group and 4.1 ± 1.1 for the HCM group, and both groups performed artistic gymnastics 12 h/week.

Ethics

The study was approved by Ethics Committee Palermo 1, Policlinico Giaccone Hospital, Palermo, Italy (Decision Number: 2/2020–19/02/2020) and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from the parents or legal guardians of each child, and each child gave verbal consent to participate in the study. The parents/guardians provided a full health history questionnaire, and the girls underwent a thorough physical examination and a structured interview.

Multidimensional Test of Self-Esteem for Body Image Perception

To evaluate body image perception, a subscale of the multidimensional test of self-esteem (Rotatori, 1994) was used. The subscale evaluates how a child feels about themselves regarding their body appearance (e.g., size, hair, skin, etc.) and consists of 25 Likert-type items, with positive and negative statements. The test was administered to the control group and the gymnast group. In the analysis of the multidimensional test of self-esteem, the control group was compared to the gymnast group (which included the gymnasts of both HPM and the HCM groups). Participants were asked to rate their agreement or disagreement with statements on a 4-point scale using anchors of absolutely true and absolutely false. The score assigned was from 4 to 1 point in the positive statements, from "absolutely true" to "absolutely false," and from 4 to 1 point in the negative statements, from "absolutely false" to "absolutely true." Higher scores indicated a higher level of body esteem.

Short Food Frequency Questionnaire

The participants were interviewed in the presence of their parents using validated questionnaires designed to report food frequency in children (Lillegaard, Overby, & Andersen, 2012; Vilela, Severo, Moreira, Ramos, & Lopes, 2019) adapted to pre-exercise eating habits. The FFQ contained questions about each of the following food items or food groups: fruit, potato chips, homemade cake, fruit drink, milk, yogurt, orange juice, savory snacks, and sweet snacks. The frequency scales used were never, once a week, 2–3 times per week, and always. One question referred to the time of consumption of the meal before the training section (90, 60, 30 min, or just before physical activity). The question about the habit of eating a pre-exercise meal required a yes or no answer.

Anthropometric Measurement

Body weight and body composition (lean mass, fat mass, bone mineral density) were measured after an overnight fast on an electronic scale (Gima 27088; Gima, Italy) calibrated to the nearest 0.1 kg. Barefoot standing height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer (Gima 27335; Gima, Italy). The coefficients of variation for the serial measurements of weight and height were 0.95 and 0.98, respectively. Body mass index (BMI) was calculated as weight, in kilograms, by squared standing height, in meters.

Bone Resorption Experimental Protocol

To avoid interference due to the circadian rhythm of excretion of the markers, the samples were collected at 8:00 in the morning (Aoshima et al., 1998; J. A. Clowes et al., 2002). A flow chart of the experimental protocol is described as miniaturized in Figure 1A. After overnight fasting, at 0 min, the first urine sample was collected. Then, the gymnasts consumed the assigned pre-exercise meal. Ninety minutes after the meal, before starting the training section, the second sample of urine was collected. Therefore, a sports session competition was simulated. At 180 min, after the end of the session, the third sample of urine was collected. At 240 min, 60 min after the end of the training session, the last sample of urine was collected. Samples were immediately frozen and kept at −80 °C until assayed.

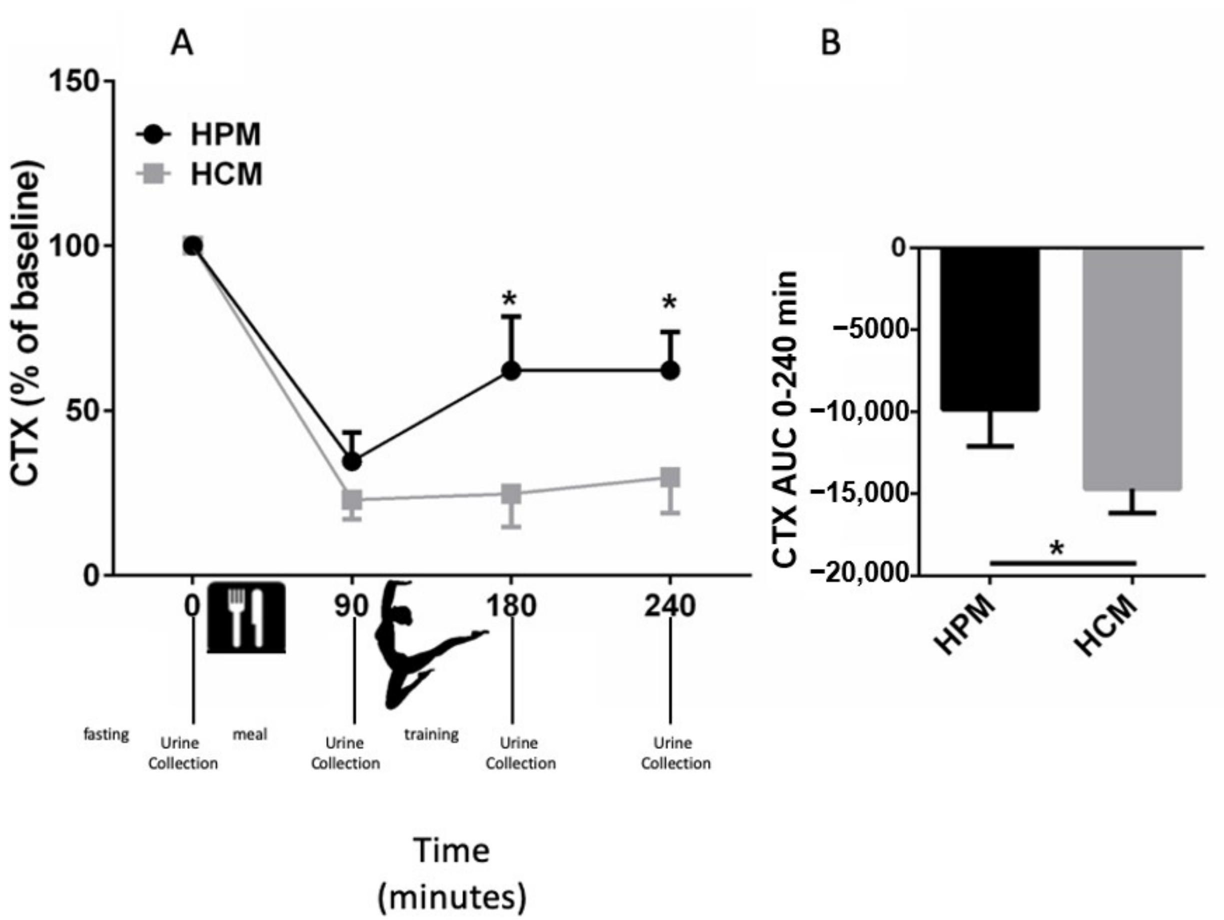


Figure 1. Effects of the pre-exercise meals on collagen type 1 (CTX) levels in female child artistic gymnasts. Urine samples were collected at 0, 90, 180, and 240 min. The meal was consumed after the first urine sample was collected (0 min). The duration of the sports session

The competition was 90 min and was simulated from time 90 to time 180 min. (**A**) Effects of the HPM and HCM on CTX levels. Mean CTX levels are shown as percent of basal level (**B**) Mean area under the curve (AUC) 0 to 240 min of CTX. Error bars depict SEM. Asterisks (*) depict statistically significant differences. Statistical significance is set at $p < 0.05$. $n = 14$ in each group.

Biochemical Measurements

Human cross-linked C-terminal telopeptides of type I collagen (ß-CTX) were measured by an enzyme-linked immunosorbent assay (Catalog Number: EH3989, Fine-Biotech), as previously reported by Baldassano et al. (S. Baldassano et al., 2019c). Assay values were corrected for urinary dilution by urinary creatinine concentration. The intra- and interassay coefficients of variation were <8% and

<10%, respectively. All samples were analyzed in duplicates in the same assay to prevent. interassay variation. Urinary creatinine was determined by a standard colorimetric method (Catalog Number: MAK080, Sigma-Aldrich, St. Louis, MO, USA).

Statistical Analysis

We calculated that a minimum of eight participants would be necessary to detect a difference of 20% in CTX with an SD of 12%, a power of 90%, a two-sided significance level of 5%, and an effect size of 80%, according to previous studies (Askov-Hansen et al., 2013; Gottschalck, Jeppesen, Holst, & Henriksen, 2008; Skov-Jeppesen et al., 2019). The CTX results are expressed as a percentage of the fasting level. Differences at time points between groups were analyzed by two-way repeated-measures ANOVA with a Sidak test for multiple comparisons. Areas under the curve (AUCs) were calculated with y = 100% as the baseline. The body image perception results were analyzed by the Student's t-test. Calculations and graphs were all made in GraphPad Prism 6 (GraphPad Software). Data are shown as averages and SEM. Differences resulting in a value of $p < 0.05$ were considered statistically significant.

Results

The self-Esteem of Body Image

The investigation regarded whether artistic gymnastics, performed at a pre-competitive level, influenced body image perceptions compared with sedentary peers. The data analysis revealed significant differences in body self-esteem between the control group and gymnasts. In particular, the gymnast group showed higher body esteem than the control group (Figure 2).

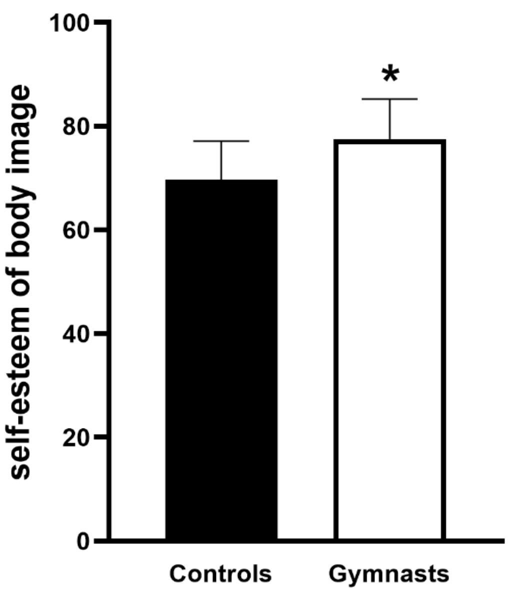


Figure 2. Body image perception of the gymnast group (which included the gymnasts of both the HPM and HCM groups) compared with the control group (the control group consisted of sedentary peers). A subscale of the multidimensional test of self-esteem was used to analyze the self-esteem of body image. Error bars depict SEM. Asterisks (*) depict statistically significant differences. Statistical significance is set at $p < 0.05$; $n = 28$ in each group.

Pre-Exercise Eating Habits

The frequency of food consumption among young gymnasts was investigated before the training section. Of the young gymnasts, 68% ($n = 19$) regularly consumed a pre-exercise meal before physical activity, and 25% ($n = 7$) of the girls had a pre-exercise meal 2–3 times/week. Only two gymnasts (7%) did not have any pre-exercise meal. The timing of meal consumption before work-out was also examined. Of the children, 39% ($n = 10$) consumed a meal ninety minutes before physical activity, 34% ($n = 9$) of the children had a meal thirty minutes before the training session and 25% ($n = 6$) of the gymnasts consumed the food sixty minutes before the training section. Only one girl (2%) had the meal just before the beginning of the workout. The gymnasts usually prefer to eat fruit ($n = 18$; 64.3%), drink fruit juice ($n = 20$; 71.4%), and eat sweet snacks ($n = 18$; 64.3%); on the other hand, they preferred a milk-based meal or savory snack at least 2–3 times per week before physical activity (Table 3).

Table 3. Consumption of the 8 food items included in the short food frequency questions (FFQ).

Food Item	Never		1/Week		2–3/Week		Always	
	n	%	*n*	%	*n*	%	*n*	%
Fruit	1	3.6%	3	10.7%	18	64.3%	4	14.3%
Potato chips	2	7.1%	4	14.3%	2	7.1%	1	3.6%
Homemade cake	2	7.1%	1	3.6%	2	7.1%	0	0.0%
Fruit drink	1	3.6%	3	10.7%	20	71.4%	2	7.1%
Milk	2	7.1%	4	14.3%	3	10.7%	2	7.1%
Yogurt	2	7.1%	4	14.3%	4	14.3%	2	7.1%
Savory snacks	2	7.1%	4	14.3%	4	14.3%	2	7.1%
Sweet snacks	1	3.6%	4	14.3%	18	64.3%	3	10.7%

Data are expressed in percent of the frequent consumption of a single item in the week. *n* refers to the number of answers for each item.

Effects of the Pre-Exercise Meal on Bone Resorption

The bone resorption marker CTX was elevated during fasting, and it was reduced after the consumption of the meals in both groups (Figure 1A, B). In particular, the effect of the HPM on bone resorption was transient, with a maximal drop in the CTX levels from the baseline at 90 min after the meal followed by a rise in CTX levels at 180 and 240 min (Figure 1A). The ingestion of the HCM also induced a maximal reduction in CTX levels 90 min after the meal. The reduction of the bone resorption marker CTX was more lasting. The effect persisted at the end of the training section and 60 min postexercise (Figure 1A), and it was significantly different in comparison with the HPM, as shown also by the AUC at 0–240 min (Figure 1B).

Discussion

Sports have a positive influence on children's perception of body image (Lepage & Crowther, 2010; Rinaldo, Zaccagni, & Gualdi-Russo, 2016). Phys- ical activity may contribute to the formation of children's personalities, to their proper perception, self-esteem, and psychological and physical well-being (Biddle et al., 2019; V. J. Poitras et al., 2016). However, so far, for children engaged in aesthetic sports, it is unclear. They may have a different ideal body image because they are subjected to stronger pressure regarding their body appearance than in other sports (Francisco et al., 2012; Lombardo, Battagliese, Lucidi, & Frost, 2012). Thus, the first purpose of this study was to assess the body image perception of premenarcheal gymnasts compared to a control group of schoolgirls. It was shown that artistic gymnasts had a positive influence on self-esteem. In particular, gymnasts were associated with a higher body perception compared with sedentary peers. Our data are in agreement with previous studies, which reported greater body satisfaction in gymnasts than in the general population (Paolo Borrione, 2013), even in young gymnasts(Zaccagni, Rinaldo, & Gualdi-Russo, 2019). A previous study has reported that the self-esteem of gymnasts 6–13 years old, competing at a school level, was significantly lower than that of their peers participating at a recreational level (AmaÁ, Anastasio, Morwick, & Yi, 2002). The discrepancy with our results may be explained because, in their study, a sample of both child and adolescent gymnasts was studied. Thus, the heterogeneity of the age range may have affected the findings of the study. No difference in self-esteem and body perception was found in athletes ("Comparison of body image between athletes and nonathletes: a meta-analytic review," 2001). However, body image dissatisfaction in gymnasts changed over time (Trevithick, Stuelcken, Mellifont, & Sayers, 2018), as former gymnasts had more positive body esteem than current athletes because they suffered more pressure to be thin from their parents. Thus, to better understand the issue, it would be interesting to follow a group of young gymnasts in a long-term study to see if changes could occur and to what extent they would have an impact on the self-esteem of body perception, dietary habits, and osteoporosis. The latter because se it is recognized that physical activity and nutrition influence bone remodeling (Amato et al., 2018a), but there is a lack of information on whether the composition of the meal and the timing of consumption is important for reducing acute bone resorption in children. The results of the study suggest that the consumption of a pre-exercise meal rich in carbohydrates 90 min before the training session attenuates bone resorption in prepubertal female gymnasts concerning a meal rich in proteins. To our knowledge, this is the first study that investigates the effects of pre-exercise meals on bone resorption in children, specifically in pre-puberty gymnasts. The pronounced bone resorption reduction observed in children following the consumption of the HCM, during the time course, suggests that the manipulation of the nutritional composition of the meal influences bone in the short term. During acute exercise, bone metabolism is unbalanced toward resorption (Lombardi, Ziemann, & Banfi, 2019). The marker of bone resorption was measured at four different time points to provide an overview of the changes in CTX levels over time. CTX was measured in urine because of the young age of the participants. A fixed quantity of water was provided to the participants to avoid differences in the hydration status and consequently urine concentration that could impact the study. The effect of meal consumption on bone resorption was analyzed in the morning, after overnight fasting, on the same day, and at the same time for all the participants. This was to avoid the day-to-day and within-day variability to which markers of bone turnover are subject, which may influence the interpretation (J. A. Clowes et al., 2002). The gymnasts consumed two different pre-exercise isocaloric meals. In particular, the HCM was rich in carbohydrates. It provided 88% carbohydrates, 9% protein, and 3% fat. The HPM provided 55% carbohydrates, 31% protein, and 13% fat. The meals were designed by considering recommended values for nutrients for young athletes, considering that children require more energy than adolescents or adults during sports activities (Benardot, Schwarz, & Heller, 1989), and the reproducibility for parents at home. Thus, the consideration is to provide a meal easy to prepare, with high palatability for young gymnasts that can be consumed easily by children. It was observed that the consumption of both HCM and HPM before exercise affects acute bone resorption. In fact, in response to feeding, there was an acute drop in resorption, seen as a decrease in CTX levels, in comparison to the fasting state. The ability to reduce the marker of bone resorption was evident with both HCM and HPM and in line with previous studies, in which it has been shown that ingestion of mixed meals, glucose, and protein results in a reduction in bone turnover markers (Bjarnason et al., 2002; Clowes, Allen, Prentis, Eastell, & Blumsohn, 2003; J. A. Clowes et al., 2002). Thus, regarding the mechanism of action for the observed effects, it is possible to speculate on the involvement of the entero-osseous axis, which coordinates bone resorption in response to nutrient intake. Both carbohydrates and protein can differentially stimulate the release of gastrointestinal hormones, such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-2 and 1 (GLP). These gastrointestinal hormones are secreted within a few minutes after the meal (S. Baldassano & Amato, 2014b)and affect acute bone remodeling by significantly reducing bone resorption assessed by the marker of bone resorption CTX (Christensen et al., 2018; D. B. Henriksen et al., 2003; Nissen et al., 2014; S. P. Schiellerup et al., 2019a). Boneresorption was measured ninety minutes after the meal, and it was more pronounced than that reported in adults in response to oral ingestion and measured for serum and urinary markers of bone resorption (Clowes et al., 2005). Specifically, in the experimental design it was observed a reduction in urinary CTX was by about 77% following the HCM and a reduction by about 62% following the HPM, whereas in adults, it was up to 50% following the meal and glucose and protein consumption camp(J. A. Clowes et al., 2002; Clowes et al., 2005). We believe that the difference observed in bone resorption in children compared with that previously reported in adults following the meal may be due to the different ages of the participants and suggests that children feeding heavily reduced acute bone resorption. Bone resorption changes as a function of age, gender, and puberty but is high in children and adolescents compared with adults (Mora et al., 1998). Moreover, it seems to be influenced in the long term by specific activity; for example, by high-intensity physical activity such as rhythmic gymnastics (Munoz, de la Piedra, Barrios, Garrido, & Argente, 2004). However, further studies will be necessary before definite conclusions can be drawn. Therefore, whether the consumption of the pre-exercise meal was able to affect bone resorption differently overtime was investigated. It was found that HCM, consumed one hundred and eighty minutes before, was still able to decrease CTX levels by about 75% at the end of the training session. Moreover, the HCM after two hundred and forty minutes was still effective in reducing the marker of bone resorption by about 70%. The reduction in CTX levels following the HPM was significantly different and less efficient rather than after the HCM. In particular, the HPM was effective, after ninety minutes of training, in decreasing CTX levels only by about 38% at the end of the workout in the young gymnasts. However, the effect of the HPM, although less pronounced than the HCM, was persistent over time. The HPM was still effective in reducing bone resorption by about 38% sixty minutes after the conclusion of the training section. These results confirm that the consumption of a pre-exercise meal with high carbohydrates reduced bone resorption in children as previously shown in adult athletes involved in an eight-day overloaded endurance training trial and individuals during and immediately after a 120 min treadmill run (de Sousa, Pereira, Fukui, Caparbo, & da Silva, 2014; Sale et al., 2015). In consideration that boneresorption is highly responsive to nutrient ingestion (D. B. Henriksen et al., 2003; Holst et al., 2007; Walsh & Henriksen, 2010) and that dietary habits could have an impact on body weight and consequently on body image perception, frequency, the timing of meal consumption and meal type consumed before physical activity were analyzed by using a short FFQ. We found that about 30% of the children do not have a regular pre-exercise meal. In addition, the difference in the timing of meal consumption was wide, ranging from 90 to 30 min before the training section. However, 96% of the girls consumed the pre-exercise meal within this time. Less than 5% of the gymnasts consumed the meal just before physical activity. The pre-exercise meal was for most of the children a fruit juice, fruit, or sweet snack. The percentage of children that consumed milk or yogurt was low, although, at a young age, physical activity and dairy consumption influenced bone mass positivity (De Smet et al., 2015). Overall, the information collected on the eating habits in these groups of children that perform an aesthetic sport suggests that the variability in preworkout meal frequency and timing needs attention to prevent inadequate eating habits. Previous studies on adolescents have demonstrated that sports-involved subjects report greater body image compared to sedentary peers (Francisco et al., 2012; Rinaldo et al., 2016). This study, although being conducted on a small sample, may contribute to shedding more light on the association between positive body image and sports. The study of the factors influencing the development of a positive body image at a young age is lacking, even though of primary importance. A positive body image is associated with more favorable eating and weight control-related attitudes and behaviors (Tylka & Wood-Barcalow, 2015), whereas body dissatisfaction is associated with disordered eating and overweight development in future life (Bratland-Sanda & Sundgot-Borgen, 2012; Cunningham et al., 2019; Dakanalis et al., 2015; Neumark-Sztainer, Wall, et al., 2018; Rohde, Stice, & Marti, 2015; Sharpe et al., 2018; Wu et al., 2019). Thus, the findings of this study might have practical implications. In fact, by doing sports, specifically artistic gymnastics, children might develop a positive body image. Moreover, artistic gymnastics, by helping to cultivate a more favorable relationship with their bodies in preadolescence, might be used as a tool to prevent poorer psychological health and disordered eating, which are associated with a greater risk of osteoporosis and overweight development. For this reason, cultural and social interventions should be programmed, starting from school, and involving the whole family to promote sports practice along with adequate nutrition. This kind of intervention may be the seed to promote the growth of a healthier population shortly.

Conclusions

In conclusion, this study showed a positive association between participation in aesthetic sports and specifically artistic gymnastic, with components of mental health and emotional well-being and in particular self-esteem of body perception. Furthermore, it was shown that a pre-exercise high-carbohydrate meal can reduce significantly bone resorption compared to a high-protein meal in gymnasts, suggesting that preworkout meal composition should be considered as a factor that preserves bone health in children.

2.3 Is the Secret in the Gut? SuperJump Activity Improves Bone Remodeling and Glucose Homeostasis by GLP-1 and GIP Pep-tides in Eumenorrheic Women
[From Vasto, Amato et al. (2021)]

Introduction

The gastrointestinal tract is the body's largest endocrine organ secreting hormones which in turn regulate whole-body homeostasis. Therefore, many dysmetabolic conditions like insulin resistance or higher risk of fractures are accompanied by altered secretion of gut peptides (S. Schiellerup et al., 2019; Zouhal et al., 2019). Gastrointestinal secretion of gut peptides is stimulated by nutrients when these reach the intestinal L cells but levels of the gut hormones seem to be influenced by an exercise bout (Broom et al., 2009; Burns, Broom, Miyashita, Mundy, & Stensel, 2007; King, Wasse, et al., 2010; Martins et al., 2007) suggesting that physical exercise could modulate gut peptides release.

There is a thigh link between gut peptides and bone. In fact ingestion of a meal induces secretion of gut peptides that act by decreasing bone resorption (S. P. Schiellerup et al., 2019b). The responsible gut peptides appear to include glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), Glucagon-like peptide-2 (GLP-2), peptide YY (PYY), and ghrelin (Proia et al., 2021). GLP-1 and GIP are also known as incretin hormones due to their role in regulating glucose homeostasis by acting on insulin release. However, the other gut peptides as GLP-2 and PYY influence glucose metabolism (S. Baldassano et al., 2017a; S. Baldassano & Amato, 2014a; S. Baldassano, Amato, Caldara, & Mulè, 2016; S. Baldassano et al., 2020a; S. Baldassano et al., 2019a). Physical exercise is indispensable to improving bone health (Amato, Baldassano, Cortis, Cooper, & Proia, 2018b; Amato, Proia, et al., 2021) and glucose metabolism (Færch et al., 2015; Fäldt et al., 2004). It can even replace glucose-lowering medication (Martins et al., 2007). However, so far, how exercise improves glucose homeostasis in humans is not fully understood and the influences of exercise on beta cell adaptations remain to be clarified.

SuperJump is an innovative activity performed on the elastic minitrampoline that can be used to be fit, maintain well-being, and counteract a sedentary lifestyle due to home confinement like during COVID-19 (A. Iannaccone, A. Fusco, S. J. Jaime, et al., 2020). We have previously shown that 20 weeks of SuperJump training reduced bone resorption and increased bone formation in eumenorrheic women acting on the key points of the regulation of bone metabolism (Vasto S, 2022). In this manuscript, it was hypothesized that gastrointestinal hormones are involved in the metabolic pathway underlying bone remodeling following SuperJump exercise in eumenorrheic women. Furthermore, since gastrointestinal hormones impact glucose metabolism it was secondary hypothesized that SuperJump may have effects on glucose homeostasis and beta cell function. Therefore, the study aimed to investigate whether the gastrointestinal hormones and specifically GLP-1, GIP, GLP-2, PYY, and ghrelin are involved in the mechanism of action that influences bone remodeling following 20 weeks of SuperJump activity and whether these changes would also impact glucose homeostasis.

Materials and Methods

Subjects and Experimental design

This study is part of a larger project (TRAMP2021). As previously described by (Vasto S, 2022) from an initial number of forty-two women, due to lack of inclusion criteria or withdrawal, twenty-four eumenorrheic women were randomized into two groups, the exercise group and the non-exercise (control group) for total of twelve women in each group. Inclusion and exclusion criteria are summarized in Table 1. Briefly, during the first visit, the participants underwent anthropometric measurements and completed a habitual dietary intake assessment (Sara Baldassano et al., 2018). Before starting the activity a blood sample (BASE) was collected; the second sample of blood was collected at the end of the 20 weeks (W20) (Figure 1).

Table 1. Inclusion and exclusion criteria of the study.

Inclusion criteria	Exclusion criteria
Women living in Italy	Bone fracture within the previous year
Age: 18–40 years	Self-reported long (> 35 days) or short (< 24 days) or irregular menstrual cycles
Currently injury free	Use of medication or suffering from any condition known to affect bone metabolism
Body mass index between 18.5 and 28 kg/m^2	Pregnancy, Breastfeeding
Menstrual cycle interval between 24 and 35 days	Current smokers
	Use of any type of hormonal contraception within the past six months
	Calcium or vitamin D supplementation in the preceding six months
	Participation in moderate and high impact-activity for ≥3 h·week before enrolling the study

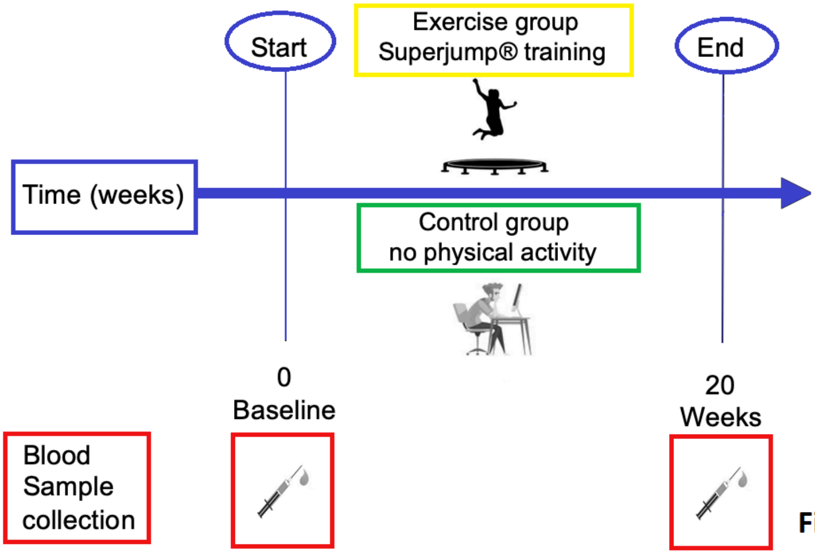


Figure 1. Overview of the experimental design. Blood samples were collected baseline, at time 0, and after twenty weeks in the two groups of study (control group and exercise group). In the exercise group, the SuperJump activity was performed for a total of 20 weeks, three times a week, for 60 minutes each session. The control group did not perform physical activity.

Workout characteristic

The exercise group performed SuperJump training (CoalSport, Rome, Italy). The intensity was 65-75% HR max and the frequency was 3 times a week for a total of 20 weeks. The session time was 60 minutes. The SuperJump training session was performed by the whole exercise group on the same days at the same time by the exercise group together. The training sessions were carried out on the mini trampoline and led by experienced instructors. Each session was divided into 5 minutes warm-up, a central phase with full-body jumping exercises, and 5 minute cool-down phase. The central phase was a circuit of 10 exercises, 50 seconds each, with 10 seconds of active recovery each time. The circuit was repeated five times per training session. The training session was entirely performed on the mini trampoline, including the recovery phase during which the subjects continued to jump on the trampoline at the minimum intensity that allowed them to perform the jump (just lifting both feet off the trampoline together). The ten resistance exercises were: 1) Isometric lateral raises 2) curl 3) Oblique 4) Adductors/ abductor 5) triceps 6) Front lifts 7) Split jump alternating drill 8) Pull to the chin 9) Jumping jack single arm 10) Standing Russian twist. All exercises were performed with dumbbells, with the weight allowing the subject to carry out the exercise for 50 seconds. SuperJump is a moderate-to-vigorous activity (sRPE = 3.1 ± 1.2), during the training session the subjects spend the47.1 ± 34.4% of the time on moderate intensity (64±76,9 % of HRmax) and 34.6 ± 39.6% of the session time on vigorous intensity (77±95,9 %of HRmax) (A. Iannaccone, A. Fusco, S. J. Jaime, et al., 2020). The performance and the effects of Superjump were recently studied (A. Iannaccone, A. Fusco, S. J. Jaime, et al., 2020; Vasto S, 2022), and the characteristics of this activity which include resistance exercise and impact activities, such as during the active recovery phase that classifies it among activities with osteogenic potential. The rationale of the SuperJump protocol was to do both resistance exercises and impact activities to exert osteogenic effects. Indeed, it's demonstrated that resistance exercise has a better osteogenic potential than just aerobic exercise (Proia et al., 2021). The active recovery phase was important to prolong "the impact stimulus" of the activity over time. The impact of a physical exercise is the combination of force magnitude and the speed at which the force is applied (KB. Gunter et al., 2012). Activities with the most osteogenic potential have ground reaction forces (GRF) greater than 3.5 times BW (per leg), with peak force occurring in less than 0.1 s (Hind & Burrows, 2007). Comparing three main activities walking, running, and jumping the last has the greatest benefits to bone mineralization (MacKelvie, Khan, Petit, Janssen, & McKay, 2003; MacKelvie, Petit, Khan, Beck, & McKay, 2004). It also seems important not only the characteristic of the movement but also the number of repetitions of 50 jumps in a session (Wiebe et al., 2008) seems to not have an osteogenic effect compared to 100 jumps (Fuchs, Bauer, & Snow, 2001; K. Gunter et al., 2008). The control group did not perform physical activity during the time of the study. Physical activity was intended as a structured activity and excluded daily life activities (like physically heavy work) and journeys on foot or by bike to go to work.

Anthropometry

Body composition and specifically lean mass and fat mass were measured by electrical bioimpedance measurement (InBody 320 Body Composition Analyzer). Body weight and Barefoot standing height were measured respectively by using an electronic scale and a wall-mounted stadiometer (Gima 27335 and Gima 27088, Italy). Body mass index (BMI) was reported as weight (kilograms) per standing height (meters squared).

Blood sample collection

Blood samples were collected by a specialist the morning after overnight fasting. For plasma samples, we used a tube containing EDTA while for serum we were allowed to clot in serum tubes at room temperature for 30 minutes before being centrifuged under the same conditions and centrifuged at 3000 rpm for 10 minutes.

Assays

To measure gut peptides, plasma samples were collected in pre-chilled EDTA-containing tubes with apoprotein (0.6 TIU/ml blood) and dipeptidyl peptidase IV inhibitor (10 µl/ml blood). Plasma was obtained by centrifugation at 5°C for 10 min at 3000 rpm for measurements of GLP-1, GIP, ghrelin, PYY, and GLP-2. All samples were immediately stored at -80°C until analyzed. All samples were analyzed in duplicates. As previously reported by (Sara Baldassano et al., 2018) human plasma peptide samples were analyzed using the following enzyme immunoassay kit: EZGLPHS-35K for active GLP-1, EZHGIP-54K for total GIP, EZGRT-89K for total ghrelin, EZHPPYYT66K for total PYY, EZGLP-237K for GLP-2, all from Millipore. The inter- and intra-assay coefficients of variation for total ghrelin were 6,62% and 1,32%; for GLP-2 were 6,62% and 5,15%; for active GLP-1 were 11,5% and 4,5%; for total PYY were 7,41% and 2,27%; and for total GIP were 3,37% and 6,45%. All samples were measured in one assay to avoid inter-assay variation. Glucose, insulin, total cholesterol, HDL-cholesterol, and triglycerides were measured by standard commercial assays supplied by Roche Diagnostics performed on the Roche COBAS c501. The HOMA2 computer model was used to estimate insulin resistance (HOMA2-IR), β-cell function (HOMA2-%B), and insulin sensitivity (HOMA2-%S) from fasting insulin and glucose concentrations calculated by the HOMA2 calculator for specific insulin version 2.2.3 available from http://www.dtu.ox.ac.uk/homacalculator. The method is an updated HOMA model and has been used extensively to measure insulin resistance β-cell function and insulin sensitivity (Ghasemi et al., 2015; Wallace, Levy, & Matthews, 2004).

Ethics

The study conducted in accordance with the Declaration of Helsinki was approved by the ethics committee 1 of the University of Palermo, Policlinico Giaccone Hospital, approval number 2-2020-27. Before the start of the study, all subjects involved provided written informed consent. In addition, the clinical study was registered on Clinicaltrials.gov under the number NCT04942691.

Statistics

Based on results of previous studies on exercise and gut peptides (Druce et al., 2005) (Martins et al., 2007) study was powered to detect a change in GLP-1 of 30% (SD 20%) considering a Type I error (α) = 0.05 (two-sided), and Type II error (β) = 0.20 (power is 80%). An *a priori* power calculation determined that ten subjects were required to achieve 80% power at $P< 0.05$ by using G Power software. The comparison between the groups was performed by one-way ANOVA followed by Tukey's post-test. A $P < 0.05$ was considered to be statistically significant by using GraphPad Prism software.

Results

The cohort under investigation did not show significant differences in body mass index or composition among the groups (control *vs* exercise) or within the groups (time zero *vs* 20 weeks). Instead, a significant difference was observed in triglycerides levels in the exercise group at W20 compared with the BASE while no differences were reported in total cholesterol, HDL-cholesterol, and LDL-cholesterol between the groups (control *vs* exercise) or within the groups (time zero *vs* 20 weeks) (Table 2). Moreover, we previously showed that there was a significant change in the markers of bone remodeling after 20 weeks of training in the exercise group. The marker of bone formation, Osteocalcin, increased from 16.2 ± 5 (BASE) to 22.2 ± 6 µg/L (W20). The marker of bone resorption, CTX, decreased from 0.44 ± 0.1 (BASE) to 0.29 ± 0.1 µg/L (W20). PTH decreased from 44 ± 15 (BASE) to 34 ± 11 ng/L (W20). Calcitonin, vitamin D, and phosphate concentrations did not change while there was a significant increase in calcium and potassium concentrations (Vasto S, 2022).

Table 2: Characteristics of the subjects measured baseline and after 20 weeks (20 W) in the two groups of women.

	Control group			**Exercise group**		
	BASE	**20 W**		**BASE**	**20 W**	
	Mean ± SD	**Mean ± SD**	**p-value**	**Mean ± SD**	**Mean ± SD**	**p-value**
BMI (kg/m^2)	22.5 ± 2.7	23.7 ± 2.9	p>0.05	22.8 ± 2.4	22.8 ± 2.8	p>0.05
LM %	74.4 ± 5.8	76.6 ± 5.2	p>0.05	73.2 ± 5.9	73.7 ± 7.2	p>0.05
FM %	25.6 ± 5.8	23.9 ± 6.2	p>0.05	26.8 ± 6	26.3 ± 7.2	p>0.05
TRIG (mg/dL)	91 ± 14	89 ± 26	p>0.05	80 ± 21	55 ±18	P=0.02
Total Chol (mg/dL)	182 ± 18	188 ± 27	p>0.05	182 ± 23	184 ± 24	p>0.05
HDL-Chol (mg/dL)	77 ± 12	75 ± 11	P>0.05	77 ± 15	80 ± 14	p>0.05
LDL-Chol	93 ± 24	98 ± 18	P>0.05	100 ± 14	89 ± 16	P>0.05

Abbreviation*: BMI, Body Mass Index; LM, Lean Mass; FM, Fat Mass; TRIG, Triglycerides; Chol, Cholesterol*

Incretins

In the control gro, up there were no significant changes in plasma GLP-1 and GIP levels at W20 compared with a BASE within the group (Figure 2). In the exercise group, GLP-1 was significantly increased at W20 compared with BASE (Figure 2 A). GLP-1 concentrations at W20 increased by 58 % from BASE (5.7 ± 1.7 *vs* 3.6 ± 0.7 pmol/l). The levels detected were within the normal range. In addition, the GIP level was significantly increased. Specifically, in the exercise group, GIP increased by 102 % at W20 compared to BASE (64.3 ± 21 *vs* 31.9 ± 12 pg/ml) (figure 2 B), and the concentrations detected were within the physiological range. There was a significant change in endogenous levels of GLP-1 and GIP in the exercise group at W20 compared to the control group (Figures 2 A and B).

Other gut hormones

In the control group there were no changes in GLP-2 (2.1 ± 0.25 *vs* 2 ± 0.26 ng/ml) PYY (58 ± 11 *vs* 56 ± 12 pg/ml) and ghrelin levels (1081 ± 491 *vs* 1036 ± 376 pg/ml) at W20 compared with BASE. Moreover, in the exercise group SuperJump training did not affect plasma GLP-2 (2.1 ± 0.20 *vs* 1.9 ± 0.16 ng/ml), PYY (55 ± 11 *vs* 52 ± 6 pg/ml), and ghrelin concentrations (947 ± 351 *vs* 1014 ± 458 pg/ml) at W20 compared to baseline. Also, the comparison between the two groups (control *vs* exercises) showed no significant changes in GLP-2, PYY, and ghrelin (Figure 3 A-C).

Markers of glucose homeostasis

In the control group there was no difference in fasting glucose (88 ± 3.1 *vs* 90 ± 4.2 mg/dL), insulin (8.1 ± 2.8 *vs* 8.6± 3.0 mUI/L) or insulin resistance (1.0 ± 0.4 *vs* 1.2 ± 0.3) at W20 compared with BASE. In the exercise group, SuperJump training significantly reduced fasting glucose (80 ± 7.4 *vs* 88 ± 4.0 mg/dL), insulin (4.7 ± 1.9 *vs* 7.9 ± 2.6 mUI/L), and insulin resistance (0.6 ± 0.2 *vs* 1.0± 0.3) at W20 compared with BASE. The comparison between the groups (control *vs* exercise) showed a significant reduction in fasting insulin, glucose, and insulin resistance in the exercise group at W20 compared to the control group (Figure 4 A-C). There was no difference in β-cell function in the control group or the exercise group at W20 compared with BASE (Figure 4 D). There was no difference in insulin sensitivity in the control group while in the exercise group there was a significant increase in insulin sensitivity at W20 compared with BASE. The comparison between the groups (control *vs* exercises) showed a significant increase in insulin sensitivity at W20 in the exercise group compared to the control group (Figure 4 E).

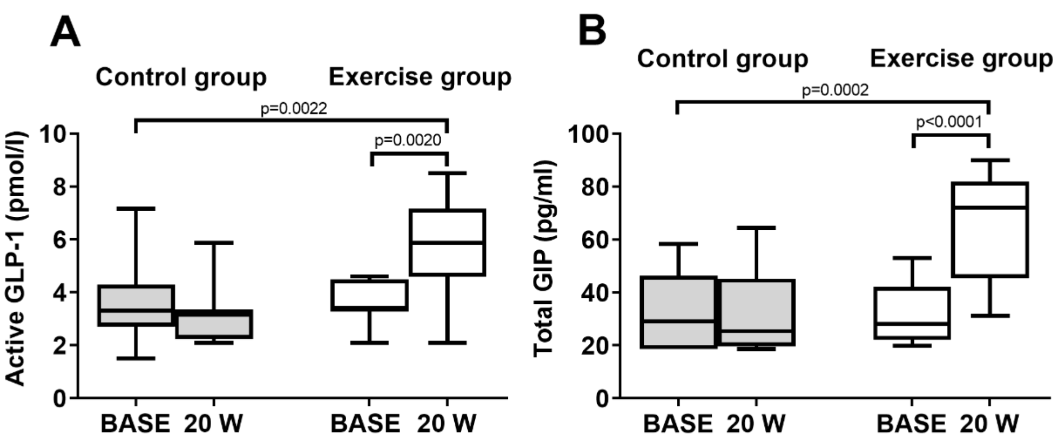


Figure 2. Endogenous Incretins levels were measured at baseline (BASE) and after 20 weeks (W 20) in the control group and exercise group. (A) Box and whisker plot of GLP-1 (B) Box and whisker plot of GIP.

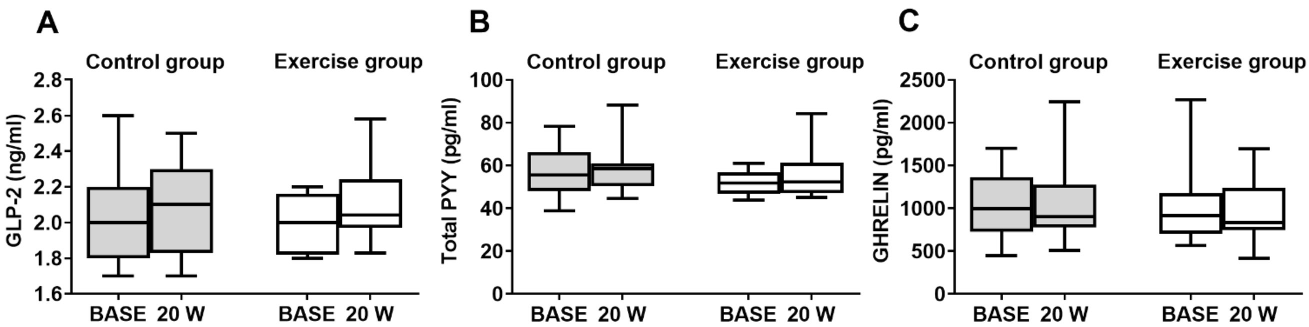


Figure 3. Endogenous gut peptide levels were measured at baseline (BASE) and after 20 weeks (W 20) in the control group and exercise group. (A) Box and whisker plot of GLP-2 (B) Box and whisker plot of PYY(C) Box and whisker plot of Ghrelin.

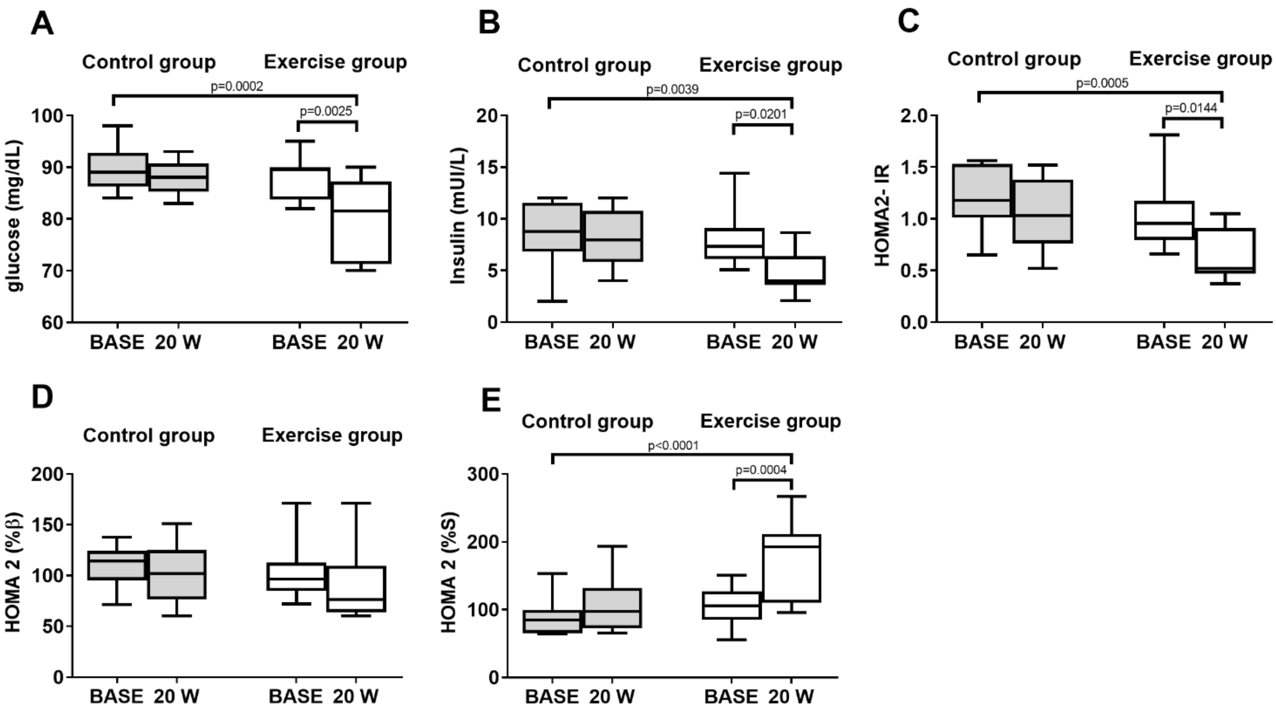


Fig. 4

Figure 4. Markers of glucose homeostasis measured baseline (BASE) and after 20 weeks (W 20) in the control group and exercise group. (**A**) Box and whisker plot of fasting glucose (**B**) Box and whisker plot of fasting insulin (**C**) Box and whisker plot of insulin resistance (**D**) Box and whisker plot of β-cell function (**E**) Box and whisker plot of insulin sensitivity.

Discussion

In previous studies, the endogenous levels of GLP-1 and GIP following physical activity have been measured at the end of a single training session (Zouhal et al., 2019) and have not investigated the potential link between gut peptides, bone remodeling, and physical activity.

This study shows that the gut peptides GLP-1 and GIP are involved in the mechanism of action that influences bone remodeling and ameliorate glucose homeostasis following 20 weeks of SuperJump training in eumenorrheic women. We previously showed that SuperJump activity exerts osteogenic action in eumenorrheic women. In fact, after 20 weeks of SuperJump training, the levels of the marker of bone resorption CTX were significantly reduced while the levels of a marker of bone formation osteocalcin were increased. We found that PTH, calcium, and potassium were involved in the mechanism of action (Vasto S, 2022). The present study showed that the SuperJump exercise program for 20 weeks significantly increased endogenous GLP-1 and GIP levels suggesting that these two incretins are part of the mechanism of action by which this type of high-impact activity influences bone remodeling in eumenorrheic women. This was confirmed by the lack of changes in the endogenous level of GLP-1 or GIP in the control group of sedentary women. We observed an increase in endogenous levels of GLP-1 and GIP that is positive for bone remodeling because it is within the normal physiological range. In fact, in women, the treatment with the long-acting agonist of the GLP-1R, liraglutide, increased P1NP (bone formation marker) and bone mineral content and reduced bone loss, indicating that GLP-1 acted by increasing bone formation (Iepsen et al., 2015). In ovariectomized rats, the treatment with liraglutide increased bone mineral density and improved trabecular thickness, number, and volume (Lu et al., 2015). Moreover, the activation of GLP-1R decreased P1NP secretion and increased cell viability in osteoblasts (E. L. Pacheco-Pantoja, L. R. Ranganath, J. A. Gallagher, P. J. Wilson, & W. D. Fraser, 2011). Also, GIP exerts anti-resorptive action and anabolic effect (E. Pacheco-Pantoja et al., 2011). GIP stimulated the expression of P1NP and ALP activity (Bollag et al., 2000) and reduced the level of CTX, the marker of bone resorption (Nissen et al., 2014). The GIP receptor is expressed in osteoblast and osteoclast-derived cell lines. Therefore, the loss of function for the GIP receptor gene, in women carrying the gene polymorphism E354Q, was correlated with decreased bone mineral density and increased risk of fractures (Torekov et al., 2014). About GLP-2, previous studies showed that GLP-2 administered subcutaneously in postmenopausal women reduced CTX, and markers of bone resorption, and had a minimal effect on bone formation (Askov-Hansen et al., 2013). In our study, GLP-2 levels did not differ between exercise and control groups suggesting that it is not involved in the mechanism of action that impacts onne remodeling in exercise women. We cannot exclude that 20 weeks of SuperJump training were not sufficient to induce differences in the endogenous levels of the peptide. However, based on previous studies, supraphysiological doses of exogenous GLP-2 are necessary to reduce bone resorption (S. Schiellerup et al., 2019). Thus, changes within the physiological range may not be sufficient to see an effect and this may account for the lack of differences. However, so far, it is still unknown if GLP-2 affects bone metabolism directly or indirectly by involving other intestinal factors. The GLP-2 receptor has not been identified in human osteoclasts or any other bone-related cell type (E. Pacheco-Pantoja et al., 2011). Evidence from human studies indicates that PYY modulates bone homeostasis (S. Schiellerup et al., 2019). The PYY increases were associated with low bone mineral density in women with weight alteration (Russell et al., 2009; Utz et al., 2008) and absence of menstrual periods (Scheid et al., 2011). In our study, we did not find any difference in PYY concentration ruling out the involvement of PYY. This is probably because our study population was constituted of eumenorrheic women with no weight alteration. We did not find any differences also in circulating ghrelin levels in the groups of study. Ghrelin is firstly a regulator of energy metabolism but seems to influence bone (Ibrahim Abdalla, 2015). However, the basal concentration of ghrelin is inversely associated with body mass index. Reduced ghrelin levels were found in obese people (McLaughlin, Abbasi, Lamendola, Frayo, & Cummings, 2004).

So far, we know that physical activity ameliorates glucose homeostasis but it is still unclear how it acts to do it. Here we suggest that GLP-1 and GIP could be part of the physiological mechanism of action that improves glucose homeostasis following a high impact of physical activity. The higher endogenous GLP-1 and GIP levels in the exercise group following 20 weeks of SuperJump activity improved glucose metabolism. In the exercise group, it was observed that reduced fasting glucose, insulin, and insulin resistance and increased insulin sensitivity. It was confirmed by the lack of changes in fasting glucose, insulin, or insulin sensitivity in the control group of the study. This agrees with the reduced gut peptide responses reported in sedentary obese people that develop insulin resistance (Koliaki, Liatis, Dalamaga, & Kokkinos, 2020). The observation of elevated GLP-1 and reduced insulin could be counterintuitive in consideration of the ability of GLP-1 to stimulate insulin release. Thus, it is necessary to point out that the incretin effect is defined as the increase in insulin response after oral ingestion of glucose. GLP-1 induces insulin secretion via the GLP-1R in a glucose-regulated manner (Meloni, DeYoung, Lowe, & Parkes, 2013). The blood samples in the groups of the study were obtained after an overnight fast. Thus, it is possible to hypothesize that fasting levels of insulin and glucose were lower thanks to a regulatory mechanism of the peptide on beta-cells that could be sensitized to secrete the minimum amount of insulin required to have accurate glycemic control. Insulin sensitivity was increased. Further studies are required.

A key strength of the present study was to analyze the effects of chronic exercise (20 weeks of Superjump exercise) on endogenous peptides concerning previous studies that have focused on the effects of acute exercise on the secretion of gastrointestinal hormones (Schubert, Sabapathy, Leveritt, & Desbrow, 2014). In fact, to our knowledge, the endogenous levels of GLP-1 and GIP following physical activity have been measured only acutely, at the end of the training session (Hallworth, Copeland, Doan, & Hazell, 2017; Howe et al., 2016; Larson-Meyer et al., 2012; Schubert et al., 2014; Ueda et al., 2013; Unick et al., 2010; Zouhal et al., 2019), and not after several weeks of a training program like in our study. Moreover, the mechanism of action and therefore the potential link between gut peptides, bone remodeling, glucose metabolism, and physical activity has not been investigated. For GLP-1 the studies measured the total and not the active form of GLP-1 like in our study (Unick et al., 2010). However, GLP-1, similar to ours, showed an increase in basal GLP-1 levels (Lund et al., 2013; Schubert et al., 2014; Unick et al., 2010). These investigations were conducted after acute exercise not only in normal-weight but also in obese trained women and suggest that endogenous levels of GLP-1 are very sensitive to physical activity. For GIP the studies have been conducted in obese/diabetic cohorts of patients or following a glucose tolerance test (Kelly et al., 2009; Larsen et al., 2017; Martins, Kulseng, Rehfeld, King, & Blundell, 2013). These researches were unconcluded and showed a decrease, increase, or no change in the GIP concentrations. We are conscious that we did not compare the Superjump group with another group that performed other forms of exercise like a different high-impact or strength training. Thus, we don't know the effects of other forms of chronic exercise on basal gut peptide release, and further studies are necessary. The exercise protocols' characteristics, like age, fitness level, BMI, and the exercise protocol, duration and intensity could differently impact gut peptide release and this is a limitation of the study. We also don't know if the observed effects were mediated by GLP-1 or GIP. We may suppose that the observed effects are mediated by a synergistic action of the two peptides but further studies are necessary to clarify the point.

Conclusions

In conclusion, the study points out the ability of physical activity by increasing endogenous GLP-1 and GIP levels to ameliorate bone and glucose metabolism suggesting that the peptides are involved in the physiological mechanism of action that improves bone and glucose homeostasis following 20 weeks of Superjump activity in eumenorrheic women.

2.4 Dare to jump: the effect of new high-impact activity " SuperJump® " on bone remodeling. [From Vasto, Amato et al. (2021)]

Introduction

Bone mineral density (BMD) reduces across the lifespan (Santos et al., 2017) and women tend to be more susceptible to bone loss and the development of osteoporosis than men (Hernlund et al., 2013b). Biochemical markers of bone remodeling reflect the cellular activities of bone formation and resorption and can be used to monitor the acute changes in bone remodeling (Johansson et al., 2014; Lester et al., 2009; Ratamess et al., 2007). They are a useful tool to verify the effects of antiresorptive treatment in osteoporotic patients. They are also used to analyze the influence of external factors, such as exercise and nutrition, on bone remodeling (Amato et al., 2018a; Maïmoun & Sultan, 2011;

Sale & Elliott-Sale, 2019). Bone remodeling is an active and dynamic process and it relies on the correct function of two different cell types responsible for bone metabolism named osteoclasts and osteoblasts. The former are multinucleated cells that destroy the bone matrix, and the latter have osteogenic functions. The interactions between osteoclasts and osteoblasts allow the maintenance of bone integrity. The imbalance between bone resorption and bone formation, in favor of resorption, results in bone loss and deterioration of bone architecture (Santos et al., 2017). Exercise has a significant influence on bone health across the lifespan and it seems to be a good candidate for preventive intervention. Several studies have shown that weight-loading exercise increases BMD (Fuchs et al., 2001; Heinonen et al., 2000; Macdonald, Kontulainen, Khan, & McKay, 2007; McKay et al., 2000; F. L. Morris, Naughton, Gibbs, Carlson, & Wark, 1997), positively influences bone development, maintaining, strength and reduces falls and risk of fractures (Kemmler, von Stengel, Engelke, Häberle, & Kalender, 2010; Robbins et al., 2007). Jumping, similar to other high-impact loading exercises, has proven to be able to generate a substantial osteogenic response (Fuchs et al., 2001) and to increase balance and bone material strength in postmenopausal women (Aragão, Karamanidis, Vaz, & Arampatzis, 2011; Posch et al., 2019; Sundh et al., 2018). Moreover, competitive female trampolinists had greater bone density, area, microarchitecture, and bone strength (Burt, Schipilow, & Boyd, 2016) concerning other disciplines. The benefits of an exercise intervention on bone health can be indicated by the markers of bone turnover other than densitometry (Wen, Huang, Li, Chong, & Ang, 2017). In many studies, the levels of bone turnover marker can be indirectly used to detect or monitor the early response of the skeleton to exercise (Amato, Proia, et al., 2021; Lester et al., 2009; Ratamess et al., 2007; Roghani et al., 2013).

Finding a new workout program able to improve bone health that positively influences well-being, and with exercise easy to standardize for participants, would be of great interest to prevent osteoporosis and lower the risk for fracture in aging women since adherence and compliance to the treatment of osteoporosis have decreased in recent years (Khosla & Shane, 2016). This along with the importance of aging in good health highlights the need of identifying new tools for osteoporosis prevention. Therefore, in the last years, the effects of physical activity have received widespread attention due to the potential for the prevention of fractures and osteoporosis (McMillan, Zengin, Ebeling, & Scott, 2017). Bone turnover may be affected by the type of training (Creighton, Morgan, Boardley, & Brolinson, 2001; Maïmoun et al., 2004; Maïmoun & Sultan, 2011; Matsumoto, Nakagawa, Nishida, & Hirota, 1997). Thus, the mechanical effects of the exercise, the components such as intensity, and volume modulate bone response (Maïmoun & Sultan, 2011). SuperJump® is a high-impact physical activity performed on trampolining that mixes aerobic and anaerobic exercises.

SuperJump® training sessions are based on total body exercises performed by jumping to the rhythm of the music on the trampoline (A. Iannaccone, A. Fusco, S. J. Jaime, et al., 2020). The effects of this workout activity on bone turnover and metabolism have not been investigated so far. We hypothesized that a controlled exercise program such as SuperJump® would promote bone formation and reduce resorption in eumenorrheic women. We imagined that markers of bone turnover would be influenced by trampoline exercise and would demonstrate changes indicative of bone remodeling in our training groups and that this would impact the essential regulators of bone metabolism like calciotropic hormones and markers of calcium metabolism. Therefore, the study aimed to investigate the effects of SuperJump® training for 20 weeks on bone turnover markers and to investigate the mechanism of action in eumenorrheic women.

Methods

A total of forty-two women volunteered in this randomized-controlled study. Participants were asked not to change their daily and diet habits during the study period. They were contacted during the study to remind them not to modify their habits until they had completed the study (Otero, Esain, González-Suarez Á, & Gil, 2017). Inclusion criteria were 1) Caucasian 2) age: 18–40 years 3) currently injury free 4) Body mass index between 18.5 and 28 kg/m^2 5) self-reported regular and frequent menstrual cycles (menstrual cycle interval between 24 and 35 days). Self-reported menstrual cycle details were assessed using a questionnaire, which included questions about menstrual frequency and length, bleeding length, and previous oral contraceptive pill use. Menstrual cycle length was defined as the number of days from the first day of menstruation to the day before the next onset of menstruation. Eumenorrhea was established if menstruation occurred at regular intervals of 24–35 days. Amenorrhoeic women (absence of menstruation for a minimum of 3 repeated months), oligomenorrheic women (menstrual cycles of 36-90 days), and women with short menstrual cycles (menstrual cycles < 24 days) were excluded from participation to ensure that existing reproductive disturbances did not affect the results (Gibbs, Williams, & De Souza, 2013). Exclusion criteria were 1) bone fracture within the previous year, 2) self-reported long (> 35 days) or short (< 24 days) or irregular menstrual cycles, 3) use of medication or suffering from any condition known to affect bone metabolism, 4) pregnancy, 5) breastfeeding 6) current smokers 7) use of any type of hormonal contraception within the past six months 8) calcium or vitamin D supplementation in the preceding six months 9) participation in moderate and high impact-activity for ≥3 h·week before enrolling the study. From a total of forty-two women, sixteen were excluded because of insufficient time to participate or did not meet the inclusion criteria. Twenty-six women were randomized into the non-exercise group (control group) and an exercise group and performed all the assessments at baseline.

One subject in the exercise group and one in the control group discontinued the study without any specific reason. Therefore, twelve women entered the exercise program and twelve were n the control group (Figure 1).

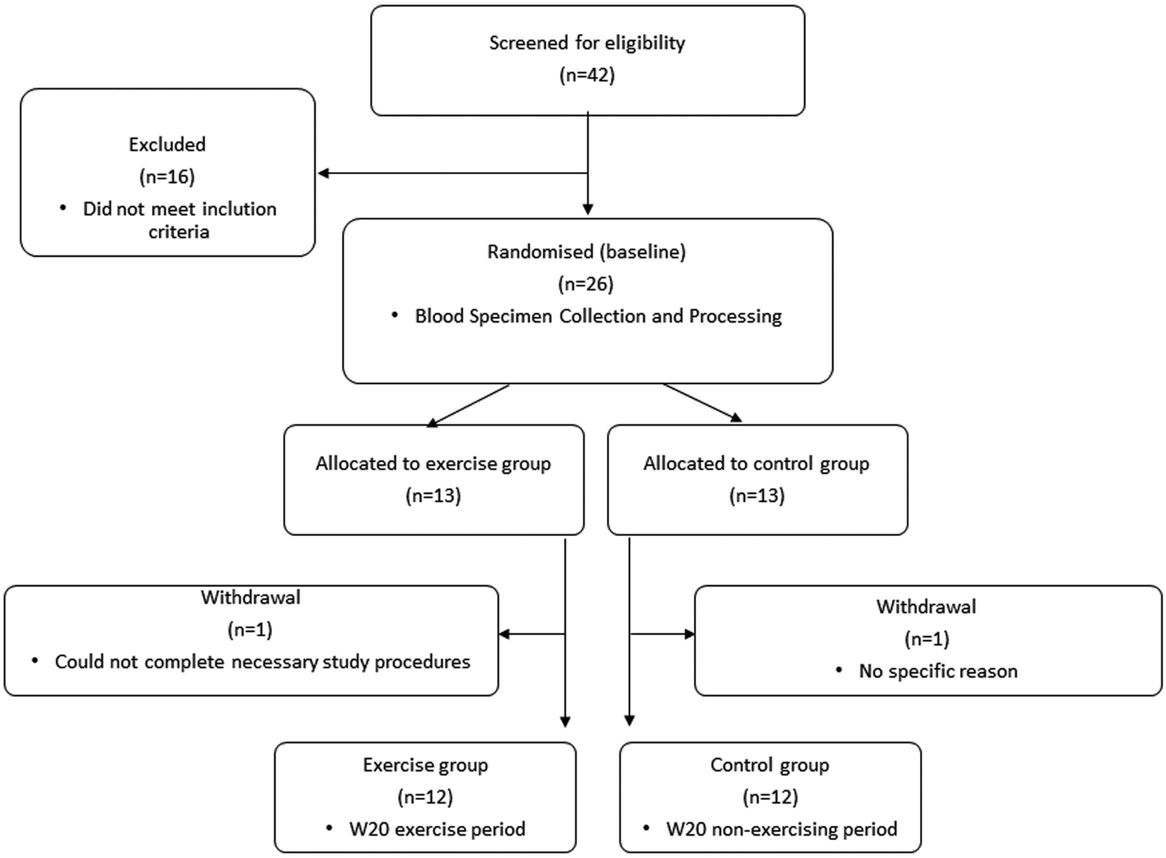


FIG. 1. Flow chart of the recruitment and selection process of subjects

Experimental design. Participants attended the first visit at the onset of menstruation, which indicated the first day of the experimental study. They completed a habitual dietary intake assessment and underwent anthropometric measurements. The blood sample (baseline sample-BASE) was collected before the experimental condition started. The control group not performed physical activity. In the exercise group, SuperJump® training was performed three times a week, each session lasting 60 minutes for a total of 20 weeks. The second sample of blood was collected, at the onset of menstruation, at the end of the 20 weeks (W20). The experimental design is described in figure 2.

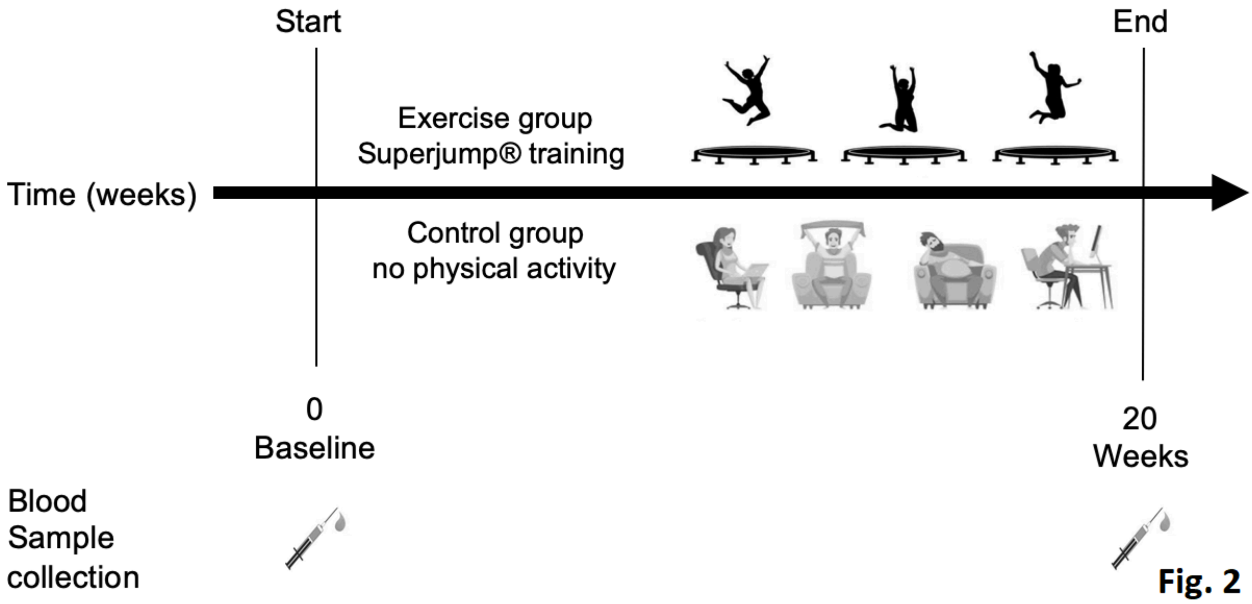


FIG. 2. Schematic overview of the study design. Blood samples were collected at time 0 (baseline) and after 20 weeks in the control group and exercise group. In the exercise group, SuperJump training was performed three times a week, each session lasting 60 minutes for a total of 20 weeks. The control group not performed physical activity.

Anthropometric measurement. Body weight was measured after an overnight fast on an electronic scale (Gima 27088; Gima, Italy) calibrated to the nearest 0.1 kg. Barefoot standing height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer (Gima 27335; Gima Italy). The coefficient of variation for serial measurements of weight and height were 0.95 and 0.98 respectively. Body mass index (BMI) was calculated as weight, in kilograms, per standing height, meters, and squared. Body composition (Lean mass, Fat mass) was measured by electrical bioimpedance measurement (InBody320 Body Composition Analyzer)

Blood sample and biochemical analysis. To minimize circadian variation, blood samples were obtained at the same time of day (between 07.30 and 08.15 h) for each woman, after an overnight fast (from 20:00 h the previous evening). Blood samples were collected in specific tubes containing EDTA for plasma and were centrifuged immediately at 1509 ×g for 10 min at 4 °C. Venous blood was dispensed into serum tubes and allowed to clot at room temperature for 30 min before being centrifuged under the same conditions. Serum samples were analyzed for Osteocalcin, PTH, CTX, Calcitonin, and Vitamin D by using an electro-chemiluminescence immunoassay (ECLIA) (Roche Diagnostics, Burgess Hill, UK) on a Cobas e601 analyzer. The method is FDA-cleared and CE-marked. Calcium, albumin Phosphate, and Potassium were measured using standard commercial assays supplied by Roche Diagnostics performed on the Roche COBAS c501. Fluctuations in protein concentrations, especially albumin, may cause total Calcium concentrations to change independently of the ionized calcium concentration, as such Calcium concentrations were 'corrected' to give an albumin-adjusted calcium (Aa Calcium) value using the following equation: (−0.8 *([Albumin] - 4)) + [Total Ca]. *Exercise program*. SuperJump® training is an innovative training performed on the elastic minitrampoline (CoalSport, Rome, Italy) (A. Iannaccone, A. Fusco, S. J. Jaime, et al., 2020). The subject performed 20 weeks of SuperJump® training, 3 times per week. All training sessions were led by experienced instructors. Each session lasted sixty minutes and included the following organization: a 5 minutes warm-up with breathing and mobility exercises from upper to lower body, a central phase with jumping exercises involving the total body, and 5 minutes cool-down phase with total body active stretching exercise. The whole training session was carried out on the mini trampoline. The central phase corresponded to a protocol performed in a circuit that is doing multiple sets of exercises, with very short recovery between exercises. Specifically, the circuit consisted of 10 exercises, lasting 50 seconds each, and 10 seconds of active recovery between the exercises. The recovery is also performed on the trampoline. Each circuit is repeated 5 times each training session. The exercise program is shown in table 1.

SuperJump® workout characteristics	
Frequency	3 times/week for 20 weeks
Intensity	65-75% of HR_{max}
Session Time	60 minutes
Exercise Time	50 seconds or 25 each side
Circuit repetitions	5 times
Recovery	10 seconds (jumping on trampoline)
Exercise type and execution sequence of the exercises	Isometric lateral raises Biceps Oblique Adductors abductor Triceps Front lifts Split jump alternating drill Pull to the chin Jumping jack single arm Standing Russian twist

Table 1: Exercise training program.

The exercise sequences, in the central phase, stimulate alternately upper and lower muscle groups to facilitate the redistribution of blood flow in muscle groups far from each other (Contro et al., 2017).

Ethics. The study was approved by the University of Palermo ethics committee Palermo 1, Policlinico Giaccone Hospital (2-2020-27). The study was performed in concordance with the Helsinki Declaration. All participants gave oral and written consent to participate. The trial is registered at Clinicaltrials.gov NCT04942691-retrospectively registered.

Statistics. Based on results of previous studies on exercise and bone remodeling and metabolism (Papageorgiou et al., 2017; Papageorgiou et al., 2018) the study was powered to detect a change in CTX of 23% (SD 13%) considering a Type I error (α) = 0.05 (two-sided), and Type II error (β) = 0.20 (power is 80%). An *a priori* power calculation determined that eight women were required to achieve 80% power at P< 0.05. Student t-tests were used to compare the baseline characteristics of the two groups. Changes between baseline and follow-up within the groups were analyzed by paired t-tests; unpaired t-tests were used to compare these differences in the two groups.

Results

Participant characteristics

Table 2 shows anthropometric measurements of the subjects at the baseline and after 20 weeks.

	Control group		Exercise group	
	BASE	20 W	BASE	20 W
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Weight (Kg)	58.5 ± 9	61.7 ± 10.5	60.3 ± 8.3	59.9 ± 8.5
Hight (cm)	161 ± 0.1	160 ± 0.1	162.3 ± 4.7	162.1 ± 4.8
Arm (cm)	28.3 ± 2.8	28.4 ± 2.9	26.5 ± 3.1	24.9 ± 6.7
Waist (cm)	73.2 ± 6.1	72.9 ± 5.7	71.8 ± 6.3	68.8 ± 3.7
Hips (cm)	100.3 ± 6.1	100.1 ± 6.6	99.1 ± 7	95.6 ± 5.3
LEAN MASS%	74.4 ± 5.8	76.6 ± 5.2	73.2 ± 5.9	73.7 ± 7.2
FATTY MASS %	25.6 ± 5.8	23.9 ± 6.2	26.8 ± 6	26.3 ± 7.2
BMI (kg/m^2)	22.5 ± 2.7	23.7 ± 2.9	22.8 ± 2.4	22.8 ± 2.8

Table 2: Characteristics of the subjects measured baseline (Begam et al.)and after 20 weeks (20 W) in the control group and exercise group.

There were no significant changes in body weight, BMI, and lean or fat body mass in the control group compared to the exercise group or within the exercise or control group neither at the beginning nor at the end of the intervention period.

Bone turnover markers. In the control group, there were no significant changes in the markers of bone remodeling at W20 compared to BASE within the groups (Figure 3). In the exercise group, plasma concentrations of the bone resorption marker CTX were significantly reduced at 20 weeks compared to BASE within the groups (figure 3 A). CTX concentrations at W20 decreased by 34% (SD ±17) from the BASE. In addition, the bone formation marker Osteocalcin was significantly increased at W20 compared to the BASE. Specifically, in the exercise group, Osteocalcin increased by 37 % (SD ± 22) at W20 compared to BASE (figure 3 B). The comparison between the groups (Control vs Exercises) showed that there were significant changes in the markers of bone remodeling after 20 weeks of training in the exercise group compared to the control group (Figure 3).

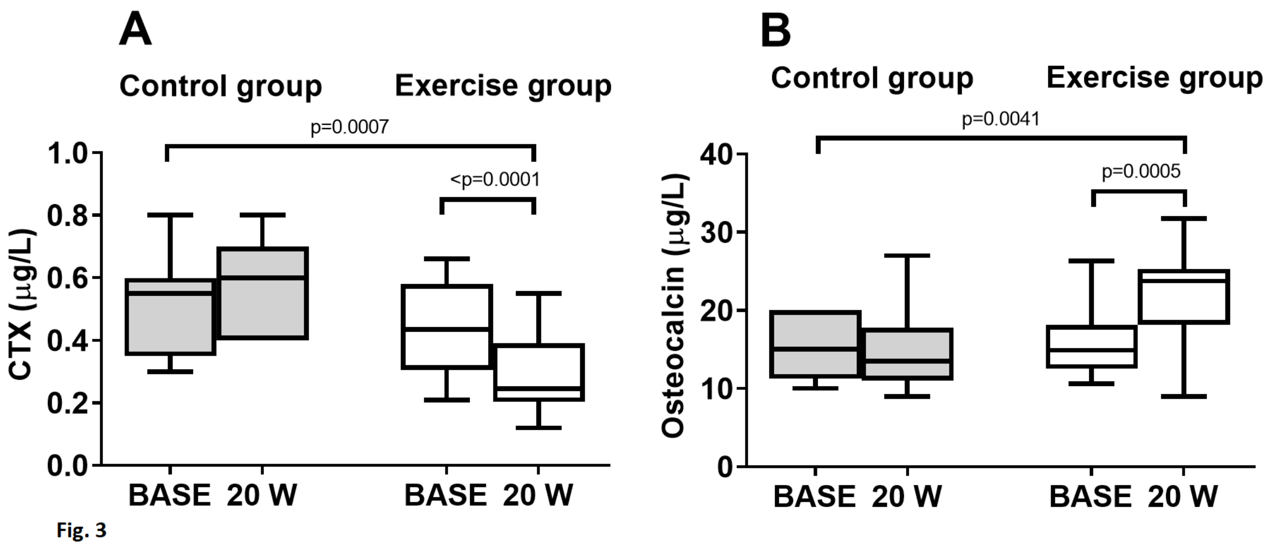


FIG. 3. Markers of bone remodeling measured baseline and after 20 weeks in the control group and exercise group. (A) Box and whisker plot of CTX, a marker of bone resorption (B) Box and whisker plot of Osteocalcin, a marker of bone formation

Markers of bone metabolism. In the control group, there were no significant changes in the markers of bone metabolism at 20 weeks compared to the baseline within the groups (Figure 4). In the exercise group, PTH concentrations decreased with time. PTH from BASE was significantly different from W20 within the groups (figure 4 A). Specifically, PTH concentrations at W20 decreased by 24% (SD ± 26) from the BASE. Moreover, Aa Calcium significantly increased at W20 compared to BASE (Figure 4B). Calcitonin, vitamin D, and phosphate concentrations did not differ between baseline and

W20 (Figure 4 C-E). The plasma concentrations of Potassium were significantly increased at 20 weeks compared to BASE (figure 4 F). The comparison between the two groups (Control vs Exercises) showed that there were significant changes in the PTH, Aa Calcium, and Potassium after W20 in the exercise group compared to the control group (Figure 4 A, B, F). Calcitonin, vitamin D, and phosphorus were not affected by the 20 weeks of training in the exercise group compared to the control group (Figure 4 C-E).

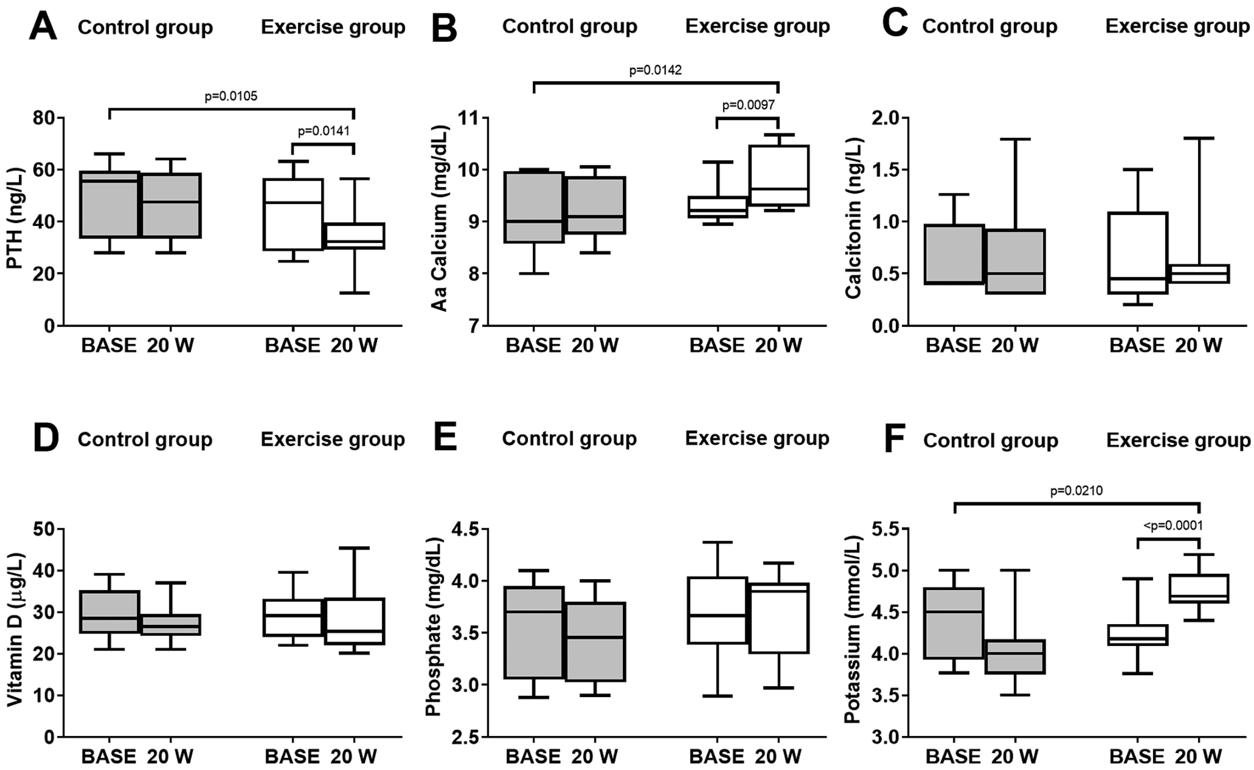


FIG. 4. Markers of bone metabolism measured at baseline and 20 weeks in the control group and exercise group. (A) Box and whisker plot of PTH (B) Box and whisker plot of Aa Calcium (C) Box and whisker plot of Calcitonin (D) Box and whisker plot of Vitamin D (E) Box and whisker plot of Phosphate (F) Box and whisker plot of Potassium.

Discussion

We investigated if a new workout activity with high impact, performed on a mini elastic trampoline, called SuperJump®, can influence bone metabolism in a short-term exercise intervention in eumenorrheic women. The study suggests that this training program exerts osteogenic action and highlights the importance of high-impact physical activity in eumenorrheic women as an essential tool for osteoporosis prevention. In fact, in the study, there were no changes in the markers of bone remodeling CTX and Osteocalcin as well as bone metabolism (PTH, ACa, Potassium, Calcitonin, Vitamin D, Phosphate) after 20 weeks in the control group. Thus, the main findings were 1) a significant increase in bone formation and a decrease in bone resorption following 20 weeks of SuperJump® workout class and 2) involvement of PTH, calcium, and potassium in the mechanism of action. The study showed that 20 weeks of the SuperJump® exercise program, three times per week for 60 minutes lasting, induced a significant reduction in the marker of bone resorption CTX and an increase in the marker of bone formation osteocalcin in eumenorrheic women. According to our results, previous studies conducted on competitive female trampolinists have reported the health benefits of the trampoline on bone. In particular competitive female trampolinists have greater bone density, area, and microarchitecture (Burt et al., 2016). Moreover, the high-impact mechanical loading of jumping exercise for 3 months increased bone material strength index (BMSi) in postmenopausal woman, suggesting that exercise loading impact bone by regulating bone material proprieties (Sundh et al., 2018). However, it is important to highlight that the response of the bone appears to be exercise modality, intensity, and age-specific (Smith et al., 2021). No effects on BMD were reported after a 12-week exercise intervention on a mini-trampoline but the markers of bone turnover were not measured. However, the intervention was highly effective in improving balance and functional mobility, strength, gait performance, and fear of falling in patients with osteopenia (Posch et al., 2019). The exercise program was different from our training program as well as the time of the intervention was shorter (12 *vs* 20 weeks). Also, the target of the participants was different. The intervention was conducted in older women with osteopenia while in our study the target population was eumenorrheic women. We don't know if the SuperJump® exercise program, with small modifications to adapt to an older group, could have an impact on bone metabolism or BMD in aging subjects, and future studies to investigate it would be of interest. Previous studies have shown that already 6 to 10 weeks of aerobic group-based exercises were able to induce a decrease in bone resorption and increase formation in post-menopausal women (Roghani et al., 2013; Wen et al., 2017). However, we focused on eumenorrheic women because although an important goal for exercise trials is to assess efficacy in populations most at risk of fracture, it is also of interest to verify the potential preventive effect of a specific physical activity. Quantifying the osteogenic potential of an exercise regimen would be beneficial to assist in developing programs that could serve to promote/maintain/reduce the rate of bone loss during the whole life to prevent injuries and delay sarcopenia that, especially in aging, are a significant burden in terms of cost and personnel. We investigated the effects on bone remodeling because it is a useful tool not only to monitor the acute effects of exercise on bone health but also to investigate the action mechanism of exercise-induced changes in bone mass (Maïmoun & Sultan, 2011). Thereafter, we attempted to provide information about the mechanism of action of the osteogenic responses that accompany the exercise intervention. In our study, it was observed a significant decrease in PTH levels after 20 weeks of SuperJump®

training whereas elevations in PTH levels are normally associated with increased bone resorption and reductions are associated with bone formation (Lombardi et al., 2020). Our study suggests that this type of high-impact activity, by reducing basal PTH level, might have a positive influence on bone turnover in eumenorrheic women by increasing bone formation and by reducing bone resorption. This is, also, following previous research, in which pre-menopausal women showed a decrease in basal PTH concentrations after high-impact exercise training performed for 6 to 12 months (Vainionpää et al., 2009). However, the underlying mechanisms influencing PTH release in response to exercise are not well understood. Differently from our study, PTH was increased after 6 weeks of endurance training in elderly men (ZERATH, HOLY, DOUCE, GUEZENNEC, & CHATARD, 1997). However, in this study, the PTH level was measured immediately (<1 min) after the end of the exercise and the participants were elderly men. Thus, the differences with our study are probably due to the analysis of PTH levels on the acute effects of exercise rather than gender. Several studies have documented PTH levels decreasing below baseline hours after exercise (Falk et al., 2016; Scott et al., 2010; J. P. R. Scott et al., 2011; Scott et al., 2014). PTH concentration increases transiently after jumping exercises and aerobics but not after acute resistance exercises (Falk et al., 2016; Scott et al., 2010; J. P. R. Scott et al., 2011; Scott et al., 2014; K. L. SHEA et al., 2014; V. D. SHERK et al., 2017) although studies found that the concertation decreased 2 h post-exercise (Ashizawa, Fujimura, Tokuyama, & Suzuki, 1997; Rogers, Dawson, Wang, Thyfault, & Hinton, 2011). However, our data support the idea that this type of regular impact training by reducing basal PTH levels acts on bone leading to osteogenic effects. The main action of PTH is to ensure optimal plasma levels of calcium, and it is under strict feedback regulation (Vainionpää et al., 2009). Therefore, PTH response would be expected to mediate variation in systemic calcium concentration (Ashizawa et al., 1997; K. L. SHEA et al., 2014; V. D. SHERK et al., 2017). In agreement with this regulatory mechanism the reduction in PTH secretion was accompanied by a significant increase in Aa Calcium level after 20 weeks of SuperJump® training moreover, it was observed a significant increase in potassium concentration, supporting the positive influence of SuperJump® exercise on bone homeostasis. In vitro studies showed that low potassium concentration can stimulate bone resorption and interventional studies in humans showed that low potassium concentration induces calcium excretion that in turn impacts bone remodeling (MacGregor & Cappuccio, 1993; Marangella et al., 2004). Thus, both calcium and potassium seem to be involved in the action mechanism that improves bone turnover following 20 weeks of SuperJump® training. Calcitonin also impacts calcium homeostasis since its production is regulated by an increase in serum calcium level. The pharmacological function of calcitonin is to inhibit bone resorption through lowered levels of circulating calcium while the physiological role of calcitonin remains unclear. It is known that calcitonin plays a significant role in protecting the skeleton under circumstances of calcium stress (R. A. Davey & Findlay, 2013; Naot, Musson, & Cornish, 2019). In our study calcitonin was not affected by SuperJump® training ruling out its involvement in the action mechanism. Recent studies suggest an inverse correlation between Vitamin D and PTH in exercising subjects. In fact, during a 32-week training program involving military recruits, there was a high frequency of stress fractures that were associated with poor vitamin D and increased circulating concentrations of PTH (T. Davey et al., 2016). In our case, this study did not observe changes in vitamin D concentration after 20 weeks of exercise. Thus, vitamin D seems not to be involved in the observed effects. Phosphate is one of the most abundant minerals in the body, and its serum levels are regulated by a complex set of processes involving not only the skeleton but also the intestine and kidneys. PTH directly causes phosphate resorption from bone and decreases its reabsorption in the proximal tubule, and indirectly by stimulating the production of calcitriol (Goretti Penido & Alon, 2012). However, similar to Vitamin D, no changes in phosphate levels were observed after 20 weeks of SuperJump® training in the woman. The presence of a control group allows for excluding changes in marker levels due only to seasonal variation (Woitge et al., 1998) and confirms that the positive effects on bone turnover in the exercise group were a result of exercise. The presence of a class activity group creates social interaction and makes it easy to learn exercises by gaining support from peers (Bulat et al., 2007; Kerschan-Schindl et al., 2002). The presence of the instructor minimizes the problems with the recollection of the exercise program, loss of motivation, and skewed image reported for how well participants have performed the exercise because the instructor controlled the adherence to the exercise and stimulate compliance.

Conclusions

In conclusion, 20 weeks of Superjump® exercise in eumenorrheic women reduced bone resorption and increased formation. PTH, calcium, and potassium seem to play a role in the osteogenic effects. Thus, the Superjump® exercise might be used as a tool for the prevention of osteoporosis in middle age women.

2.5 Effects of a resistance training protocol on physical performance, body composition, bone metabolism, and systemic homeostasis in patients diagnosed with Parkinson's disease: A pilot and feasibility studies.

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Bone metabolism is defined as the balance between the resorption activity, by osteoclasts, and the deposition of new bone matrix by osteoblasts. It is regulated by the endocrine signaling of hormones that regulate calcium, including parathyroid, calcitonin, and 1α, 25-dihydroxy vitamin D3, as well as by mechanical stimuli (Sato, Kaji, Tsuru, & Oizumi, 2001). The alterations of resorption-formation mechanism balance are associated with pathologies characterized by motor impairment (Vaidya et al., 2021) as in Parkinson's disease (PD), in which motor symptoms are associated with a decrease in bone mineral density (Choi et al., 2021).

Parkinson's disease (PD) is a neurodegenerative disorder, increases in prevalence with age, that causes motor and non-motor symptoms such as depression/psychosis but also emotional and communicative changes like the typical "facial masking" and emotional speech (i.e., dysarthria) that could result in "social symptoms" such as stigma, dehumanization, and loneliness, with a consequent worst quality of life(Prenger, Madray, Van Hedger, Anello, & MacDonald, 2020).

To classify the PD neurologists use the visual examination of motor tasks and semi-quantitative rating scales divided into five stages by Hoehn–Yahr (Hoehn & Yahr, 1967). The incidence of PD cases in 2019 was 1.1 million camp(Ou et al., 2021). The economic burden of Parkinson's was estimated to be $ 52 billion in 2017 in the USA but these costs are underestimated. This is because intervention to delayed disease progression, and incidence and alleviate symptoms may reduce the future economic cost for society, patients, caregivers, and payers. (Yang et al., 2020). To make the diagnosis the subject must present distal resting tremor of 3 to 6 Hz, rigidity, bradykinesia, and asymmetrical onset(Riccò et al., 2020). According to the AAN guideline in 2002 (Miyasaki, Martin, Suchowersky, Weiner, & Lang, 2002), the therapeutic options to relieve motor symptoms in the early stages of PD are based on enhancing dopaminergic tone with levodopa, monoamine oxidase inhibitors, dopamine agonists (DAs), or a combination thereof. The choice of treatment is influenced by the potential neuropsychiatric adverse effects associated with DAs. The recent AAN ISSUES GUIDELINE FOR TREATMENT OF EARLY PARKINSON'S DISEASE(Pringsheim et al., 2021) recommends treatment of motor symptoms with levodopa medications over treatment with dopamine agonists more than DAs.

Physical activity is a key stimulus for symptoms management, it also produces lactate and myokines that contribute to brain health (Adams, 2021; Amato, Ragonese, et al., 2021; Proia, Di Liegro, Schiera, Fricano, & Di Liegro, 2016). Several factors influence bone metabolism during physical activity such as nutrition as meals influence bone resorption through mechanisms still under study such as the secretion of gut hormones (Amato et al., 2018a; Amato, Proia, et al., 2021; Sara Baldassano et al., 2022; S. Baldassano et al., 2022; A. Iannaccone, A. Fusco, SJ. Jaime, et al., 2020; Messina et al., 2019; Proia et al., 2021; Vasto S, 2022; S. Vasto, Amato, Proia, & Baldassano, 2022).

However, this mechanical stimulus must have a specific range within which the bone tissue response is activated, specifically from 50 to 100 microstrain (με), defined as minimum effective strain (MES) (H. M. Frost, 1990).

Exercise seems to be effective to stimulate bone formation if it has certain characteristics such as impact with the ground or other surfaces (Sonya Vasto et al., 2021). In addition, a mechanism that explains the importance of mechanical loading is that this loading, through mRNA stimulation, regulates the activity of osteogenic and bone resorption factors while maintaining the properties of the tissue (Guo et al., 2015). There are receptors such as those of collagen, and mechano-sensitive integrins present in connective tissues that are activated exclusively upon mechanical stimulus (Schleip, 2015). Therefore, the reduction or complete absence of mechanical stimulus causes alteration and loss of bone tissue, predisposing to diseases related to it. The loss of mechanical stimulus is the main cause of bone disease in PD due to decreased daily life physical activity. Typically people with PD, frequently adopt a sedentary lifestyle as a result of their reduced postural stability, gait disturbances, and decreased strength. However, reduced physical activity, although the most obvious, is not the only cause of reduced bone mineral density (BMD) in PD: Calcium metabolism regulatory hormones and vitamin D are extremely deficient in PD patients (Fink et al., 2008; Handa et al., 2019); High-dose treatment with the dopamine precursor, levodopa, is the current standard drug for PD, and is associated with low BMD due to the deleterious effects of homocysteine (Hcy), a toxic metabolite that inhibits the bone formation (Handa et al., 2019; Postuma & Lang, 2004; Sato et al., 2001; van Meurs et al., 2004). Moreover, it has been shown that one of the possible causes of the onset of PD is genetic (Deng et al., 2018; Lesage & Brice, 2009; Zimprich et al., 2004). Some genes whose altered expression is linked to PD have been identified: the "Parkinson-designated genes" (PARKS). The PARKS are expressed not only in the brain but also in other tissues including bone cells (Xiong et al., 2021). They play a key role in bone tissue homeostasis, they act on osteoblastic differentiation of Bone marrow stromal cells (BMSCs), they have a role in osteogenesis and osteoclast genesis and they indirectly contribute to increasing bone thickness (Berwick et al., 2017; Cui et al., 2021; Deng et al., 2018; Kim et al., 2017; Lesage & Brice, 2009; Miyake et al., 2012; Xu et al., 2021; Zimprich et al., 2004).

Beyond drug therapy, few or no "tools" have been identified in the literature that can help to reduce the resulting BMD loss in people with PD, including which exercise would be best suited for this purpose. To date, there are no studies that analyze the characteristics that physical exercise could have to better stimulate bone metabolism in people with neurodegenerative disease. Recent literature tends to simply associate daily physical activity with BMD through the use of accelerometers or data collected by self-report questionnaires (Khlebtovsky et al., 2017; Lorefält, Toss, & Granérus, 2007). Consequently, the purpose of our study was to evaluate the effects of a resistance training protocol on physical performance, body composition, bone metabolism, and systemic homeostasis. Additionally, this study intends to pave the way to understand whether physical exercises could be supportive of drug therapy to slow and manage the symptoms of disease progression and improve the quality of life of people with Parkinson's disease.

Materials and Methods

We recruited 13 subjects (age 64,83 ± 5,70) diagnosed with Parkinson's disease according to the criteria of the "United Kingdom Parkinson society Brain Bank"(Postuma et al., 2015). All patients were recruited from the neurological center "Paolo Giaccone" University of Palermo by a call in October 2021 from patients being treated at that hospital facility. Of the 13 patients, 11 (n = 84.6%) were male and the median disease duration in years was 5,7 ± 3,08. The inclusion criteria were: 1) Parkinson's diagnosis; 2) no dementia; 3) more than 50 years old; 4) self-sufficient walking; 5) being able to carry out our motor performance tests independently; 6) attending all the training sessions.

One subject was drop out because he didn't attend all the training sessions. Twelve subjects completed all training sessions and assessments. The participants performed motor performance tests, postural and static analysis, body composition analyses, and blood sample analyses at baseline (T0) and after 11 weeks of training (T1). They joined and completed the short-term intervention study with excellent compliance. The "Waterloo Footedness questionnaire (WFQ-R)" was administered to determine their dominant leg (van Melick, Meddeler, Hoogeboom, Nijhuis-van der Sanden, & van Cingel, 2017). All patients were evaluated with the Hoehn & Yahr (H&Y)(Goetz et al., 2004) staging and the Unified Parkinson's Disease Rating Scale (UPDRS)(Ramaker, Marinus, Stiggelbout, & Van Hilten, 2002). To exclude patients with dementia, those who scored less than 21 on Montreal cognitive assessment (MoCA)(Biundo et al., 2013) test were excluded from the current study. The Levodopa Equivalent Daily Dose (LEDD)(Julien et al., 2021) was also calculated for each patient to take into account the daily therapeutic load.

Motor performance tests

All the motor performance tests were performed at the Sport and Exercise Sciences Research Unit laboratory at the University of Palermo (Italy). Each participant was asked to come early in the morning. After the motor evaluations, within two-three days, the subjects would go to the neurology center in Palermo to have their blood drawn. All initial evaluations were done in November 2021

Postural and static analysis

The postural (also known as stabilometry) and the static (also known as baropodometry) analyses were performed with the FreeMed posturography system (produced by Sensor Medica, Guidonia Montecelio, Roma, Italy).

For the postural analysis, the subjects stood on the platform under two conditions: open (OE) and closed eyes (CE) for 51,2 seconds each with an angle of 30° between the feet. We have taken into consideration the Sway area (cm^2), the area which contains the trajectory of the center of pressure (COP); the sway paths length (mm), the length of the distance traveled by the COP, and the Sway average speed (mm/s), the average speed at which the COP moves in its whole trajectory with OE and CE. As regards the static analysis all participants stood on the platform for 10 seconds with the most comfortable position of the feet and the following parameters were evaluated: the foot area (cm^2), forefoot, and hindfoot load (%) for the left and right foot.

Cervical ROM

The cervical range of movement (ROM) was evaluated with an accelerometer (Sensor Medica) to measure the lateral rotation (°), lateral inclination, flexion, and extension (°)

Timed Up and Go

The Timed Up and Go (TUG) test is a simple test that enables the measurement of the level of functional mobility and dynamic balance skills. It measures the time it takes a person to get up from a chair, walk 3 meters, turn around, go back to the chair and sit down again. A score of 10 seconds or less indicates normal mobility; times between 11 and 20 seconds are within normal limits for elderly with frailty e disabled patients; times longer than 20 seconds indicate that the person needs external assistance. A score greater than 30 seconds suggests that the person has a high risk of falls (Bohannon, 2006). Alternatively, a recommended reference value as the limit between a normal capacity and one below normal is 12 seconds (Bischoff et al., 2003).

Five times sit to stand (5TSTS)

For standardized administration of the 5TSTS, we followed the Academy of Neurologic Physical therapy guideline (therapy). The participant was asked to get up and sit back in the chair 5 times. The time taken to complete the test was recorded.

Functional reach test

This test evaluates the static balance through maximal forward reach from a fixed base of support (P. W. Duncan, Weiner, Chandler, & Studenski, 1990). For the starting position, the subject stood with the dominant upper limb side of the body against the wall and the dominant upper limb extended at a 90 ° angle between the torso and the limb. The difference between the arm's length and maximal forward reach is measured, the test was repeated three times and was considered the best measure.

Grooved pegboard test

The grooved pegboard test evaluates manual dexterity, i.e., the skills that allow us to coordinate the hands and fingers to manipulate objects essentially in daily life (Wang et al., 2011). This ability is often compromised in neurological diseases. We used the Lafayette Instrument Grooved Pegboard Test tool. At the start signal, the subject placed pegs from left to right for the right-hand task and in opposite direction for the left hand. The dominant hand trial is administered first, followed by the nondominant hand trial. The time was measured with a digital chronometer. All participants performed the test two times for both dominant e non-dominant hands, but we took into consideration just the results of the dominant limb.

Handgrip test

The Handgrip test measures the maximum strength of the muscles of the hand and forearm through the use of a digital dynamometer (Karatrantou, 2019). The subject holds the handgrip keeping the arm extended at the bodyside; at the operator's signal, the participant must compress the handgrip as tightly as possible, for 3 seconds. The test must be repeated 3 times for each limb. The dynamometer used has three calibrations expressed in kg: 20 (for children), 40 (for women), and 80 (for men). The best result of the three trials for each limb was considered.

Eye-hand reaction and the foot-reaction time test

We performed the eye-hands reaction test and the foot-reaction time test to evaluate the reaction skills. The tests are based on the measurement of the time interval between the presentation of a visual or acoustic stimulus and the execution of a response (pressing a specific button or lifting the foot off the floor). The tests were performed in the “simple task” condition, in which there was only one stimulus to which one must be given only one answer. For the eye-hands reaction test, the participant was seated in a chair with the dominant limb placed on the table in front (90 ° between arm and forearm) where the computer was placed, with the index finger resting on the answer button. The subject had to press the button with the dominant limb once the light on the screen turned green. The test includes five evaluations, the average was calculated from the five scores obtained, expressed in seconds. To perform the foot-reaction time test we used the optical system OPTOJUMP (MICROGATE) and the OPTOJUMP NEXT software. The subject was standing with only one limb inside the platform, the OPTOJUMP NEXT software emitted a sound stimulus after which the subject had to lift the foot completely off the floor as quickly as possible and put it back in the shortest possible time. The test was carried out alternately with the right and left limbs for three times. The average of the reaction times recorded by the software was calculated to obtain the final value for each subject.

2.2. Body composition analysis

We analyzed the body composition by electrical bioimpedance measurement at 50 kHz, single frequency (BIAlight, DS MEDICA) to evaluate the following parameters before and after the training period: Body mass index (BMI), Total body water (TBW) (l), Free fat mass (FFM) (Kg), Fat (Kg), Skeletal muscle mass (SM) (Kg), Skeletal muscle mass index (SMI) (kg/m^2), Basal metabolic rate (BMR) (Kcal). The BIA was performed fasting, the subject was lying for 10 minutes before starting the assessment with the right hand and foot bare to allow the electrodes to be attached and without metal objects on him.

2.3. Blood sample analysis

A venous whole blood sample was collected in VACUTAINER tubes and EDTA-K3 between 7:00 and 8:00 AM (Sara Baldassano et al., 2018; S. Baldassano et al., 2019c). The blood sample was collected fasting at T0 and T1, to monitor the changes in bone markers and systemic homeostasis. Whole blood samples were collected in tubes without any anticoagulant and fractionated by centrifugation at 1.300 g for 15 min at room temperature to obtain serum. The serum was used to measure the following chemical markers using automated procedures according to standard commercially commercial assays from Roche Diagnostics performed on the Roche COBAS c501: glucose, insulin, total cholesterol, HDL-cholesterol, triglycerides, triiodothyronine (FT3), thyroxine (FT4), thyroid stimulating hormone (TSH). To evaluate insulin resistance and insulin sensitivity we used a specific computer model described in (S. Vasto et al., 2022; Sonya Vasto et al., 2022) updated method from Wallace et al. (2004) and Ghasemi et al. (2015) (Ghasemi et al., 2015; Wallace et al., 2004). Serum samples were analyzed for C-terminal telopeptide (CTX), a marker of bone resorption, osteocalcin (also referred to as bone-GLA-protein, BGP), a marker of bone formation, and the markers of bone metabolism parathyroid hormone (PTH), calcitonin, albumin-adjusted calcium (Aa calcium), vitamin D.

BGP, PTH, CTX, and Vitamin D were measured by electro-chemiluminescence immunoassay (ECLIA) following the same method used in Vasto et al. (Sonya Vasto et al., 2021), and fluctuations in protein concentrations were 'corrected' to give an albumin-adjusted calcium (Aa calcium) value using specific equation (Sonya Vasto et al., 2021).

2.4. Training protocol

The training period ran from December 2021 to February 2022. The training protocol lasted 11 weeks triweekly, it was performed at the participant's home using digital platforms with the help of two physical exercise experts. Each training session lasted 90 minutes and had 3 stages: the warm-up, the main workout, and the cool-down. The warm-up and the cool-down were the same for the entire duration of the study. The warm-up included breathing exercises and mobility exercises for the scapula-humeral, ankle, and hip joints, and the cervical spine and pelvic region. Each movement was performed for 10 repetitions or (if the movement was unilateral) 5 repetitions on each side with a rest period between 30'' and 40'' for each set. The difficulty of the exercises was increased with the use of different tools from one week to the next (Table 1). The cooldown included stretching exercises for the cervical spine, torso, upper limbs, and lower limbs. Each stretch position was maintained for 15 seconds. After the warmup, the main workout included three different weekly sessions (A, B, and C) of resistance training with intensity progressively increasing. Figure 1 is a general representation of each exercise performed by participants. The protocol, with the progression and the tools used, is detailed in table 1. Figure 1. Graphical representation of each exercise performed by study participants in the three different weekly sessions.

Figure 1. Graphical representation of each exercise performed by study participants in the three different weekly sessions. Note, each exercise could be performed with the support of a chair if the participant needed it.

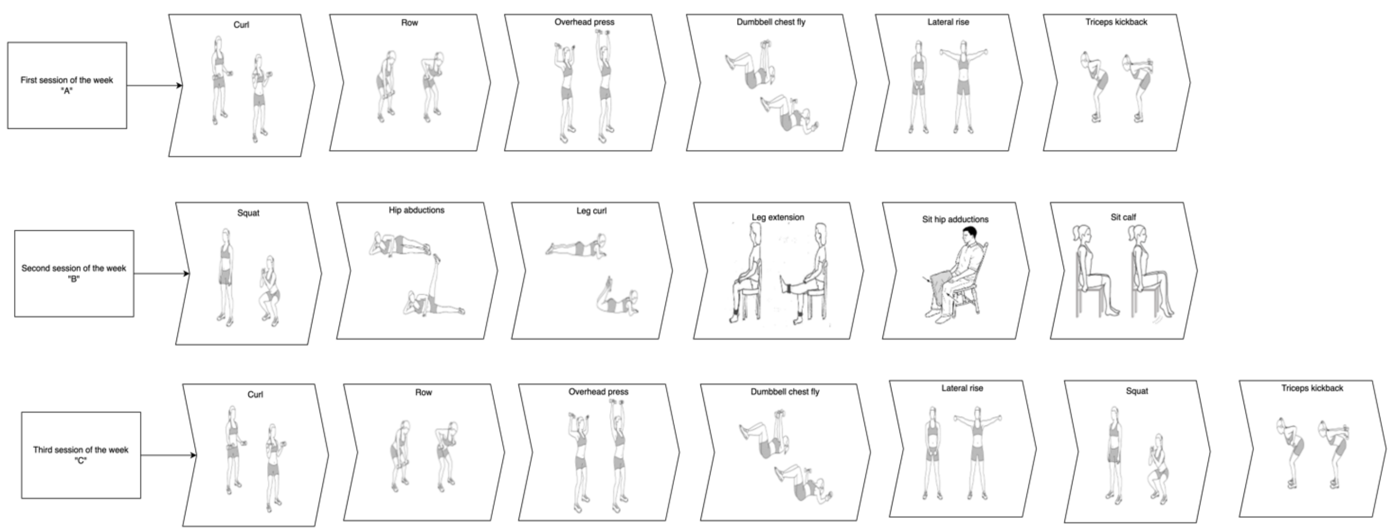


Table 1. Training sessions (A, B, and C) exercises and progressions.

SESSION	WEEKS OF TRAINING				
A	1 Set X reps ^{tool}	2, 3 Set X reps ^{tool}	4-6 Set X reps ^{tool}	7-10 Set X reps ^{tool}	11 Set X reps ^{tool}
CURL	3x15^{ws}	3x15^{ws}	2x15^{d}	3x15^{d}	3x20^{d}
ROW	2x15^{ws}	2x15^{ws}	2x10^{d}	2x15^{d}	2x20^{d}
OVERHEAD PRESS	2x10^{ws}	2x10^{ws}	2x6^{d}	2x8^{d}	2x15^{d}
DUMBBELL CHEST FLY	3x10^{bw}	3x10^{bw}	3x8^{d}	3x10^{d}	2x15^{d}
LATERAL RAISE	3x10^{bw}	3x10^{bw}	3x6^{d}	3x8^{d}	2x20^{d}
TRICEPS KICKBACK	2x10^{bw}	2x10^{bw}	2x6^{d}	2x6^{d}	2x10^{d}
B					
SQUAT	2x10^{bw}	2x10^{bw}	2x10^{d}	2x15^{d}	2x15^{d} + 10’’ isometric
HIP ABDUCTIONS	2x10^{bw}	2x10^{bw}	3x8^{bw}	3x12^{bw}	3x15^{eb}
LEG CURL	2x10^{bw}	2x10^{bw}	3x8^{bw}	3x12^{bw}	3x12^{eb}
LEG EXTENSION	2x10^{bw}	2x10^{bw}	3x8^{bw}	3x12^{bw}	3x15^{bw}
SIT HIP ADDUCTIONS	2x10^{p}	2x10^{p}	3x8^{p}	3x12^{p}	3x15^{p}
SIT CALF	2x10^{ws}	2x10^{ws}	3x8^{ws}	3x12^{ws}	3x15 (stand)
C					
CURL	3x15^{ws}	3x15^{ws}	2x15^{d}	3x15^{d}	3x20^{d}
ROW	2x15^{ws}	2x15^{ws}	2x10^{d}	2x15^{d}	2x20^{d}
OVERHEAD PRESS	2x10^{ws}	2x10^{ws}	2x6^{d}	2x8^{d}	2x15^{d}
DUMBBELL CHEST FLYS	3x10^{bw}	3x10^{bw}	3x8^{d}	3x10^{d}	2x15^{d}
LATERAL RAISE	3x10^{bw}	3x10^{bw}	3x6^{d}	3x8^{d}	2x20^{d}
SQUAT	2x10^{bw}	2x10^{bw}	2x10^{d}	2x15^{d}	2x15^{d} + 10’’ isometric
TRICEPS KICKBACK	2x10^{bw}	2x10^{bw}	2x6^{d}	2x6^{d}	2x10^{d}

Superscript letters indicate the tool used to perform the exercise: bw= body weight; d= dumbbell (two for 1 kg each one); eb= elastic band; ws= wooden stick; p= pillow.

2.6. Ethics

The principles expressed in the Declaration of Helsinki were met in our study. The ethics committee commission from the University Of Novi Sad Faculty Of Sport And Physical Education, gave us the following approval number:46-06-02/2020-1 Novi Sad, Serbia. All participants were informed about the study before the start and they signed written informed consent.

3. Statistical analysis

We used the Shapiro–Wilk test to assess the normality of data. To analyze the difference between the mean of the variables that resulted in not being normally distributed we performed the non-parametric test Wilcoxon rank and we used the paired t-test to analyze the differences between the normally distributed variables. Pearson’s coefficient was calculated to analyze the correlations between bone metabolism markers. An r value < 0.5 was considered with a low correlation, values between 0.5 and 0.7 moderate correlation, and values > 0.7 indicated a strong correlation. Statistical analysis was performed with R-Studio (© 2009-2022 RStudio, PBC) and SPSS (IBM SPSS Statistics, version 23) software.

Results

The anthropometric and clinical characteristics of patients are summarized in the following table (table 2)

Table 2. Clinical and anthropometric characteristics of patients with PD (mean, SD).

Parameters	Mean ± SD
Number of participants	*13*
Age	*64,83 ± 5,70*
Height	*171,58 ± 7,30*
weight	*76,93 ± 13,54*
Disease duration	5.7 ± 3.08
Hoehn and Yahr	*1.5 ± 0.5*
UPDRS 1	7 ± 2.4
UPDRS 2	23.2 ± 4.5
UPDRS 3	25.2 ± 7.1
MoCA	24.5 ± 2.3
LEDD	288.5 ± 109.7

H&Y= Hoehn & Yahr; UPDRS= Unified Parkinson's Disease Rating Scale; MoCA= Montreal cognitive assessment; LEDD= Levodopa Equivalent Daily Dose.

Motor performance test and Body composition analysis

The changes in Motor performance and body composition variables before and after the training time are shown in table 3 as mean and standard deviation. We noted an improvement in the following tests: Timed up and Go (s)(V = 78, p-value = 0.00); Sit to stand test (s) (V = 75, p-value = 0.00); dominant peg-board Test (s) (V = 68, p-value = 0.02);Dominant foot-reaction time (V = 76.5, p-value = 0.00). Functional reach test (t = -3.13, df = 11, p-value = 0.01) and a change in pressure feet distribution: Left foot area (cm^2) (V = 58, p-value = 0.03); Left forefoot load (%) (t = 3.44, df = 11, p-value = 0.01); Left hindfoot load (%) (t = -3.44, df = 11, p-value = 0.01). Also the R lateral bending (°) changed significantly (t = -2.53, df = 11, p-value = 0.03).

Table 3. Motor performance and body composition variables at baseline (T0) and after 11 weeks (T1). Data from motor performance and body composition were collected at time 0 (baseline) and the end of the 11 weeks.

	T0		**T1**		**P-value**
	Mean	Std. Deviation	Mean	Std. Deviation	
Postural analysis					
Sway area OE (cm^2)	155.63	133.96	285.57	317.60	0.30

Sway paths length OE (mm)	649.09	147.48	827.91	286.62	0.03^
Sway average speed OE (mm/s)	13.15	2.93	16.68	5.80	0.04^
Sway area CE (cm^2)	429.90	516.67	287.15	333.63	0.30
Sway paths length CE (mm)	740.45	141.64	888.92	338.72	0.06
Sway average speed CE (mm/s)	14.90	2.70	17.87	6.84	0.06
Static analysis					
L foot area (cm^2)	93.58	17.50	83.08	20.05	0.03^
R foot area (cm^2)	94.75	21.11	88.67	23.18	0.22
R forefoot load (%)	57.75	13.47	53.75	14.16	0.10
L forefoot load (%)	47.25	12.14	40.00	12.53	0.01*
R hindfoot load (%)	42.25	13.47	46.25	14.16	0.10
L hindfoot load (%)	52.75	12.14	60.00	12.53	0.01*
Cervical ROM					
R lateral rotation (°)	54.98	9.14	59.84	16.47	0.18
L lateral rotation (°)	55.10	16.21	59.77	14.18	0.13
R lateral bending (°)	25.85	8.67	29.51	9.64	0.03*
L lateral bending (°)	26.46	12.37	28.18	11.15	0.36
Flexion (°)	43.05	9.29	45.53	12.88	0.47
Extension (°)	38.62	17.70	47.77	10.98	0.49
Motor function tests					
Timed up and go (s)	12.47	2.69	9.29	2.16	0.00^^
5x sit-to- stand test (s)	21.86	8.52	14.19	4.22	0.00^^
Functional reach test (cm)	31.37	7.06	38.73	5.34	0.01*
D peg board test (s)	137.00	49.61	126.87	51.59	0.02^
D hand-grip (n)	34.71	11.07	34.53	11.81	0.78
ND hand-grip (n)	31.88	10.53	33.28	11.22	0.18
D eye-hands reaction test (s)	0.54	0.36	0.41	0.11	0.07
ND foot-reaction time (s)	0.83	0.28	0.61	0.09	0.05
D foot-reaction time (s)	0.72	0.15	0.61	0.08	0.00^^
Body composition					
BMI	25.99	3.63	25.88	3.68	0.61
TBW (l)	39.99	6.35	40.78	8.16	0.60
FFM (Kg)	53.58	8.25	54.73	10.65	0.57
Fat (Kg)	23.33	9.34	21.84	8.61	0.45
SM (Kg)	28.57	5.80	28.62	6.61	0.95
SMI (kg/m^2)	9.63	1.44	9.63	1.75	0.98
BMR (Kcal)	1527.33	178.18	1552.00	229.99	0.57

The significative difference between T0 and T1 for the Wilcoxon rank test it's indicated with " ^" for P < 0,05 and "^^" for P<0,01; "*"= significative difference (P < 0,05) between T0 and T1 for the paired t-test; OE=open eyes, CE=closed eyes, L=left, R=right, D=dominant limb, ND=non dominant limb, BMI= body mass index, TBW= total body water, FFM= free fat mass, SM= skeletal muscle mass, SMI= Skeletal muscle mass index, BMR=basal metabolic rate.

Blood sample analysis

Changes between T0 and T1 in all parameters analyzed with blood analysis are shown in table 4. there were no changes after 11 weeks of resistance training in lipid and glucose metabolism. Also, FT4 and TSH were not affected, while there was an increase in FT3 values (p=0.00) suggesting that it impacts thyroid function. As regards bone markers we had an increased value for the osteocalcin (p=0.01), PTH (p=0.00), CTX (p=0.00), and calcium (p=0.00) concentration and a reduction in vitamin D (p=0.04) (tab.4).

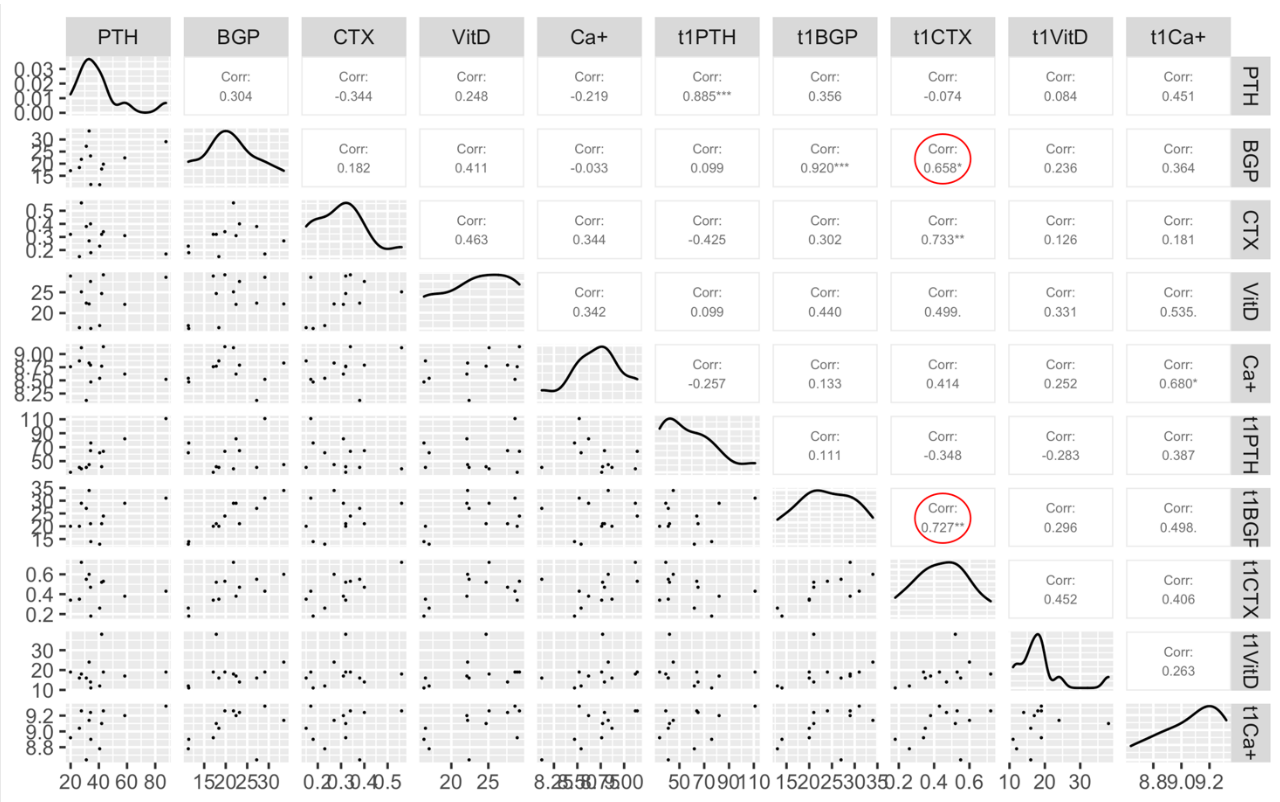
Table 4. Values of glucose metabolism, thyroid function, and bone metabolism markers at baseline and after 11 weeks of training. Blood samples were collected at time 0 (baseline) and the end of the 11 weeks (T1).

	T0		**T1**		**p-value**
	Mean	Std. Deviation	Mean	Std. Deviation	
Glucose metabolism markers					
Glycemia (mg/dL)	88.50	12.17	93.08	7.42	0.20
Insulin (mUI/L)	12.05	5.15	11.05	3.81	0.43
Insulin Sensitivity (%)	77.26	32.66	78.61	30.06	0.85
Insulin resistance	1.53	0.64	1.44	0.50	0.57
Lipid metabolism markers					
Total Cholesterol (mg/dl)	166.67	33.34	170.42	32.77	0.37
HDL Cholesterol (mg/dl)	49.33	9.59	52.08	10.13	0.11
Total HDL Cholesterol (mg/dl)	3.50	0.95	3.43	1.10	0.68
Triglycerides (mg/dl)	103.33	32.82	99.42	48.90	0.58
LDL Cholesterol (mg/dl)	96.67	30.79	98.45	30.00	0.58
LDL/HDL Cholesterol (mg/dl)	2.05	0.79	2.01	0.87	0.74
Thyroid function markers					
FT3 (ng/L)	3.25	0.70	4.64	0.47	0.00^^
FT4 (ng/L)	1.24	0.14	1.29	0.16	0.27
TSH (mlU/l)	1.79	1.05	1.80	0.87	0.79
Bone metabolism markers					
PTH (ng/L)	39.84	17.99	58.50	22.86	0.00^^
Osteocalcin (mcg/dL)	21.08	6.66	23.58	6.58	0.01*
CTX (ng/L)	0.30	0.12	0.44	0.15	0.00**
Vitamin D (mcg/dL)	23.38	4.83	18.58	7.04	0.04*
Calcium (mg/dL)	8.71	0.28	9.07	0.21	0.00**

Data are expressed as means and standard deviations. The significative difference between T0 and T1 for the Wilcoxon rank test it's indicated with " ^" for P < 0,05 and "^^" for P<0,01; "*"= significative difference (P < 0,05), "**"= significative difference (P < 0,01) between T0 and T1 for the paired t-test.

Correlation analysis between T0 and T1 for bone metabolism markers shows a strong positive correlation (red circle figure 2) between CTX T1 with BGP T0 (r=0.66, p<0.05) and with BGP T1 (r=0.73, p<0.01). Correlations are shown in Figure 2.

Figure 2. Correlations between all bone markers at baseline (T0) and after 11 weeks of training (T1).



"*"= significative difference (P < 0,05), "**"= significative difference (P < 0,01) between T0 and T1 for Pearson's correlation. PTH= parathyroid hormone, BGP= bone-GLA-protein (osteocalcin), CTX= C-terminal telopeptide, VitD= vitamin D, Ca+= calcium

Discussion

In the literature, the effects of resistance training in subjects with Parkinson's disease have been evaluated in many studies (Carvalho et al., 2015; Corcos et al., 2013; Demonceau et al., 2017; DiFrancisco-Donoghue et al., 2012; Helgerud et al., 2020; Hulbert et al., 2021; Silva-Batista et al., 2020). More specifically, some of these evaluate the effect of this training on respiratory capacity(Alves et al., 2019; Claus et al., 2021; Sapienza, Troche, Pitts, & Davenport, 2011; Troche et al., 2010; Troche, Rosenbek, Okun, & Sapienza, 2014), on the risk of falls and balance camp(Cherup et al., 2019; M. E. Morris et al., 2012; M. E. Morris et al., 2011; M. E. Morris et al., 2015; M. E. Morris et al., 2017; Shen & Mak, 2012, 2014; Silva-Batista et al., 2018), and the quality of life and depression camp(de Lima et al., 2019; Papaioannou et al., 2003; Silva-Batista et al., 2017). However, none of them evaluates the effect of resistance training on the bone metabolism of these subjects. Individuals with PD have a high predisposition to lose bone tissue, and the lack of scheduled exercise contributes to this (Fink et al., 2008; Handa et al., 2019; Xiong et al., 2021). Since, resistance training results in an increase in site-specific bone density more than other training (Benedetti, Furlini, Zati, & Letizia Mauro, 2018; E. C. Haakonssen et al., 2015; Harding et al., 2020), the purpose of this study was to evaluate the effect of home-based strength training on physical performance, body composition, bone metabolism, and systemic homeostasis in people with Parkinson's disease. In the context of evaluating the effect of this training on physical performance, we also wanted to assess whether neck stiffness, a hallmark of PD(MacMahon Copas, McComish, Fletcher, & Caldwell, 2021), varies as a result of resistance training because neck rigidity seems to be caused by the impossibility of voluntary control of the long-latency proprioceptive reflexes(Anastasopoulos, Maurer, Nasios, & Mergner, 2009) and training is associated with improved corticospinal plasticity for motor learning (Hotting & Roder, 2013; Hu, Avela, Kidgell, Piirainen, & Walker, 2022; Schlaffke et al., 2014; Singh, Neva, & Staines, 2016). Our results have been showing, although in the absolute value, an improved trend in all movements of the cervical ROM with a statistically significant improvement in the Right lateral bending (°) (p-value = 0.03). The axial tone was related to functional performance. Particularly, Frazen et al. showed how the neck tone is strongly correlated with balance and mobility disorders in individuals with PD(Franzén et al., 2009) and our resistance protocol influenced balance, evident in static analysis: it shows a better feet pressure distribution (%) between forefoot and hindfoot at T1, significant for the left foot which perfectly meets the reference parameters of weight and maximum pressure distribution (Ohlendorf et al., 2020) and the load distribution (%) for the right foot after the training period is closer to the reference parameters than T0 (forefoot: p=0.01; hindfoot: p=0.10) (Ohlendorf et al., 2020); the physiological pressure distribution of body weight at the feet is 60% on the rearfoot and to 40% midfoot and forefoot (Ohlendorf et al., 2020). Alterations of the connective tissue, in age atrophy or diseases such as Parkinson's which see an altered distribution of plantar pressure, modify the normal characteristics of the forefoot (Ohlendorf et al., 2020). People with Parkinson's disease have impaired control of their balance during gait and static posture, often related to injuries resulting from falls and consequently a worse quality of life. Tsakanikas et al. (2021) showed that the spatial static distribution of the Center of Pressure can provide important information about the status of the patient both for static balance and gait monitoring (Tsakanikas et al., 2021). The changes on feet pressure distribution affected, with significant improvements, the functional tests TUG test (dynamic balance), (T0= 12.47± 2.69, T1=9.29± 2.16, p =0.00), and Functional reach test (static balance), (T0=31.37± 7.06, T1=38.73 ± 5.34, p=0.01). Therefore, we hypothesized that reprogramming of postural tone, after the strength training period, could influence the pressure distribution at the feet and thus balance, and this influences the results of functional tests "TUG" and "functional reach".

Several studies found that people with Parkinson's disease needed more time to perform Reaction Time tests compared to healthy controls (Genoves et al., 2021; Rahman et al., 2021). Furthermore, Morrison et al with a similar reaction test for upper and lower limbs found a correlation between the risk of falling and slowing reaction time in Parkinson's disease (Morrison et al., 2021). However, this skill could be trained and improved by simple or dual-task practice as demonstrated by recent studies (Firouzi et al., 2021; Monaghan, Finley, Mehta, & Peterson, 2021). Nevertheless, no studies showed so far, the effect of strength training on reaction time and, consequently, by reducing the reaction time, its possible impact on the risk of falls. In our study the reaction time assessment, both to visual and acoustic stimulus, had an improving trend in the eye-hand reaction test (s) (from T0=0.54±0.36, to T1=0.41±0.11, p=0.07) and non-dominant foot-reaction time (s) test (T0= 0.83± 0.28, T1=0.61± 0.09, p= 0.05). A significant decrease in reaction time to the acoustic stimulus was found in the dominant foot-reaction time (s) test (T0=0.72±0.15, T1=0.61±0.08, p =0.00).

Manual dexterity is the skill that allows us to manipulate objects thanks to eye-hand coordination and it is a mark of motor function (Gershon et al., 2010). Usually, manual dexterity is evaluated as the time to complete the pegboard test (s) that in our study reduced from 137± 49.61 (T0) to 126.87±51.59 (T1) seconds.

It's established that people with PD have a worst pegboard test performance than healthy adults of a similar age group (Earhart et al., 2011). The increase in the time it takes to complete the pegboard test is correlated with declining psychomotor processing speed indicator of pathophysiological changes in top-down visual and motor control pathways. The pegboard test is also a predictor of the activities of daily living dysfunction (Hinkle & Pontone, 2021). In addition, our results show that the subjects after the 11 weeks of training passed from a score of 21,86 ± 8,52 (s) at T0 to a 14,19 ± 4,22 (s) (p=0.00) in the 5x sit-to-stand test. It's demonstrated that 16 seconds to complete 5TSTS discriminates the fallers from the non-fallers, particularly more than 16 seconds indicating risk for falls, as mentioned before after the training period subjects reduced their 5TSTS under 16 seconds (R. P. Duncan, Leddy, & Earhart, 2011).

However, this training for 11 weeks doesn't change body composition parameters (Tab.3) and this could result useful for these subjects who usually tend to quickly reduce their weight and their lean mass with the progression of the disease (Cumming, Macleod, Myint, & Counsell, 2017; Lorefält, Toss, & Granérus, 2009; S. Song et al., 2021; Uc et al., 2006). Regarding bone metabolism, our results suggest that could be an influence of resistance training on bone turnover, positive for osteocalcin (formation marker), which from 21.08 ± 6.66 (µg/L) at baseline increases significantly (p<0.05) in the post-training period to 23.58 ± 6.58 (µg/L). However, this increased formation activity, also evidenced by an increase in calcium (p< 0.01), seems to be balanced by the concomitant increase in reabsorption markers CTX (p< 0.01), PTH (p< 0.01), and the reduction at T1 of Vitamin D (p< 0.05). In contrast, the correlation (r= 0.727) at T1 between CTX T1 and osteocalcin T1 indicates that this process is in balance and corresponds to the normal balanced bone turnover process.

Thus, physical activity has a positive effect because meanwhile the two processes of formation and resorption are matched as they should in physiological conditions and there is no increased tendency for resorption as there is in disease conditions such as PD. In addition, besides the increase in osteocalcin and calcium, indicators of bone formation, the increase in PTH after physical activity may indirectly be indicative of bone formation (Proia et al., 2021). However, the role of PTH in response to physical activity is still unclear even more so in the presence of subjects with pathology.

Limitations

This study has limitations: first of all, the lack of a control group that did not perform physical activity although we evaluated each of the patients at baseline and the end of the training, in future studies a control group is necessary. Another limitation is the small number of participants. The training set and the pilot nature of the project made us unable to work with a larger number of subjects. Therefore, studies with a larger number of subjects are required. Another limitation was the lack of follow-up. We evaluated the effect of resistance training on bone metabolism in PD after 11 weeks of training. Given the chronic nature of the disease we don’t know, stopping the activity, for how long the benefits of the intervention were present and if continuing it longer they could further improve their condition. Once again further investigations are required.

Conclusions

In conclusion, this resistance training for people with Parkinson's is effective in improving physical performance and this is evident in the improvements of functional tests and would result in better daily living functions.

Also, this protocol with this frequency and progression seems to affect bone metabolism, certainly not increasing resorption at the expense of formation. Indeed, these two processes are shown to be in balance. Therefore, home-based resistance training could be a strategy to support drug therapy to prevent and reduce BMD loss associated with PD, maintain healthy body composition and improve functional physical performance.

3. Chapter 3 – Overview of the Ph.D. activities

3.1. Research areas of interest

During the "Health Promotion and Cognitive Sciences" Ph.D. program Alessandra Amato had two main research interests whose meeting point will be the goal of future works: the relationship between genetics and physical activity and the effect of the latter on bone metabolism in healthy and unhealthy subjects. Specifically for the first-mentioned research interest, starting from her technical skills in sports, Miss Amato investigated the influence of genetics on the performance of professional basketball players, and from this master's dissertation project she published four original articles useful for understanding how to exploit the knowledge of performance-enhancing polymorphisms (PEPs) in athletic training and injury prevention. For the second and current research interest, Miss Amato followed several projects about the effect of specific high-impact activity on bone formation and resorption markers and body composition, but also about the influence of nutrition on bone metabolism before and after exercise deepening the study of the characteristics that physical activity must have to obtain a good osteogenic potential even in pathologic subjects, particularly with Parkinson's and Multiple Sclerosis disease. Miss Amato's future goal will be to understand how physical exercise and genetics can cooperate to maintain adequate bone health in subjects who have BMD loss among their signs such as neurodegenerative diseases.

3.2. International Mobility

From November 2021 to April 2022 Miss Amato spent her abroad period at the University of Colorado, Boulder, United States of America. She was a visiting scholar in the Neurophysiology of Movement Laboratory of the Integrative Physiology department, under the supervision of prof. Roger Enoka. During this period Miss Amato was involved in three different research projects:

the first project investigated how tempo-controlled, moderate-intensity resistance training of the hip abductors and ankle dorsiflexors could influences force steadiness, strength, and postural sway, in older adults. Particularly, Amato was involved in the months of November and December 2021 in the evaluation and training sessions; in the months of January and February 2022, she worked on the revision of the abstract of the project presented to the American Society of Biomechanics (ASB) annual conference at Rocky Mountain national park on April 8-9 2022. The successive manuscript entitled: "Tempo-controlled resistance training of the hip abductors and ankle dorsiflexors with light loads does not improve postural sway in older adults" has been published in the Journal "Experimental Brain Research".

The second project aimed to investigate the correlation between the pegboard test performance (evaluated with a force transducer) to the signals of the "Earable" headband device. This is a wireless

system using 6 active dry electrodes, to record the EEG and EMG signals, an inertial measurement unit (IMU), and also the tracking of the eye blinking through the EOG signal.
The objective of the third project was to identify the physiological mechanisms responsible for differences in the time it takes middle-aged and older adults to complete a pegboard test of manual dexterity. prior studies indicate that the most consistent explanatory variable is force steadiness. However, the maximal strength of this association and the underlying mechanisms remain to be determined. She took part also in monthly meetings of KAGET and the International MotoNeuron Society and weekly lab meetings with professor Roger Enoka. Furthermore, she followed the "Advanced Statistics and Introduction to Research Methods" course in the Integrative Physiology department with prof. Steven Hobbs (University of Colorado, Boulder)**.** In September 2022 she spent one week at Granada University where she attended some evaluation sessions with tools like DEXA and QCT and she had a meeting on his latest work on the effect of resistance training in Parkinson's disease under the supervision of Dr. Luis Gracia Marco.

3.3 Skills and tools

Thanks to her main research topic, Miss Amato has learned to use tools for the assessment of body composition such as BIA both mono and multifrequency, and equipment for the assessment of motor performance: Opto-jump, handgrip, pegboard, Gyko, Polar system, T-force, lactate sampling from the fingertip. Furthermore, she used other tools for postural and balance control evaluation such as baropodometric and stabilometry analysis. She spent a research period at a biology laboratory under the supervision of prof. Patrizia Proia (University of Palermo) during which she performed DNA extraction, polymerase chain reaction (PCR), and electrophoretic mobility shift assay (EMSA) for the project performance-enhancing polymorphisms (PEPs) and sport. She had acquired skills in data management and the use of statistical software such as "SPSS", "And GraphPad". Furthermore, she followed "The Advanced Statistics and Introduction to Research Methods" course learning the use of programming, computing, and graphics platforms such as "R" and MATLAB. Furthermore, during her time in the prof. Enoka's laboratory has learned to use different tools and devices such as EMG, the "Spike 2" program to assess force steadiness, the wireless inertial sensors (Opals V2, APDM), "Earable" headband device, she also learned to make subcutaneous electrodes.

3.4 Abstract of published papers during the three years Ph.D. course

1. Bianco, A., Ravalli, S., Maugeri, G., D'Agata, V., Vecchio, M., D'Amico, A. G., … Musumeci, G. (2019). The "Journal of Functional Morphology and Kinesiology" Journal Club Series: Highlights on Recent Papers in Overtraining and Exercise Addiction. *Journal of Functional Morphology and Kinesiology*, *4*(4), 68. doi:10.3390/jfmk4040068

We are glad to introduce the seventeenth Journal Club. This edition is focused on several relevant studies published in the last years in the field of Overtraining and Exercise Addiction, chosen by our Editorial Board members and their colleagues. We hope to stimulate your curiosity in this field and to share with you the passion for the sport seen also from the scientific point of view. The Editorial Board members wish you an inspiring lecture.

2. Proia, P., Amato, A., Puleo, R., Arnetta, F., Rizzo, F., Di Grigoli, L., …& Messina, G. (2019). Efficacy of 12 weeks of proprioceptive training in patients with multiple sclerosis. Journal of human sport and exercise, 14(5proc), s1986-s1992. doi:10.14198/jhse.2019.14.Proc5.19

This pilot study aims to investigate if a specific training protocol can improve the quality of life in people with multiple sclerosis through the improvement of some impaired abilities like balance. We enrolled in our project 20 subjects (age: 34,88 ± 4,45; height: 168,25 ± 8,66 cm; weight: 72,31 ± 17,28 kg) but only 5 completed the study. A proprioceptive training was administered for 12 weeks, 2 times a week, an hour for each session. At the beginning (T0) and the end of the study (T1), static and dynamic balance parameters were tested. Statistical analyzes were performed using IBM SPSS Statistics 22. Changes in balance and strength between T0 and T1 were evaluated using t Student test for paired data. Pearson linear correlation coefficient was used to investigate the correlations between all parameters analyzed. As regards static baropodometry, there was a statistic significant different between left forefoot load (T0 54,25 ± 2,5 % and T1 59,5± 1,73%; P<0,05) and left rearfoot load (T0 45,75± 2,5% and T1 40,5± 1,73%; P<0,05). In dynamic baropodometry we gave more attention to three parameters: left length gait line (T0 176±23,58 and T1 215±18,64 (mm); P<0,05), right surface (T0 106,75±14,97 and T1 149±11,58 (cm); P<0,05) and right pressure point (T0 1050± 130,90 and T1 537,25±65,46 (gr/cm); P<0,05). In conclusion, it is possible to hypothesize that the application of proprioceptive treatment in patients with multiple sclerosis can improve gait stability and therefore walking. However, further studies, with a greater number of subjects, are necessary to confirm this trend.

3. Messina, G., Amato, A., Brighina, F., Lo Monaco, A., Giustino, V., Brusa, J., Iovane, A., & Proia, P. (2019). Lactate level and handgrip test in migraine and fibromyalgia after self-myofascial release treatment. Journal of human sport and exercise, 14(5proc), s2019-s2025. https://doi.org/10.14198/jhse.2019.14.Proc5.23

The study aimed to determine the effect of self-myofascial release (SMFR) treatment on handgrip test scores in patients with fibromyalgia and migraine, investigating also if the lactate levels influenced the result. Twenty-five subjects were affected by migraine (age 49.7±12.5; height 164.9±6.62 cm; weight 68.6±16.9 kg) and ten subjects were affected by fibromyalgia (age 43.7±21.2; height 158±5.65 cm; weight 70±28.9 kg) were enrolled in the study. The assessments included a handgrip test, to evaluate the isometric strength of both hands and forearm muscles and the lactate levels evaluation. The treatment was performed on the three most painful trigger points indicated by subjects as concerned with fibromyalgia and the suboccipital muscles for migraineurs subjects. The handgrip test score, at T0, showed respectively in fibromyalgia and migraine groups a result of 22,82 ± 10,58 and 24,74 ± 9,13 (kg) for the right hand and 23 ± 8,59 and 22 ± 9,13 (kg) for the left hand without any statistical significance difference. At T1 the handgrip test score showed a positive trend in increase in migraineurs (23,23 ± 8,18 (kg) and 21,03 ± 8,57 left hand (kg)) but without a statistical significance difference; vice versa in fibromyalgic subjects (24,95± 8,65 (kg) 22,29 ± 10,90 right hand (kg)). Given the interesting preliminary results, it needs further studies to confirm the trend, increasing the number of subjects and extending the treatment for a longer time.

4. Cataldi, S., Amato, A., Messina, G., Iovane, A., Greco, G., Guarini, A.,… & Fischetti, F. (2020). Effects of combined exercise on psychological and physiological variables in cancer patients: a pilot study. Acta medica mediterranea. 36: 1133. DOI: 10.19193/0393-6384_2020_2_174

This study aimed to investigate the effect of a short-term combined exercise intervention program on perceived self-efficacy, fatigue, lower back flexibility, balance, and task-specific functional mobility in cancer patients. Fifteen patients met all the eligibility criteria and were assigned to a single training group (range age, 22-75 years) that performed an 8-week intervention program (~60min, 2d·wk-1). Each session included progressive training in cardiorespiratory, resistance, flexibility, and postural education exercises. Measures pre-intervention and post-intervention included psychological and physiological measurements. Adherence to training was high (92.3±5.2%) and no major health problems were noted in the participants over the 8 weeks. Measures of fatigue have significantly decreased ($p<0.001$; -27.7%) and perceived capability to regulate negative affect ($p<0.001$; +18.2%) and to express positive emotions ($p = 0.003$; +11.8%) improved between the pre and post-study measurements. Highly significant increases were observed in the trunk lateral flexibility test (L: $p<0.001$; -13.2%; R: $p<0.001$; -12.8%), stork balance stand test (L: $p<0.001$, +30.1%; R: $p<0.001$,

+66.7%), and in the number of standing up and sitting down from a chair within 30 seconds ($p<0.001$; +20.4%). Results suggest that a short-term combined exercise program may improve the physical fitness, functional capacity, and capability to manage emotional life, and reduce levels of perceived fatigue in cancer patients providing important support to deal with the physiological and psychological side effects. Specialists in Adapted Physical Education need to be involved in the biomedical staff because they are the only ones able to manipulate the training variables for the health and well-being benefit of the special populations.

5. Amato, A., Baldassano, S., Proia, P., Messina, G., D'amico, G. (2020). Effects of protein supplementation in the fitness world: a 12-week cross-over studio. Journal of human sport and exercise, 15, 308-314. https://doi.org/10.14198/jhse.2020.15.Proc2.22

This project aimed to evaluate the effect of isolated protein supplementation in young amateur athletes. Sixteen subjects aged between 20 and 30 were recruited for this study. Before starting sports performance was assessed at T0, in all subjects, using a physical performance test and evaluated body composition. Therefore, the subjects were randomly assigned to two groups (groups A and B) of 8 subjects each. Group A started to intake 30 g of protein powder diluted in water after each training session (3 times a week) for six weeks (T0) whilst group B was the placebo. After 6 weeks (T1), the measurement was repeated for all subjects, group A became a placebo, and group B started with the supplementation. At the end of 6 weeks (T2), we carried out all the tests performed again. Results showed a significant improvement in almost all tests between T0 and T2 within the same group ($p < .05$), but no significant difference was found between the start and the end of the protein intake period in both groups. In conclusion, supplementation did not affect performance and body composition significantly. Instead, training seems to influence performance more the supplementation.

6. Messina, G., Francavilla, V.C., Giustino, V., Mingrino, O., Genovesi, F., Amato, A., Proia, P. (2020). Use of nutritional supplement to improve performance in professional soccer players: A case report. Journal of Human Sport and Exercise, 15(2proc), S289-S298. doi:https://doi.org/10.14198/jhse.2020.15.Proc2.20

The study aimed to investigate the intake percentage and the satisfaction level of some nutritional supplements used by professional soccer players. Twenty-nine professional soccer players (age: 24.6 ± 5.2 years, body weight: 79.2 ± 4.9 kg, body height: 1.83 ± 0.05 m) belonging to a team of Serie A were interviewed on: their frequency of use, tolerability, and acceptance of the supplements (creatine, β-alanine, whey protein, nitrates, vitamin D3, caffeine) proposed by the nutritionist team. This survey revealed great interindividual variability in the intake of the proposed supplements. All respondents (n = 29) said they take cholecalciferol (vitamin D3), 17 out of 29 creatine, 14 out of 29 whey protein, and 10 out of 29 dietary nitrates. No participants declared to assume β-alanine or caffeine anhydrous. Cholecalciferol resulted in the most accepted supplement, followed by creatine and whey protein. Study participants prefer to take dietary nitrates through the consumption of vegetable juices, primarily from fennel and celery juice, and only two out of twenty-nine regularly take concentrated beet juice. Since none of the twenty-nine participants interviewed uses β-alanine and caffeine in anhydrous form, the daily contribution of caffeine is mainly guaranteed by the consumption of coffee.

7. Vitello, S., Di Liegro, I., Ricciardi, M.R., Verga, C., Amato, A., Schiera, G.,… & Proia, P. (2020). Correlation between polymorphism of tyms gene and toxicity response to treatment with 5-fluorouracil and capecitabine. Eur j transl myol. 4;30(3):8970. Doi: 10.4081/ejtm.2020.8970. Pmid: 33117504; pmcid: pmc7582406.

Tumorigenesis is a multiphasic process in which genetic alterations guide the progressive transformation of cancer cells1. To evaluate the possible correlation between some gene variants and the risk of the toxicity development onset, two of the polymorphisms of the thymidylate synthase (TYMS), rs34743033 (2R/3R) and rs16430 (DEL/INS) were investigated. We enrolled in our study 47 patients from the Hospital of Sicily. Our preliminary findings suggest that there could be a linkage between the genotypes discussed and the development of toxicity following the chemotherapy treatment. These results need to be confirmed by further studies, however, this short paper offers some initial insight into the relationships between genetic background and the better outcome for patients.

8. Amato, A., Messina, G., Giustino, V., Brusa, J., Brighina, F., Proia, P. (2021). A pilot study on non-invasive treatment of migraine. Eur j transl myol 31 (1): 9646, 2021 doi: 10.4081/ejtm.2021.9646

This paper aimed to determine the effect of self-myofascial release (SMFR) on postural stability and to analyze if it can influence migraine conditions. Twenty-five subjects (age 49.7± 12.5) affected by migraine were enrolled. Assessments included a stabilometric analysis to evaluate balance and plantar support, with eyes open (OE) and closed (CE); cervical ROM measurement; evaluation of upper limb strength through handgrip. All the analyses were carried out before and after the administration of a single SMFR protocol, using medium-density small balls laid in the three most painful trigger points in migraine patients: trapezius, sternocleidomastoids, and suboccipital muscles. Performing a T-test for paired samples, there was a significant increase in two ranges of the stabilometric analysis: ellipse surface, both with open and closed eyes (p-value EO = 0.05; p-value EC = 0.04) and length of the sway path, but just with closed eyes (p-value = 0.05). SMFR might have a positive impact on postural stability in subjects with migraine. Further investigation should be conducted to confirm the hypothesis.

9. Amato, A., Proia, P., Caldara, G.F., Alongi, A., Ferrantelli, V., Baldassano, S. (2021). Analysis of body perception, pre-workout meal habits and bone resorption in child gymnasts. Int. J. Environ. Res. Public health 18, 2184. Https://doi.org/10.3390/ijerph18042184

The beneficial effects of physical activity on body image perception and bone are debated among artistic gymnasts. Gymnasts seem to be at greater risk of developing body dissatisfaction, eating disorders, and osteoporosis due to inadequate nutrition and attention to the appearance of the body. The objective of this work was to investigate the association between the artistic gymnast and a more favorable body image compared to their sedentary peers and if a pre-workout high-carbohydrate meal (HCM; 300 kcal, 88% carbohydrates, 9% protein, 3% fat) or high-protein meal (HPM; 300 kcal, 55% carbohydrates, 31% protein, 13% fat) can attenuate bone resorption in young rhythmic gymnasts. Twenty-eight preadolescent female gymnasts were examined. Self-esteem tests were used to analyze body image perception. Preworkout eating habits were examined by short food frequency questions (FFQ) validated for children. The biomarker of the bone resorption C-terminal telopeptide region of collagen type 1 (CTX) was measured in the urine (fasting, postmeal, and postworkout). Gymnasts reported higher satisfaction with their body appearance compared to sedentary peers. Of the gymnasts, 30% did not have a pre-workout meal regularly, and the timing of the consumption was variable. Bone resorption was decreased by the HCM, consumed 90 min before the training, concerning the HPM. The study suggests that playing artistic gymnastics is associated with a positive body self-perception in a child. The variability in pre-workout meal frequency and timing needs attention to prevent inadequate eating habits in light of the ability of the HCM to reduce acute bone resorption.

10. Proia, P., Amato, A., Drid, P., Korovljev, D., Vasto, S., Baldassano, S. (2021). The impact of diet and physical activity on bone health in children and adolescents. Frontiers in endocrinology 12, 1080. DOI=10.3389/fendo.2021.704647

There is growing recognition of the role of diet and physical activity in modulating bone mineral density, bone mineral content, and remodeling, which in turn can impact bone health later in life. Adequate nutrient composition could influence bone health and help to maximize peak bone mass. Therefore, children's nutrition may have lifelong consequences. Also, physical activity, adequate in volume or intensity, may have positive consequences on bone mineral content and density and may preserve bone loss in adulthood. Most of the literature that exists for children, about diet and physical activity on bone health, has been translated from studies conducted in adults. Thus, there are still many unanswered questions about what type of diet and physical activity may positively influence skeletal development. This review focuses on bone requirements in terms of nutrients and physical activity in childhood and adolescence to promote bone health. It explores the contemporary scientific literature that analyzes the impact of diet together with the typology and timing of physical activity that could be more appropriate depending on whether they are children or adolescents to assure optimal skeleton formation. A description of the role of parathyroid hormone (PTH) and gut hormones (gastric inhibitory peptide (GIP), glucagon-like peptide (GLP)-1, and GLP-2) as potential candidates in this interaction to promote bone health is also presented.

11. Amato, A., Messina, G., Feka, K., Genua, D., Ragonese, P., Kostrzewa-Nowak, D.,… & Proia P. (2021) Taopatch® combined with home-based training protocol to prevent sedentary lifestyle and biochemical changes in ms patients during covid-19 pandemic. Eur j transl myol. Doi: 10.4081/ejtm.2021.9877. Epub ahead of print. Pmid: 34498450.

In Multiple sclerosis (MS) it is important to preserve the residual physiological functions of subjects. The present study investigates the influence of nanotechnological device treatment combined with a home-based training program (TP) on lactate level, hand grip strength, and cervical mobility in MS patients. Seventeen MS patients were enrolled in the study and randomly assigned to an experimental group (EG) in which the Taopatch® nanotechnological device was applied or to a control group (CG). All the participants carried out a cervical range of motion (1) assessment and the hand grip test at baseline (T0) and after TP (T1), also investigating the lactate levels to figure out if there could be a correlation with the possible changes in the investigated parameters. The results showed no significant differences in both groups for ROM. As regards the hand grip test, EG showed a statistically significant improvement in strength for both hands, dominant ($p = 0.01$) and non-dominant ($p = 0.04$), while the CG showed an improvement only for the non-dominant hand ($p = 0.001$). No correlation was found between baseline lactate level and cervical ROM change. We can definitely conclude that exercise and Taopatch® can help to improve and maintain hand strength in

MS subjects and also can prevent a sedentary lifestyle during the COVID-19 pandemic time. These are preliminary results that need further investigations, possibly increasing the sample size and lengthening the time of intervention.

12. Amato, A., Ragonese, P., Ingoglia, S., Schiera, G., Schirò, G., Di Liegro, C.M.,… & Proia P. Lactate threshold training program on patients with multiple sclerosis: a multidisciplinary approach. *Nutrients*. 2021; 13(12):4284. Https://doi.org/10.3390/nu13124284

Physical activity could play a key role in improving the quality of life, particularly in patients with nervous system diseases such as multiple sclerosis (MS). Through lactacid anaerobic training, this study aims to investigate the effects at a bio-psycho-physical level to counteract the chronic fatigue associated with the pathology and to improve mental health at a psychological and neurotrophic level. Eight subjects (age: 34.88 ± 4.45 years) affected by multiple sclerosis were involved. A lactate threshold training program was administered biweekly for 12 weeks at the beginning of the study (T0), at the end of the study (T1), and 9 months after the end of the study (T2), with physical, psychological, and hematochemicals parameters, and dietary habits being tested. The results obtained confirmed that Lactaid exercise can influence brain-derived neurotrophic factor (BDNF) levels as well as dehydroepiandrosterone sulfate (DHEAS) levels. In addition, levels of baseline lactate, which could be best used as an energy substrate, showed a decrease after the protocol training. Self-efficacy regarding worries and concerns management significantly increased from T0 to T1. The eating attitudes test (EAT-26) did not highlight any eating disease in the patients with a normal diet enrolled in our study. Physical exercise also greatly influenced the patients psychologically and emotionally, increasing their self-esteem. Lactate threshold training, together with dietary habits, appears to exert synergic positive effects on inflammation, neural plasticity, and neuroprotection, producing preventive effects on MS symptoms and progression

13. Vasto, S.$^{\delta}$, Amato, A.$^{\delta}$, Proia, P., Caldarella, R., Cortis, C., & Baldassano, S. (2022). Dare to jump: The effect of the new high-impact activity SuperJump on bone remodeling. A new tool to maintain fitness during COVID-19 home confinement. Biology of Sport, 39(4), 1011-1019. https://doi.org/10.5114/biolsport.2022.108993

SuperJump is a high-impact activity performed on an elastic trampoline that mixes aerobic and anaerobic exercises already proposed as a home-based activity for preventing a sedentary lifestyle. We determined in a randomized controlled trial whether 20 weeks of SuperJump activity would promote bone formation and reduce resorption in eumenorrheic women. Twenty-four women were randomized to a non-exercise group (control group) or an exercise group that performed SuperJump activity three times a week for 20 weeks. Blood samples were collected in both groups at baseline and the end of the 20 weeks and compared within and between the groups for C-terminal telopeptide (CTX), a marker of bone resorption, osteocalcin, a marker of bone formation, and the markers of bone metabolism parathyroid hormone (PTH), calcitonin, albumin-adjusted calcium (Aa calcium), vitamin D, phosphate and potassium. After 20 weeks of SuperJump activity, levels of CTX were significantly reduced while levels of osteocalcin were increased. PTH, calcium, and potassium were involved in the mechanism of action because PTH was reduced while calcium and potassium were increased. Calcitonin, vitamin D, and phosphate levels did not change. These data suggest that SuperJump activity can reduce bone resorption and improve bone formation by acting on essential regulators of bone metabolism. They also suggest that SuperJump training may be used as a valuable intervention to prevent the occurrence of osteoporosis in aging because it improves bone homeostasis in favor of bone formation and could counteract a sedentary lifestyle, such as during COVID-19 home confinement, which could itself contribute to the variation of bone metabolism.

14. Vasto, S., Amato, A., Proia, P., & Baldassano, S. (2022). Is the Secret in the Gut? SuperJump Activity Improves Bone Remodeling and Glucose Homeostasis by GLP-1 and GIP Peptides in Eumenorrheic Women. Biology, 11(2), 296. https://doi.org/10.3390/biology11020296

We showed that twenty weeks of SuperJump activity, an innovative workout training performed on an elastic minitrampoline, reduced bone resorption and increased bone formation in eumenorrheic women acting on the key points of the regulation of bone metabolism. The present study analyzed whether gastrointestinal hormones are involved in the mechanism of action and if it has an impact on glucose homeostasis. The control group was composed of twelve women, similar to the exercise group that performed the SuperJump activity for twenty weeks. The analysis was performed on blood samples and investigated GLP-1, GIP, GLP-2, PYY, ghrelin, glucose, insulin, insulin resistance, β-cell function, and insulin sensitivity. The results showed that the activity contributes to raising the GLP-1and GIP levels, and not on GLP-2, PYY, and ghrelin, which did not change. Moreover, SuperJump activity significantly reduced fasting insulin, glucose, insulin resistance, and increased insulin sensitivity but did not affect beta-cell function. These data suggest that GLP-1 and GIP are involved in the mechanism of action that improves bone and glucose homeostasis following 20 weeks of SuperJump activity in eumenorrheic women.

15. Ficarra, S., Di Raimondo, D., Navarra, G.A., Izadi, M., Amato, A., Macaluso, F.P., Proia, P., Musiari, G., Buscemi, C., Barile, A.M., Randazzo, C., Tuttolomondo, A., Buscemi, S.,

Bellafiore, M. (2022). Effects of Mediterranean Diet Combined with CrossFit Training on Trained Adults' Performance and Body Composition. J Pers Med. 12(8):1238. doi: 10.3390/jpm12081238.

CrossFit is a high-intensity training discipline increasingly practiced in recent years. Specific nutritional approaches are usually recommended to maximize performance and improve body composition in high-intensity training regimens; notwithstanding, to date there are no targeted nutritional recommendations for CrossFit athletes. The Mediterranean Diet (MD) is a diet approach with a well-designed proportion of macronutrients, using only available/seasonal food from the Mediterranean area, whose health benefits are well demonstrated. No studies have evaluated this dietary strategy among CrossFit athletes and practitioners; for this reason, we tested the effects of 8 weeks of MD on CrossFit athletes' performance and body composition. Participants were assigned to two groups: a diet group (DG) in which participants performed CrossFit training plus MD, and a control group (CG) in which participants partook in the CrossFit training, continuing their habitual diet. Participants were tested before and after the 8 weeks of intervention. At the end of the study, no significant difference was noted in participants' body composition, whereas improvements in anaerobic power, explosive strength of the lower limbs, and CrossFit-specific performance were observed only in the DG. Our results suggest that adopting an MD in CrossFit athletes/practitioners could be a useful strategy to improve specific strength, endurance, and anaerobic capacity while maintaining overall body composition.

16. Carzoli, J.P., Koger, K., Amato, A., Enoka, R.M. (2022). Tempo-controlled resistance training of the hip abductors and ankle dorsiflexors with light loads does not improve postural sway in older adults. Exp Brain Res. 2022 Nov;240(11):3049-3060. doi: 10.1007/s00221-022-06477-6. Epub 2022 Oct 13. PMID: 36227344.

The force steadiness capabilities of the hip abductors and ankle dorsiflexors can explain a significant amount of the variance in postural sway during four types of standing balance tests. Control over balance, as well as force steadiness, generally worsens with aging, although the latter can be improved with unique training interventions. The purpose of our study was to assess how tempo-controlled, light-load resistance training of the hip abductors and ankle dorsiflexors influences performance in clinical movement tests, postural sway, muscle strength, and force steadiness in older adults. Participants ($n = 28$, 70 ± 7 years, 8 men) completed nine training sessions for either the hip abductors or ankle dorsiflexors in the nondominant leg. The training involved lifting a load equal to 15% of the maximal force achieved during an isometric contraction. Linear mixed-effects models revealed no changes ($p > 0.05$) in the Sit-To-Stand test, Timed Up-and-Go test, maximal voluntary contraction (MVC) torque, or postural sway from before to after either training intervention. Only the dorsiflexor group significantly improved nondominant leg dorsiflexion force steadiness, but this did not translate to any other tasks. However, absolute and relative measures of MVC torque and force steadiness of the hip abductors and ankle dorsiflexors in the dominant and nondominant legs could predict sway-area rate in each of the four standing balance conditions. The responsiveness of leg muscles to light-load steadiness training in older adults appears to depend on the type of exercises performed during the intervention.

17. Amato, A., Baldassano, S., Vasto, S., Schirò, G., Davì, C., Drid, P., Dos Santos Mendes, F.A., Caldarella, R., D'Amelio, M., and Proia, P. (2022). Effects of a resistance training protocol on physical performance, body composition, bone metabolism, and systemic homeostasis in patients diagnosed with Parkinson's disease: A pilot and feasibility studies. Int. J. Environ. Res. Public Health 2022, 19, 13022. https://doi.org/10.3390/ijerph192013022

Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor impairments and it is correlated with loss of bone mineral density. This study aimed to analyze the effects of resistance training on bone metabolism, systemic homeostasis, body composition, and physical performance in people with PD. Thirteen subjects (age 64.83 ± 5.70) with the PD diagnosis were recruited. Participants performed neuromuscular tests, body composition assessment, and blood sample analysis at baseline and after an 11 weeks-training period. Each training session lasted 90 minutes, three times a week. The participants had significant improvements in the timed up-and-go ($P<0,01$), sit-to-stand ($P<0,01$), dominant peg-board ($P<0,05$), dominant foot-reaction time ($P<0,01$) functional reach tests ($P<0,05$). They showed better pressure foot distributions in the left forefoot ($P<0,05$) and hindfoot ($P<0,05$) and increased cervical right lateral bending angle ($P<0,05$). The protocol affects bone metabolism markers osteocalcin ($P<0,05$), calcium ($P<0,01$), PTH ($P<0,01$), the C-terminal telopeptide (CTX) ($P<0,01$) and the vitamin D ($P<0,05$). Eleven weeks of resistance training improved manual dexterity, static and dynamic balance, reaction time, cervical ROM, and reduce bone loss in people with PD.

Appendix

a) Conferences attended during the three years Ph.D. course

- <u>ESNS 2018, sports nutrition: from Lab to the field held 16-17 November, Roma.</u>

  Oral presentation (**Awarded**) and poster presentation:

"Relationship between energy metabolism and bone metabolism: a pilot study on gymnasts".

- XI Congresso Nazionale SISMeS 2019, Research and Training Applied to Movement and Sport Sciences. Bologna, 27 29 September 2019; Sport Sci Health (2019) 15 (Suppl 1):S1–S117.

  Poster presentation:

  "Carbohydrates consumption pre-exercise attenuate bone resorption marker in young female gymnasts".
- ESNS international congress Madrid 16-17/11/2019.

  Poster presentation:

  "Nutrition supplementation and athletic performance improvement in equestrian athletes of Jumping and Dressage".
- The 3rd International Electronic Conference on Environmental Research and Public Health — Public Health Issues in the Context of the COVID-19 Pandemic 11/01/2021 - 25/01/2021 Infectious Disease, Mental Health, Reproductive Health, Covid, Global Health, Child Health, Occupational Health, Oral Health, Environmental Exposure.

  Abstracts submitted:

  "Can specific pre-workout meals reduce bone resorption in young gymnasts?" (doi:10.3390/ECERPH-3-09002);

  "Effect of training with Supejump® on bone metabolism in women"(doi:10.3390/ECERPH-3-09082)
- 1st International Electronic Conference on Biomedicine. Session-Translational Biomarkers in Clinical Biomedicine and Precision Medicine Personalized training to prevent osteoporosis: Submission ID: sciforum-044542

  Abstracts submitted:

  "What about jumping with music on a mini trampoline?";

  "Is the wellness of the gut the wellness of the whole body? Gut peptides, glucose homeostasis, and training with SuperJump®".

- 93° Congresso SIBS, Sessione: 8 scienza e medicina dello sport e dell'esercizio fisic. Palermo 22-25 Aprile 2021.

  Oral presentation:

  "Effect of long-term superjump® training on bone remodeling and metabolism in women";

  Abstract submitted:

"Is hypoxia-inducible factor (hif-1) alpha involved in taravana syndrome?"

- XII Congresso Nazionale SISMeS 2021, Research and Training Applied to Movement and Sport Sciences. Padova 8-10 Ottobre 2021

  Poster presentation:

  "The efficacy of anaerobic training on multiple sclerosis symptoms management"

- Rocky Mountain American Society of Biomechanics. Rocky Mountain, Colorado (USA), 8-10 April 2022
  Oral presentation: "Tempo-controlled hip abduction and ankle dorsiflexion resistance training does not improve postural sway in older Adults"

- The 24th annual European Congress of Endocrinology™ (ECE) will take place in Milan, Italy between 21-24 May 2022

  Abstract submitted and Poster presentation:

  "SuperJump training in eumenorrheic women and gut peptides: a randomized controlled study about the mechanism of action on bone and glucose homeostasis"

- 94° Congresso della Società Italiana Biologia Sperimentale. Torino, 6-9 Aprile 2022

  Abstract submitted:

  "Development of a home-based training program for patients with Parkinson's disease: neurobiological and motor skills effect"

- XIII Congresso Nazionale SISMeS 2022, Research and Training Applied to Movement and Sport Sciences. Milano 4-6 Novembre 2022

  "Resistance training for resistant bones: how to slow bone mineral density loss in Parkinson's disease"
  "Relationship between CrossFit workout performance and power output variables of 30-second Wingate test in CrossFit Athletes"

c) Co-tutor master's thesis

School of Humanities and Cultural Heritage
Study course in sciences and techniques of preventive and adapted motor and sports activities class LM 67:

• "Everesting 8488 challenge accepted: dietary supplementation to support performance"

• "Everesting 8848" challenge accepted: metabolic impact analysis "
• "Genetic predisposition to potency in youth volleyball: a pilot study"
• "Genetic predisposition to potency in young basketball players: a pilot study"
• "Analysis of the genetic profile of elite point fighting athletes and correlations with sports performance"
• "Bee pollen and resistance activity: evaluation of oxidative stress and performance."
• "Body composition: comparison between assessment methods"
• "Effects of a resistance training protocol on body composition and bone metabolism in subjects with Parkinson's disease"
• "Effects of a resistance training protocol on physical performance in individuals with Parkinson's disease"
• "The effects of a resistance training protocol on postural balance and mobility of the cervical spine in individuals with Parkinson's disease"

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