
Post-Graduate Program in Movement Science

PEDRO HENRIQUE NARCISO PICCHI

**IMPACT OF HIIT ON BONE HEALTH IN ADOLESCENT GIRLS: CLINICAL TRIAL
ON THE INFLUENCE OF MECHANICAL IMPACT**

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Master thesis presented to Postgraduate program in Movement Sciences of School of Technology and Sciences of São Paulo State University (UNESP) - Presidente Prudente as part of the requirements for obtaining the title of Doctor in Movement Sciences.

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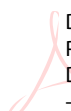
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“Life is an eternal dance and like a dance, the more rigid I became, the harder it got. [...] It is this eternal dance that separates human beings from angels, from demons, from gods. And I must not forget, we must not forget that we are human beings”

(Ren Gill)

RESUMO

O objetivo desta tese foi analisar o efeito do exercício intervalado de alta intensidade, com e sem impacto gravitacional, nos marcadores de remodelação óssea e osteocinas de adolescentes do sexo feminino não treinadas. Além disso, analisamos o efeito de cada modalidade (com e sem impacto) em citocinas e adipocinas potencialmente associadas aos marcadores de remodelação óssea e osteocinas. Por fim, avaliamos como cada modalidade de exercício influencia a percepção afetiva das adolescentes e qual delas foi mais agradável. Este foi um estudo de design cruzado, no qual 11 adolescentes do sexo feminino participaram de dois testes de exercício intervalado de alta intensidade (corrida intervalada de alta intensidade [HIIR] e ciclismo intervalado de alta intensidade [HIIC]) em ordem aleatória. A composição corporal, ingestão nutricional e histórico de atividades físicas relacionadas aos ossos foram avaliados utilizando métodos específicos. As participantes realizaram um teste incremental progressivo para determinar a carga máxima de trabalho na esteira e no cicloergômetro. Em dias diferentes, pela manhã, elas completaram os testes, que envolveram oito séries de um minuto de esforço máximo, seguidas por um minuto de recuperação passiva entre as séries. Amostras de sangue foram coletadas antes do exercício (linha de base), 5 minutos e 60 minutos após o exercício. O sangue sérico foi utilizado para analisar marcadores ósseos de formação (como P1NP e OPG) e reabsorção (como CTX-I), osteocinas (como esclerostina, OPG e RANKL), citocinas (como IL-6, IL-10 e TNF- α) e cortisol. Adipocinas (como leptina e adiponectina) e outros marcadores metabólicos (como glicose e LHD) foram medidos no soro. A percepção afetiva foi registrada pela Escala de Sentimentos (FS) antes e depois de cada teste, e o nível de prazer foi registrado pela Escala de Aproveitamento da Atividade Física (PACES) após cada teste. A análise estatística incluiu modelos mistos lineares (LMM) para avaliar o efeito fixo do tempo (pré, 5 minutos e 60 minutos após o exercício), modalidade (HIIR e HIIC) e interação entre os fatores (tempo*modalidade), considerando o efeito aleatório de cada indivíduo. Covariáveis (densidade mineral óssea, massa gorda, atividade física prévia relacionada aos ossos e valores dos marcadores sanguíneos na linha de base) foram testadas individualmente para cada modelo, considerando a correlação com o resultado. A diferença na pontuação total do PACES e nos itens individuais entre as modalidades foi testada usando um teste t independente. O Capítulo 4 mostra que a HIIR induziu uma expressão significativamente maior de P1NP em comparação à HIIC, embora CTX-I e outras osteocinas tenham sido diferentes entre os grupos. Além disso, a HIIR foi mais agradável do que a HIIC, e a percepção afetiva foi negativamente impactada pela última. O Capítulo 5 mostra que tanto a HIIR quanto a HIIC tiveram respostas semelhantes em marcadores anti-inflamatórios e adipocinas; no entanto, a HIIR induziu maior secreção de TNF- α , possivelmente devido ao impacto mecânico e ao possível maior dano tecidual. Assim, a HIIR e a HIIC induziram respostas semelhantes em adolescentes do sexo feminino não treinadas, apesar de pequenas diferenças em P1NP e TNF- α . Mais importante ainda, a HIIR foi percebida como mais agradável, com menor impacto na percepção afetiva das adolescentes. Portanto, a HIIR parece ser a melhor estratégia para melhorar os resultados de saúde relacionados aos ossos e à inflamação em adolescentes do sexo feminino.

Palavras-chave: adolescência, exercício aeróbico, osteocinas, citocinas

ABSTRACT

The aim of this thesis was to analyze the effect of high-intensity interval exercise with and without gravitational impact on bone turnover markers and osteokines of untrained adolescent females. Additionally, we analyzed the effect of each mode (with and without impact) on cytokines and adipokines that are potentially associated with bone turnover markers and osteokines. Lastly, we assessed how each mode of exercise impacts the affective perception of the adolescents and which mode was more enjoyable. This was a cross-over design study, where 11 adolescent females participated in two HIIE trials (high-intensity interval running [HIIR] and high-intensity interval cycling [HIIC]) in random order. Body composition, nutritional intake, and bone-specific physical activity background were assessed using specific methods. They performed a progressive incremental test to determine the peak workload for the treadmill and cycle ergometer. On different days, in the morning, they completed the trials, involving eight bouts of one minute of maximal effort, followed by one minute of passive recovery between bouts. Blood samples were collected before exercise (baseline), 5-min, and 60-min post-exercise. Serum blood was used to analyze bone markers of formation (i.e., P1NP and OPG) and resorption (i.e., CTX-I), osteokines (i.e., sclerostin, OPG, and RANKL), cytokines (i.e., IL-6, IL-10, and TNF- α), and cortisol. Adipokines (i.e., leptin and adiponectin) and other metabolic markers (i.e., glucose and LHD) were measured in serum. Affective perception was reported by the Feeling Scale (FS) before and after each trial, and enjoyment was reported by the Physical Activity Enjoyment Scale (PACES) after each trial. Statistical analysis included linear mixed models (LMM) to assess the fixed effect of time (pre, 5-min, and 60-min post-exercise), mode (HIIR and HIIC), and interaction between factors (time*mode), while accounting for the random effect of each individual. Covariates (areal bone mineral density, fat mass, previous bone-specific physical activity, and blood markers values at baseline) were tested individually for each model considering the correlation with the outcome. Difference in PACES total score and their individual items between modes was tested using an independent t-test. Chapter 4 shows that HIIR induced significant greater expression of P1NP compared to HIIC, although CTX-I and other osteokines were different between groups. In addition, HIIR was more enjoyable than HIIC, and the affective perception was negatively affected by the latter. Chapter 5 shows that both HIIR and HIIC similar response in anti-inflammatory markers and adipokines; however, HIIR induced greater secretion of TNF- α , potentially because of the mechanical impact and eventual greater tissue damage. Thus, HIIR and HIIC induced similar responses in untrained female adolescents, despite small differences in P1NP and TNF- α . More importantly, HIIR was perceived as more enjoyable, with a lower impact on affect perception of the adolescents. Thus, HIIR seems to be the best strategy to improve health outcomes related to bone and inflammation in adolescent females.

Keywords: adolescence, aerobic exercise, osteokines, cytokines

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GLOSSARY OF TERMS

APHV – Age at Peak Height Velocity

BALP – Bone Alkaline Phosphatase

BMC – Bone Mineral Content

BMD – Bone Mineral Density

BMI – Body Mass Index

BPAQ – Bone-specific Physical Activity Questionnaire

BTM – Bone Turnover Markers

CI – Confidence Interval

CTX-I – C-terminal Telopeptide of Type I Collagen

DXA – Dual-energy X-ray Absorptiometry

EDTA – Ethylenediaminetetraacetic Acid

ELISA – Enzyme-Linked Immunosorbent Assay

ES – Effect Size

FDR – False Discovery Rate

FM – Fat Mass

FS – Feeling Scale

FX – Fracture Strain

HIIC – High-Intensity Interval Cycling

HIIE – High-Intensity Interval Exercise

HIIR – High-Intensity Interval Running

HR – Heart Rate

IL-6 – Interleukin 6

IL-10 – Interleukin 10

IOF – International Osteoporosis Foundation

LDH – Lactate Dehydrogenase

LMM – Linear Mixed Models

LST – Lean Soft Tissue

MESm – Minimum Effective Strain for Modeling

MESp – Minimum Effective Strain for Pathological Loading

MESr – Minimum Effective Strain for Remodeling

OPG – Osteoprotegerin

P1NP – Procollagen Type I N-Terminal Propeptide

PA – Physical Activity

PACES – Physical Activity Enjoyment Scale

PBM – Peak Bone Mass

PHV – Peak Height Velocity

RPE – Rate of Perceived Exertion

RANKL – Receptor Activator of Nuclear Factor κ B Ligand

SD – Standard Deviation

SEM – Standard Error of Mean

TBLH-aBMD – Total Body Less Head areal Bone Mineral Density

TNF- α – Tumor Necrosis Factor alpha

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1. THEORETICAL FOUNDATION

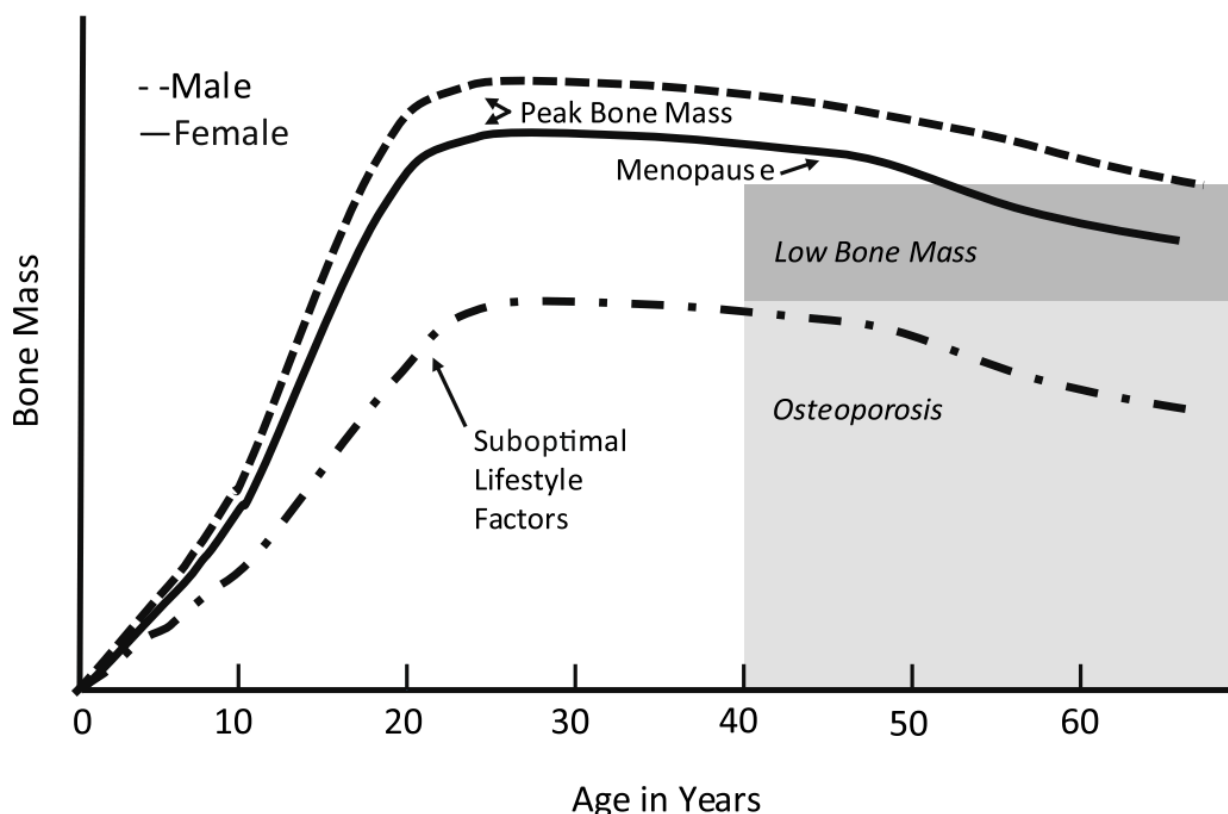
Osteoporosis is a systemic disease and is characterized by changes in bone micro and macroarchitectur, decreased mass and stiffness and, consequently, increased bone fragility (GÓMEZ et al., 2021). This can result in fractures that are frequently seen in the forearm, hip, and spine, which have significant implications for public health (more hospitalizations, reduced quality of life, loss of functional capacity, and potentially lower life expectancy) (CLYNES et al., 2020; DALY et al., 2019; KANIS et al., 2019). Considering its significant impact on the quality of life during old age, added to the trend of aging of the world population, there is a growing concern on the impact of osteoporosis in society (SHEN et al., 2022)

The incidence of osteoporosis does not only increase with age, but it impacts more significantly women than men (CLYNES et al., 2020; GUERRA et al., 2016; KANIS et al., 2019; SHEN et al., 2022; ZERBINI et al., 2015). This is because changes in reproductive hormones during menopause, such as decreased estrogen production, negatively affect bone metabolism, contributing to increased bone fragility among women (CAULEY, 2015; ZALLONE, 2006). Data from 1990 to 2019 shows that women consistently experienced higher rates of disease burden (101.4% vs 86.2%) and mortality (116.9% vs 105.3%) due to low bone mineral density (BMD) compared to men (SHEN et al., 2022). In Brazil, the number of fractures among women is expected to increase by 63% between 2015 and 2030, with an estimated 24% mortality rate within one year following hip fractures (AZIZIYEH et al., 2019; GUERRA et al., 2016), highlighting the gender disparities related to bone health.

Additionally, the risk of osteoporosis is also influenced by peak bone mass (PBM), which is the greatest amount of bone accrued until early adulthood (WEAVER et al., 2016; ZERBINI et al., 2015). Although genetics play a major role in PBM, 38–54% is influenced by external factors, primarily mechanical loading through body weight and physical activity (BONJOUR et al., 2009; KRALL; DAWSON-HUGHES, 1993). Furthermore, 95% of adult bone mass is achieved after 4 years following the peak in bone accretion (BAXTER-JONES et al., 2011). During this period of mass gain, bone is more susceptible to external influences; thus, it is a critical moment for optimizing peak bone mass (WEAVER et al., 2016). For girls, this is particularly significant because these gains during growth represent twice the amount of bone mass that will be lost

subsequently during the postmenopausal years (BAXTER-JONES et al., 2011). Therefore, understanding the best strategies to enhance peak of bone mass of adolescent girls is essential to reduce their risk of developing osteoporosis later in life (**FIGURE 1.1**).

Figure 1.1 - Bone mass across the lifespan with optimal and suboptimal lifestyle choices.



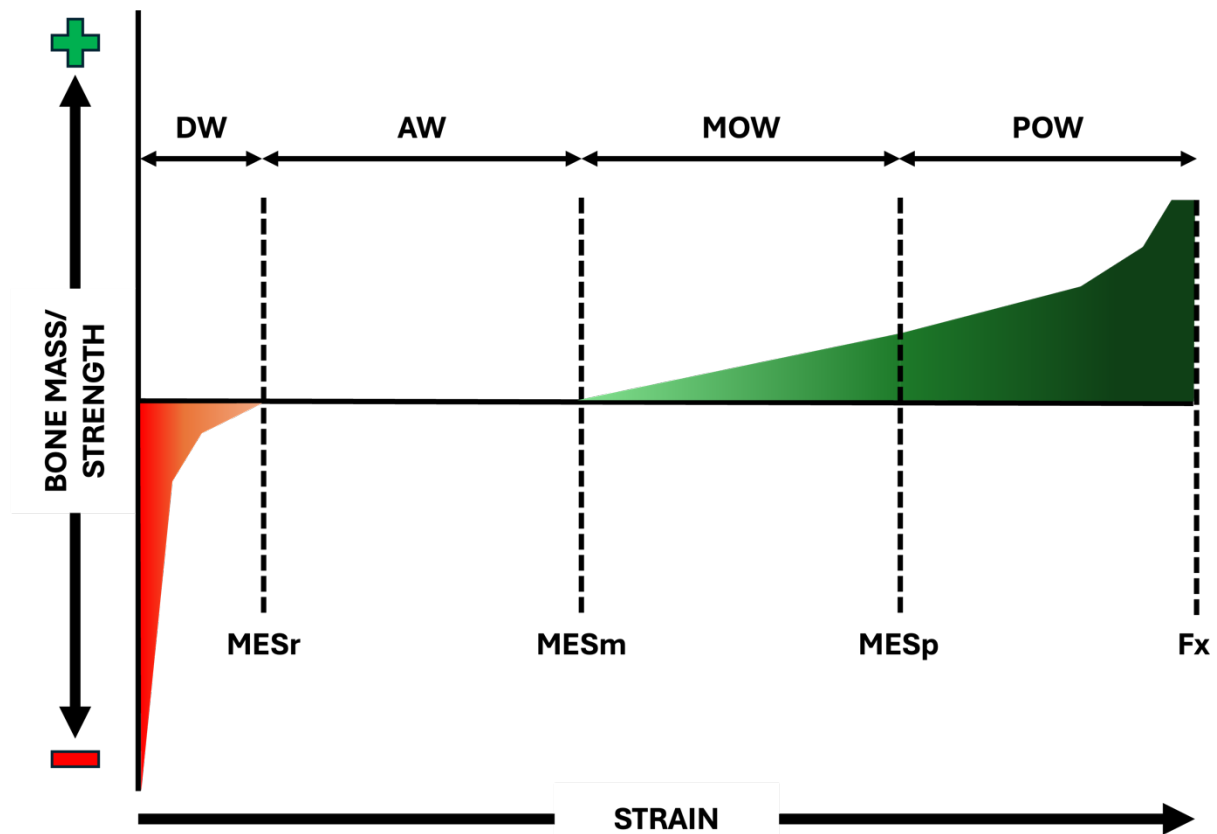
Note: Reproduced from (WEAVER et al., 2016) under the Creative Commons CC-BY License (Attribution-Noncommercial).

Physical activity (PA), i.e., bodily movement produced by skeletal muscles that results in energy expenditure (CASPERSEN; POWELL; CHRISTENSON, 1985), is one of the most important determinants of bone health across life (KOHRT et al., 2004). The mechanical forces involved in PA stimulate bone adaptations through direct impact loading (e.g., ground reaction forces from activities such as jumping and running) and indirect loading via muscle contractions (BONNET; FERRARI, 2010). Studies have shown that adults who were physically active during adolescence present an 8-10% greater bone mineral content (BMC) compared to their less active peers (BAXTER-JONES et al., 2008). Furthermore, among girls, maintaining or increasing PA levels throughout adolescence resulted in greater gains in BMC and bone mineral density (BMD) compared to the girls whose PA levels declined during the same period (RAUTAVA et al., 2007).

These changes in bone structure are referred to as mechanoadaptation, a process that occurs in response to mechanical demands, as described by Wolff's Law (WOLFF, 1893).

Also known as "Mechanostat Theory", this model posits that bone adapts to mechanical loads based on specific thresholds (FROST, 2003). When strain exceed the modeling threshold, it initiates an adaptive process that enhances the strength of the stressed area by reorganizing the bone microarchitecture or adding additional tissue. However, excessive stress may lead to microdamage and eventually fractures may occur to the bone; in contrast, when loads fall below a remodeling threshold (i.e., conditions of disuse), bone resorption accentuates, and its strength and mass consequently decrease (FROST, 2003). In this sense, bone adaptation would be a consequence of the balance between bone modeling (bone formation independent of osteoclast resorption) and remodeling (coupled action of osteoclasts and osteoblasts resorbing and forming bone, respectively, within a multicellular unit), and the latter would be the dominant mechanoadaptive response in bone (FROST, 2000) (**FIGURE 1.2**).

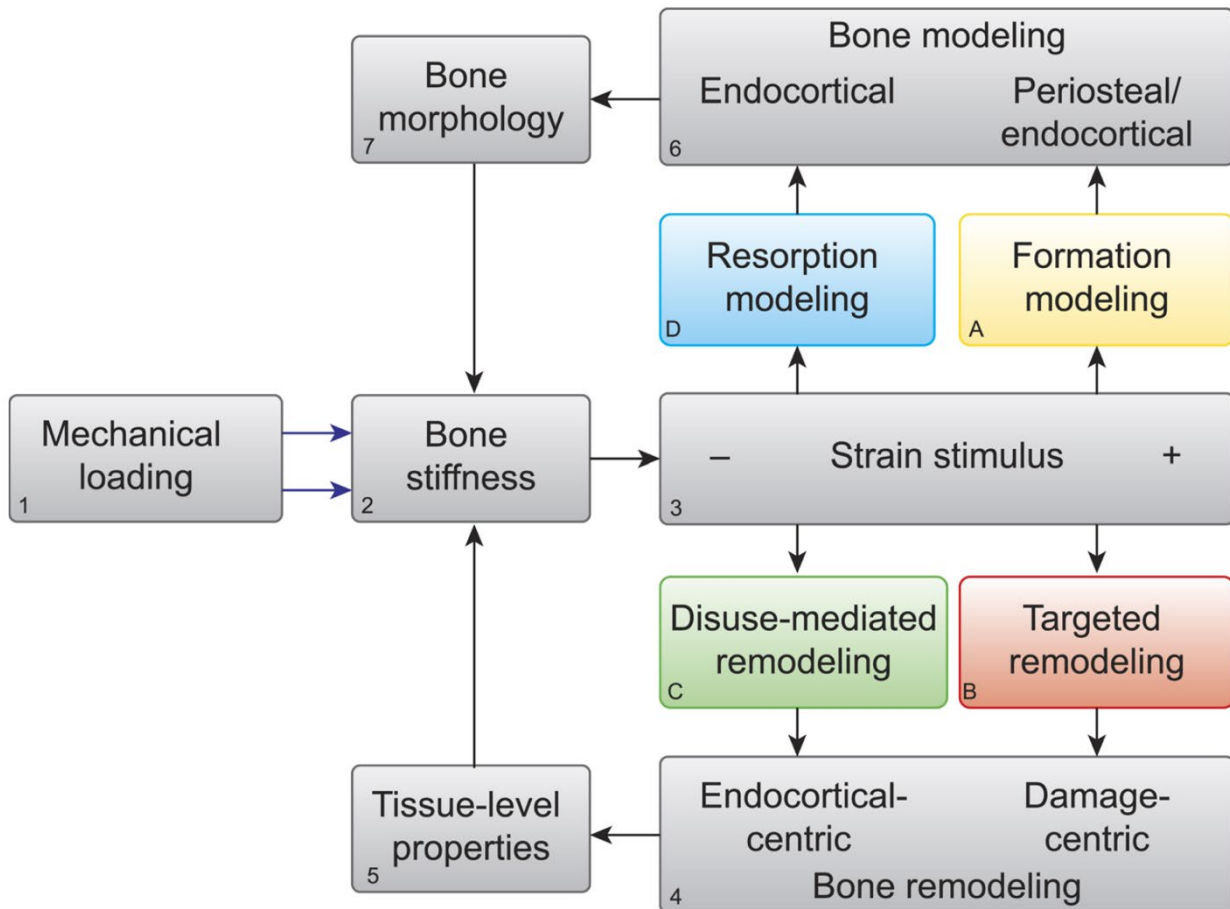
Figure 1.2 - Bone (re)modeling thresholds.



Note: The bottom line represents typical peak bone strains, ranging from zero to fracture strain (FX), and the location of the remodeling, modeling and microdamage strain thresholds (MESr, MESm, and MESp, respectively). The horizontal axis indicates a balance between gain and loss in bone mass or strength. When strains are below this line, thus, below the MESr range, bone remodeling resorbs bone. When the strains enter or exceed the MESm range, modeling would increase bone strength and mass. If strains exceed the MESp, bone fracture may occur. DW = disuse; AW = the adapted window as in normally adapted adults; MOW = the mild overload window as in healthy growing mammals; POW = the pathological overload window. Source: adapted from (FROST, 2000).

However, recent advances in bone mechanobiology have suggested that both bone modeling and remodeling may occur as a response to heightened mechanical loading (i.e., formation modeling and targeted remodeling) and as a response to a state of disuse (i.e., resorption modeling and disuse-mediated remodeling). In either case, bone modeling and remodeling could lead to distinct bone adaptations (morphological and tissue-level changes), which would depend on the mechanical loads applied to the bone and its inherent mechanical properties (e.g., bone stiffness) (Hughes et al., 2020). **(FIGURE 1.3)**. This framework is centered on the role of the osteocytes, the mechanosensory cells in the bone, and their influence on the osteoblasts and osteoclasts.

Figure 1.3 - Theoretical framework of bone functional adaptation at the diaphysis.



Note: Mechanical loading [1] on a bone of a given stiffness [2] creates a strain stimulus [3]. Bone formation modeling [A] and targeted remodeling [B] may be triggered when the stimulus is greater than customary. Formation modeling results in new bone deposited on the endocortical and periosteal surfaces within the diaphysis, while targeted remodeling aims to removed bone from the damaged area. In a scenario of disuse or unloading, disuse-mediated remodeling [C] and resorption modeling [D] may be triggered on or near the endocortical surface of the diaphysis. In the disuse-mediated remodeling, there is a couple action of resorption and formation, while resorption modeling occurs independent of formation. Remodeling affects mainly tissue-level mechanical properties [5] and modeling alters bone morphology [7]. Consequently, all these processes impact bone stiffness [2], thereby affecting the strain response to subsequent bouts of mechanical loading. Reproduced with permission from Hughes et al., (2020) under the Creative Commons CC-BY License (Attribution-Noncommercial).

The osteocytes are the most abundant cell type within the bone matrix. Through their extensive lacuna-canalicular network, they serve as key mechanosensory cells, transducing mechanical signals such as fluid shear stress, hydrostatic pressure, electric fields, and tissue strain (i.e., shear, compression, and torsion forces) into biochemical signals, i.e., osteokines, that coordinate the activity of osteoblasts and osteoclasts, thereby regulating bone formation and resorption (JAALOUK; LAMMERDING, 2009; ROBLING; CASTILLO; TURNER, 2006; YOU et al., 2008).

Osteocytes almost exclusively express sclerostin, a glycoprotein that acts as an antagonist in the Wnt signaling pathway to inhibit osteoblast activity under unloading conditions (LIN et al., 2009; ROBLING et al., 2008). Studies have shown that mechanical loading is responsible to inhibit sclerostin in osteocytes, facilitating bone formation (ROBLING et al., 2008). Osteocytes also promotes bone resorption via secretion of Receptor Activator of Nuclear Factor κ B Ligand (RANKL), a factor that binds to its receptor RANK on the membrane of the osteoclast's precursors, facilitating osteoclastogenesis (YOU et al., 2008). Secretion of RANKL is an example of targeted-remodeling, commonly observed in fatigue-loaded bone by apoptosing osteocytes surrounding the damaged area (KENNEDY et al., 2012). However, osteocytes can also regulate bone resorption via expression of osteoprotegerin (OPG), a decoy receptor that can bind to RANKL and block osteoclastogenesis (ROBLING et al., 2008; YOU et al., 2008). In this sense, the balance between OPG and RANKL (OPG:RANKL ratio) is frequently used as a marker of bone anabolism.

While osteokines may indicate whether an intervention is inducing a signaling response favoring formation of osteoblasts or osteoclasts, the activity of these cells can be measured assessing bone turnover markers (BTMs). The BTMs are biochemical markers found in blood or urine that reflect the rate of bone formation and resorption (SCHINI et al., 2023). They have been used to monitor acute adaptations from interventions either in clinical or research settings. The International Osteoporosis Foundations (IOF) recommends that Procollagen I N-propeptide (PINP) and Type I Collagen C-Telopeptide (CTX-I) should be used as primary markers of osteoblasts and osteoclasts activity, respectively (VASIKARAN et al., 2011). Roughly, increased serum PINP would be a consequence of increased bone formation, while increased serum CTX-I would be the result of increased bone resorption, and the balance between them would express the bone turnover (SCHINI et al., 2023). Together with the osteokines (e.g., sclerostin, RANKL, OPG), BTMs would provide a more comprehensive understanding of the effect PA on bone modeling and remodeling. However, studies investigating the effect of PA on bone markers present high heterogeneity and are mostly focused on adult men and the elderly (DOLAN et al., 2020, 2022, 2024), limiting our understanding of how those markers respond and adapt to mechanical loading, mainly in younger population.

The impact of PA - including exercise, a structured and planned form of PA - on bone markers is influenced by different factors, including age, sex and training variables, i.e., duration,

intensity and gravitational impact (DOLAN et al., 2022; KOHRT et al., 2004; SCHINI et al., 2023). Kish et al. analyzed the acute effect of plyometric exercise on bone turnover markers (bone alkaline phosphatase [BALP], OPG, RANKL, and CTX-I) of boys and men and found that although the intervention positively affected those markers, the pattern of increase differed between groups (KISH et al., 2015). This corroborates the “window of opportunity” concept for building stronger bones during growth (HAAPASALO et al., 1998; PEARSON; LIEBERMAN, 2004). Additionally, high-impact exercise induces an osteogenic effect in boys and girls; however, this response might occur through different signaling pathways, which, in girls, would involve the suppression of catabolic pathways, i.e., inhibition of RANKL expression and CTX-I (DEKKER et al., 2017; KLENTROU et al., 2018; KURGAN et al., 2020).

On the other hand, engaging in exercise modalities that do not involve gravitational impact may not benefit the bone tissue. Research indicates that cyclists and swimmers exhibit similar or lower bone mineral density to their non-athletic peers, with lower bone density than athletes in high-impact sports (AGOSTINETE et al., 2020a, 2020b; GONZÁLEZ-AGÜERO et al., 2017; OLMEDILLAS et al., 2018). However, recent studies on the impact of cycling on bone turnover markers have shown that, when exercise is performed at high-intensity, it may exert an anabolic effect on bone (BORZOOEIAN et al., 2023; KOUVELIOTI et al., 2019a, 2019b). The long duration of exercise (>60min), usually at moderate-to-vigorous intensity, would lead to a disturbance in calcium metabolism that seems to be responsible for increasing osteoclasts activity, thus increasing bone resorption (BARRY et al., 2011; WHERRY; SWANSON; KOHRT, 2022). Thus, shortening the exercise length and increasing intensity using a high-intensity interval exercise (HIIE) strategy would benefit an anabolic response on bone as this type of training is known to impose great tension on musculoskeletal system, consequently, increasing load on bone tissue (BUCHHEIT; LAURSEN, 2013). Yet, only one study has compared the effect of HIIE with and without impact on bone metabolic markers, using a cross-over design.

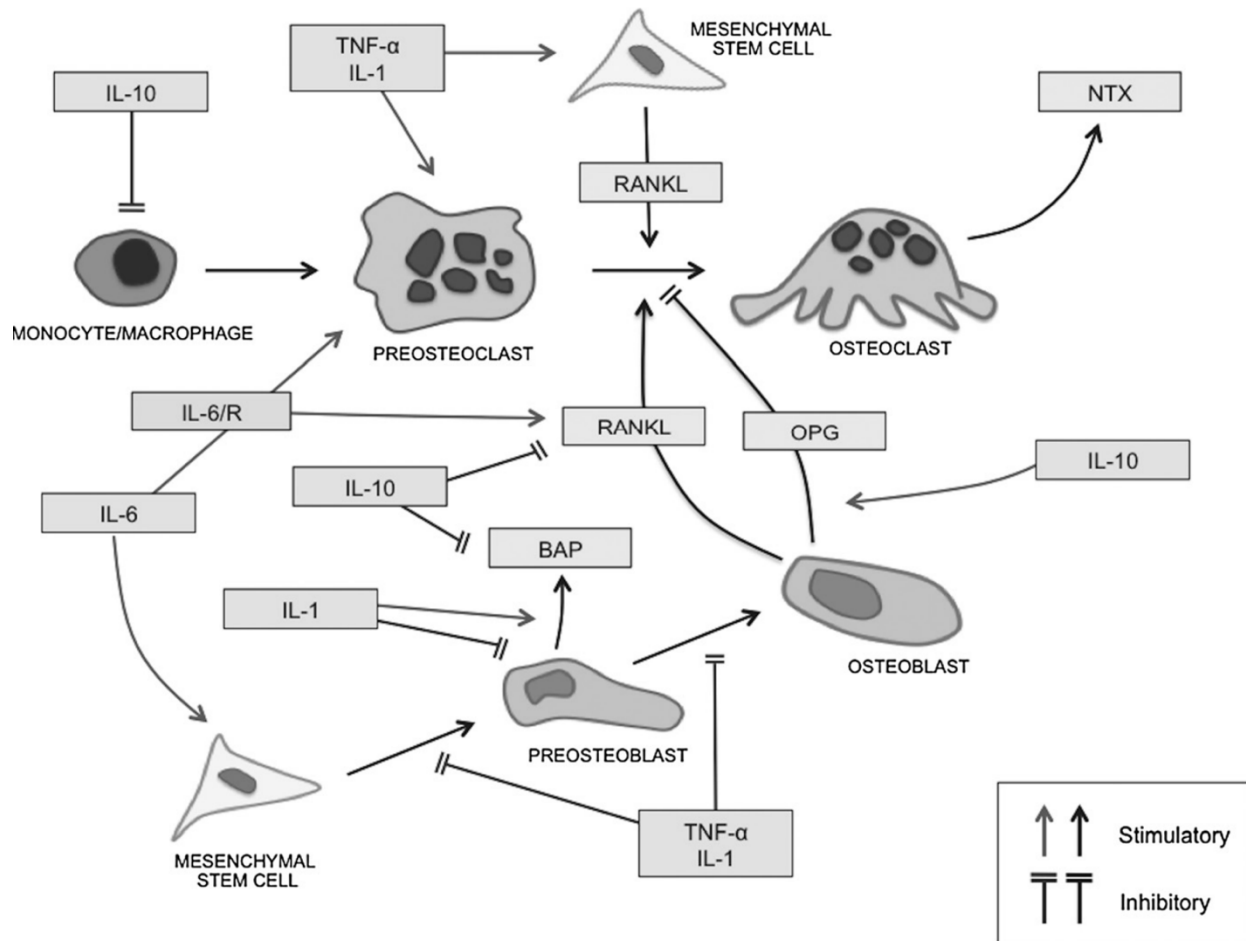
In this study, recreationally active women were submitted to two sessions of HIIE on a bicycle and on a treadmill, and the acute response of sclerostin, P1NP and CTX-I were measured through serum before and after each session. Different from what was expected, the authors did not observe any differences in markers of formation and resorption following both HIIE, although sclerostin has increased significantly 5-min post-exercise and decreased to baseline levels 1h post-

exercise (KOUVELIOTI et al., 2018a). Thus, not only the effect of high-intensity exercise with and without impact remain controversial, but it remains unclear whether those modes of exercise (cycling and running) could benefit adolescent females whose bones are still growing. Furthermore, individuals not engaged in any exercise routine may present a bone that is less adapted to mechanical loading, increasing its responsiveness to exercise (HUGHES et al., 2020).

Potentially, HIIE is a great strategy for bone tissue, as it combines short periods of high intensity and high muscle demand, intervaled with periods of recovery, important to reestablish the responsiveness of bone cells to the mechanical stimuli applied (BUCHHEIT; LAURSEN, 2013; ROBLING et al., 2002). Additionally, high-intensity exercise may impact bone metabolism via non-mechanical pathways, such as modulation of cytokines and inflammation (FURMAN et al., 2019; KURGAN et al., 2020; NEMET et al., 2002; PETERSEN; PEDERSEN, 2005; TAKAYANAGI, 2007), potentially mediated by the adipose tissue and skeletal muscle (KIRK et al., 2020).

In this scenario, RANKL is one of the main mediators of the relationship between immune and bone cells (TAKAYANAGI, 2007; YOU et al., 2008). This is because RANKL, which is secreted by osteocytes and osteoblasts, also belongs to the TNF family and is secreted by activated T-cells (ANDERSON et al., 1997). IL-6 and TNF- α are pro-inflammatory cytokines (although muscle-derived IL-6 plays an anti-inflammatory role) that are negatively associated with bone by acting to stimulate the expression of RANKL, thus increasing osteoclasts' differentiation and bone resorption (UDAGAWA et al., 1995). Conversely, the anti-inflammatory cytokine IL-10 suppresses the expression of both TNF- α and IL-6, blocking the signaling of RANKL, therefore limiting bone resorption (FURMAN et al., 2019; PETERSEN; PEDERSEN, 2005) (**FIGURE 1.4**).

Figure 1.4 - Interaction between cytokines and bone cells.



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Adipose tissue plays an important role in energy metabolism and systemic inflammation through the secretion of adipokines, the main ones being leptin and adiponectin (KERSHAW; FLIER, 2004). The first is a key regulator of energy balance, but high-levels of leptin is associated with a pro-inflammatory state via increased expression of IL-6 and TNF- α . Adiponectin, on the other hand, has an anti-inflammatory effect, as it increases insulin sensitivity and promotes IL-10 expression (KERSHAW; FLIER, 2004; REN et al., 2022).

The effect of exercise on these cytokines and adipokines is dependent on intensity and type of exercise. For instance, intermittent and aerobic exercise promote a greater increase in IL-10 compared to resistance exercise (CABRAL-SANTOS et al., 2019). Kouvelioti et al. (2019) compared the effects of high-intensity interval running (HIIR) and cycling (HIIC) on IL-10 and

TNF- α in active young adults, finding distinct responses between the two exercises (KOUVELIOTI et al., 2019b). Regarding adipokines, the effect of exercise on them is more complex. Studies on trained adults have showed that short-term intense exercise declined leptin (JÜRIMÄE; JÜRIMÄE, 2005) and increased adiponectin concentrations (JÜRIMÄE; PURGE; JÜRIMÄE, 2005); however, this effect could be influenced by training status and amount of muscle involved in the activity (BOUASSIDA et al., 2010).

Although it's widely spread that bone adaptations depend on the gravitational impact, other non-mechanical pathways may influence bone response to exercise as well. Beyond impact, HIIR and HIIC also differ in the number of muscles recruited (greater in running) and muscle strain (greater in cycling), which could impact mediators of bone remodeling, such as cytokines (i.e., IL-6, IL-10 and TNF- α) and adipokines (i.e., leptin and adiponectin). Exploring the differences and potential benefits of high-intensity exercise without gravitational impact may benefit adolescents with injury-related limitations or who require impact-free exercise. Additionally, assessing the preference of the adolescents for the type of intervention (HIIR vs HIIC) may also help the professionals responsible for prescribing exercise for this population.

Considering that the practice of PA among adolescents has been decreasing over the years (COOPER et al., 2015), designing the strategy that promotes motivation and adherence to exercise in this population, mainly through feelings of enjoyment and well-being, seems to be as important as identifying the dose (e.g., intensity) that promotes the greatest health benefit (DISHMAN; SALLIS; ORENSTEIN, 1985; EKKEKAKIS; PARFITT; PETRUZZELLO, 2011). A disadvantage for HIIE is that it may lead to unpleasant feelings, which would affect negatively the adherence in physical activity (BIDDLE; BATTERHAM, 2015; EKKEKAKIS, 2009; RHODES; KATES, 2015). The Dual-Mode Theory explains this phenomenon, stating that exercise affects emotions through a combination of cognitive processes (like self-efficacy and goals) and physical sensations (such as breathing, acidity levels, and core temperature) (EKKEKAKIS, 2009). However, despite the arousal in perceived feelings, a positive feeling of pleasure, activated by the reward system, may occur as a result of increased self-efficacy and excitement after completing an intense exercise session (MALIK et al., 2018; ROSE; PARFITT, 2007). In addition to the contrasts in the literature on this topic, no study was found that compared the effect of HIIC and HIIR on the affective perception and enjoyment of adolescent girls.

To the best of our knowledge, no study has investigated the effect of HIIT and HIIC on bone markers, osteokines, cytokines, and adipokines in adolescent females not engaged in any exercise program using a cross-over design trial. Moreover, we believe that in addition to investigating the effect of different exercise modes on health outcomes, it is important to understand exercise mode preference and how each of them can affect mediators of adherence to physical exercise, such as affective perception and enjoyment. We hypothesize that HIIE and HIIC would impact bone through different pathways, but leading to similar bone turnover (i.e., ratio between bone formation and resorption markers) and inflammatory response. However, HIIT would be less detrimental to the affective perception of the exercise, while it would be more enjoyable.

2. THESIS OBJECTIVES

2.1. General objective

To investigate the effect high-intensity interval exercise with and without impact on markers of bone metabolism, and potential mediators of these markers, such as pro- and anti-inflammatory cytokines and adipokines, assessing the impact of each mode of exercise on affective perception and enjoyment.

2.2. Specific objectives

- I)** To compare the effect of HIIR and HIIC on bone turnover markers (i.e., P1NP, CTX-I, and sclerostin) and osteokines (i.e., RANKL and OPG) of adolescent girls (**Chapter 4**).
- II)** To investigate the impact of each mode on their affective perception and enjoyment (**Chapter 4**).
- III)** To compare the effect of HIIR and HIIC on inflammatory markers (i.e., IL-6, IL-10 and TNF- α) and adipokines (i.e., leptin and adiponectin) of adolescent girls (**Chapter 5**).

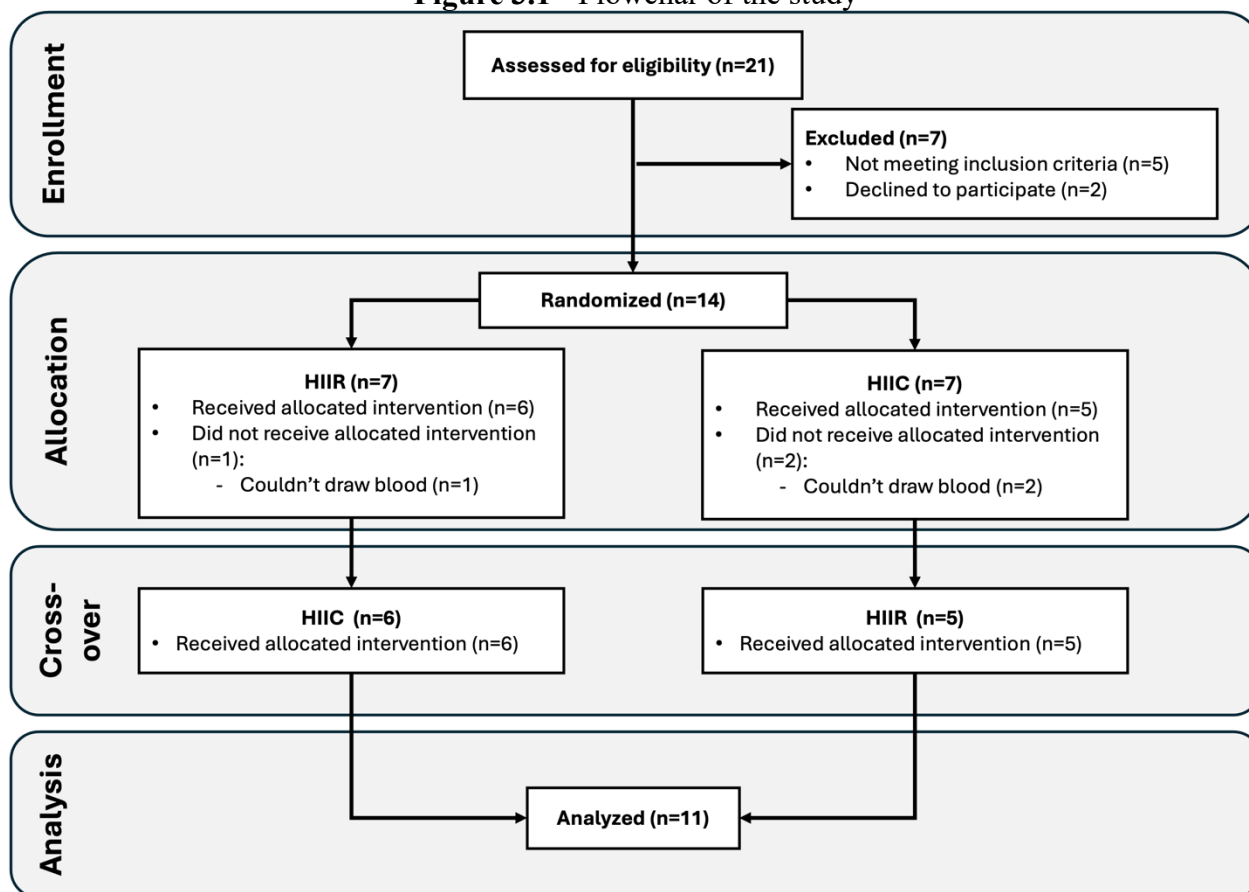
3. METHODS

3.1. Participants

Female adolescents were recruited for the study through digital media and visits to public and private schools in the city of Presidente Prudente-SP/Brazil. Inclusion criteria were: (i) aged between 14 and 19 years old; (ii) non-smokers; (iii) having already gone through menarche; (iv) not presenting any pre-diagnosed osteometabolic disease; (v) free of musculoskeletal injury that would impair exercise, (vi) not having used glucocorticoids for more than 3 months (COSMAN et al, 2014); (vii) not engaged in any sport modality or exercise routine in a structured way (more than 3 hours of weekly training) in the previous 6 months; (viii) presented the consent and assent form signed by the adolescent and their legal guardian. Exclusion criteria were: (i) not passed 2 years from PHV, (ii) could not draw blood.

A total of 21 adolescents were assessed for eligibility; however, 7 adolescents did not meet the inclusion criteria (e.g., participation in organized sports or resistance training programs [5] or declined to participate [2]), and 3 adolescents withdrew after being assigned to the study (e.g., fear of blood draws [1], inability to draw blood [2]). Consequently, 11 adolescents successfully completed the study (**FIGURE 3.1**).

Figure 3.1 - Flowchar of the study



Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling. Source: from the author.

3.2. Ethical aspects

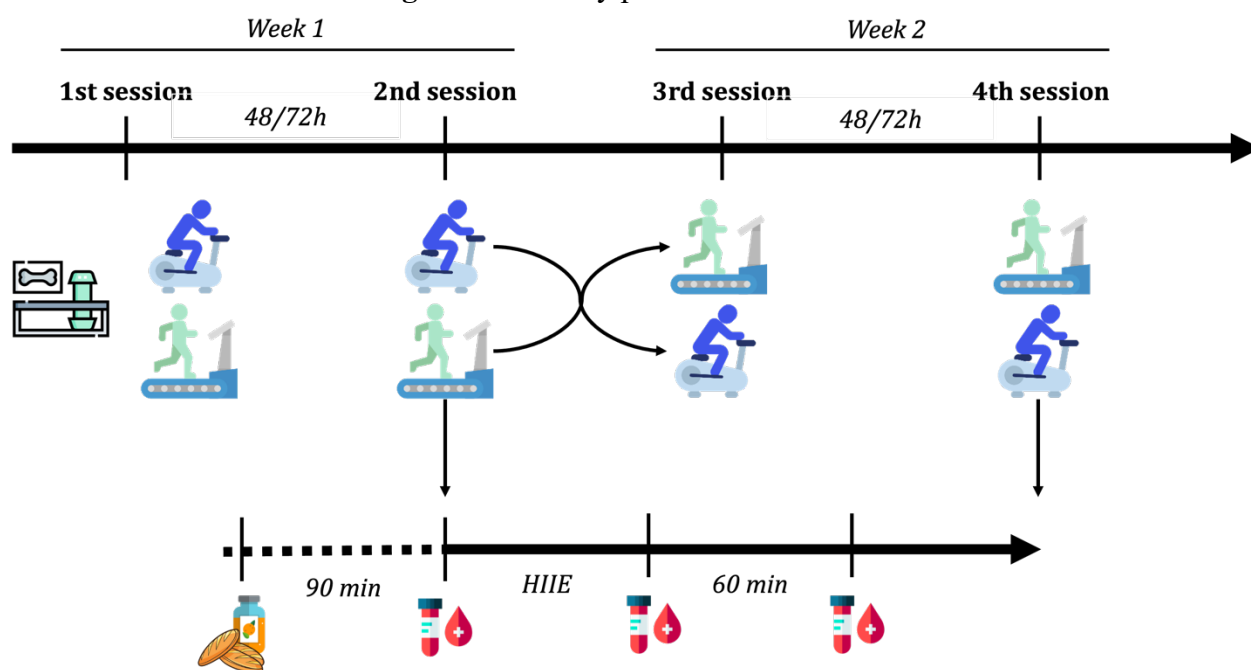
The study was conducted per the Declaration of Helsinki and received ethics approval from the Research Ethics Boards of the Sao Paulo State University (CAAE 67621222.8.0000.5402 / Approval 5.951.255).

3.3. Study design

This was a cross-over randomized designed study. Participants assigned to the study performed two high-intensity exercise trial in random order: a high-intensity interval running (HIIR) trial on the treadmill and a high-intensity interval cycling (HIIC) trial on a cycle ergometer. The order of trials was block randomized using the Excel software. Participants had to visit the laboratory at São Paulo State University twice over two weeks. During their first visit, participants completed baseline assessments such as body composition and anthropometrics, answered

questionnaires, and performed an incremental test on either the bicycle or treadmill according to their assigned exercise mode. The second visit happened 48h to 72h later, when they performed the first high-intensity interval trial of the same exercise mode as the incremental test performed earlier in the week. The third and fourth visits followed the same procedures – with the exception of the baseline assessments – but alternating the exercise modes (cycling and running) (**FIGURE 3.2**).

Figure 3.2 - Study protocol and timeline.



Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling. Source: from the author.

3.4. Intervention protocol

Before the incremental test performed during the first and third visits, the protocol was explained in detail, security information was given, and they were allowed to familiarize themselves with running or cycling at different intensities. In the treadmill, participants warmed up for 5-min at 5 km/h with 1% slope. Then, speed was increased to 6km/h and increments of 1km/h were done every minute until voluntary fatigue. Similarly, the incremental test on the bicycle started with a 5-min warm-up at 35 W, followed by an increase to 60 W and increments of 25 W every minute until voluntary fatigue. Heart rate (HR) was continuously monitored with an OH1 Sensor (Polar®), and rate of perceived exertion (RPE) was evaluated using the Borg Scale, recording maximum values.

After 48 to 72 hours of the incremental test, in the morning (between 07:30 and 10:30) the participants performed the high-intensity interval trial in the exercise mode (running or cycling) they were tested in that week. Both trial started with a 4-min warm-up followed by 8 bouts of 1-min effort at the maximal workload achieved in the incremental test, separated by 1-min passive recovery intervals.

The morning before each trial, participants were given a standardized breakfast containing orange juice and whole wheat bread (butter was allowed upon request). On the morning of each trial, blood samples were collected pre-exercise (1.5 hours post-prandial), 5-min and 60-min post-exercise.

3.5. Baseline measurements

3.5.1. Body composition

The height (cm) of the adolescents were measured using a wall stadiometer (E120A, Tonelli, Brasil) with 0.1 cm accuracy, and body mass (kg) using a digital scale (Welmy, model W300A), with 0.1 kg accuracy. Based on the values obtained, the peak height velocity (PHV) of these adolescents, an important indicator of somatic maturation, was estimated based on the mathematical models (MOORE et al., 2015). The equation provides the time (in years) left (negative values) or past (positive values) for the PHV, then, subtracting the value of age, we can estimate the age at which PHV occurred (APHV).

The bone density (BMD [g/cm²]) and content (BMC [g]) were measured by the bone densitometry technique [dual-energy X-ray absorptiometry - DXA], (General Electrics brand, model WH - Prodigy Primo). The device was calibrated before the beginning of the measurements, following the manufacturer's recommendations, and three examinations were performed: total body, lumbar spine, and femoral neck. The measurements were conducted by a single, previously trained evaluator and, during the exam, all participants were wearing light clothing, barefoot, with no metal objects attached to their bodies. The total body analysis, besides providing the variables of bone density and content, also provided the values of lean soft tissue and body fat, both in percentage and absolute values.

3.5.2. Questionnaires

The adolescents answered the Bone-specific Physical Activity Questionnaire (BPAQ) (WEEKS; BECK, 2008). It collects information regarding historical bone-relevant physical activity in two domains: exercise loading history from birth (past-BPAQ [pBPAQ]) and from the previous 12 months (current-BPAQ [cBPAQ]).

Over the two weeks of assessments and tests, a trained dietitian applied the 24-hour multiple-pass dietary recall with participants via online calls. Participants were previously instructed to follow their usual diet. The calls were made on Mondays, Wednesdays, and Saturdays, accounting for two weekdays and one weekend day, and they were asked to report all foods and beverages consumed on the day before the call. A photographic manual was used to reduce memory bias of portion sizes. The information collected was used to estimate their mean energy intake over the week and the quantities of micronutrients and macronutrients consumed in the week preceding each trial, using a food composition table (BARANOWSKI, 2012).

In addition, information about the date on which menarche occurred and whether the adolescents are on contraceptive methods were collected. If they did, information about the method used was collected.

3.6. Outcome variables

3.6.1. Blood analysis

Approximately 3 mL of whole blood was collected into pre-chilled Vacutainer SST tubes and EDTA from participants' median cubital vein in the antecubital fossa while lying in a supine position. Samples were collected using a standard venipuncture technique by a trained nurse during each session at pre-exercise, and 5 min and 60 min post-exercise. Blood samples were inverted 8-10 times before sitting for 30 min at room temperature. The samples were then centrifuged at 3000 g for 15 min at 4° before the serum and plasma were aliquoted into 1.5ml microcentrifuge tubes (Eppendorfs®) and stored at -80°C until analysis.

3.6.2. Bone turnover markers

The analyses of PINP and CTX-I were carried out by a private laboratory that adheres to the Brazilian standards set by the Ministry of Health of the Brazilian government. PINP was

analysed from serum and the CTX-I, from the plasma EDTA; both used the electrochemiluminescence technique.

Additionally, OPG, RANKL and sclerostin were analysed from serum by the enzyme-linked immunosorbent assay (ELISA) with the following kits: #DSST00, P416285; #DY626, P412568; #DY805, P356096 (R&D System, Minneapolis, USA). The intra-assay coefficient of variation for the standard curve in each assay were 3,7% for sclerostin; 0.9% and 1.1% for RANKL; and 0.7% and 1.0% for OPG.

3.6.3. Cytokines and adipokines

Serum IL-6, IL-10 and TNF- α were measured in triplicate with ELLA single assay kits (cat.# SPCKBPS-003028, ST01B-PS-000276, ST01B-PS-002803, respectively; ProteinSimple, San Jose, CA). The inter-assay coefficients of variation were 9.9% for IL-6, 6.6% for IL-10 and 8.7% for TNF- α . The intra-assay coefficients of variation for IL-6, IL-10 and TNF- α were 4.6%, 6.69% and 2.54%, respectively. Serum concentrations of leptin and adiponectin were acquired by the enzyme-linked immunosorbent assay (ELISA) using commercial kits (R&D System, Minneapolis, USA).

3.6.4. Metabolic markers

In order to control for potential differences in the metabolic demand between the exercise trials, serum glucose and LDH from were measured using the colorimetric method with Labtest commercial kits (Lagoa Santa, Minas Gerais, Brazil).

3.6.5. Affective perception and enjoyment

Before and 5 min after each trial, the adolescents answered the Feeling-Scale (FS), a 11-point single-item bipolar measure ranging from +5 to -5 (ALVES et al., 2019). The difference between pre- and post-exercise was used to estimate exercise impact on self-reported affect and arousal from exercise. Then, 10 min after completing each trial, the adolescents answered the Physical Activity Enjoyment Scale (PACES), which quantifies enjoyment of physical activity through a 17-item bipolar scale that is separated by a 7-point scale (e.g., 1 = “I enjoy it”; 7 = “I hate it”, 4 = “neutral”) (ALVES et al., 2019).

3.7. Statistical Analysis

Normality and variability of the residuals were tested using graphic visualization (i.e., histograms and Q-Q plots). Variables that violated the normality of the residuals were log-transformed. Linear mixed models were used to assess the fixed effects of exercise mode and time (repeated measure) and the interaction between these factors on the outcomes, while accounting for the individual variability (random effect). Kenward-Roger's method was set to adjust the degrees of freedom in the model. Baseline level of the outcome was included as a covariate in the model, and total-body less head BMD and pBPAQ were also tested as covariates. For the models where adipokines were the outcomes, body fat (%) was also included as a covariate. When interaction was significant, False Discovery Rate (FDR) was used to adjust the p-values for multiple comparisons. An independent T-test was used to analyze differences in nutritional variables between weeks and differences between modes on PACES score. Additionally, we also tested the effect of each mode on each item of the PACES questionnaire. Effect size was calculated using Cohen's-d: . Statistical significance was set at an alpha level of 0.05 and analysis were carried using "stats", "lme4", "emmeans" and "effectsizes" packages in R.

4. ACUTE EFFECT OF HIGH-INTENSITY INTERVAL CYCLING AND RUNNING ON BONE TURNOVER MARKERS AND OSTEOKINES IN FEMALE ADOLESCENTS

4.1. Abstract

The purpose of this study was to compare the acute effect of high-intensity interval exercise running (HIIR) and cycling (HIIC) on bone turnover markers and osteokines in adolescent girls, assessing the impact of the exercise on affective perception and enjoyment. In a cross-over design trial, 11 adolescent females, aged 15 to 19 years, completed two high-intensity interval trials (HIIR and HIIC) in random order. They performed a progressive incremental test to determine the workload in peak velocity output and peak power output, which were subsequently used to prescribe the HIIR and HIIC trials, respectively. Each trial consisted of 8 bouts of 1 min of effort at maximal workload with 1 min of passive recovery between bouts. Blood samples were collected pre-exercise (i.e., baseline), 5- and 60-min post-exercise. Serum blood was used to analyze bone markers of formation (i.e., P1NP and OPG) and resorption (i.e., CTX-I, sclerostin and RANKL), and cortisol. The adolescents answered the Feeling Scale (FS) before and after each trial, and the Physical Activity Enjoyment Scale (PACES) 10-min after each trial. P1NP significantly peaked 5-min post-HIIR (Interaction effect: $\beta = 11.418$, $p = 0.027$; $ES = 0.28$) but not following HIIC. Neither HIIR nor HIIC impacted CTX-I (Time effect: $\beta = 0.009$, $p = 0.653$; $ES = 0.09$). In contrast, sclerostin increased immediately after HIIR and HIIC, returning to baseline levels 60-min post exercise (Time effect: $\beta = 10.889$, $p = 0.011$; $ES = 0.89$). HIIR and HIIC did not change OPG (Time effect: $\beta = 48.164$, $p = 0.329$; $ES = 0.07$), RANKL (Time effect: $\beta = 29.316$, $p = 0.132$; $ES = 0.07$), or the logOPG:RANKL ratio (Time effect: $\beta = -0.174$, $p = 0.207$; $ES = 0.11$). Regarding affective and enjoyment, FS score decreased following HIIC ($\Delta = -4.1 \pm 0.9$, $p < 0.001$, $ES = 1.01$). PACES total score was higher in HIIR compared to HIIC (75.6 ± 5.7 vs 67.2 ± 6.3 , $p = 0.004$, $ES = 1.48$). Cortisol levels did not change following any exercise mode (Time effect: $\beta = 0.016$, $p = 0.992$; $ES = 0$). Overall, even in high-intensity exercise, bone formation seems to be conditioned to the presence of impact-loading, as observed in the effect of HIIR in P1NP. Even though the response in the other markers was similar between the exercise modes, HIIC has a negative impact on affective perception and is also less pleasurable than HIIR.

4.2. Introduction

Osteoporosis is a chronic disease defined as weakening of bones due to a decrease in bone mass and strength, consequently increasing the risk of fractures (GÓMEZ et al., 2021). Recent evidence indicates that the prevalence of osteopenia (i.e., reduced bone mass) and osteoporosis is 40% and 19.4% worldwide, respectively (XIAO et al., 2022). In addition, women, especially in postmenopausal period, are at greater risk of developing osteoporosis (WEAVER et al., 2016; XIAO et al., 2022). Although sexual hormones changes play a significant role in the rapid loss of bone mass post-menopause (CAULEY, 2015; ZALLONE, 2006), women tend to have a lower peak bone mass (PBM) in early adulthood (BAXTER-JONES et al., 2011).

Among the factors capable of affecting PBM is physical activity (BAXTER-JONES et al., 2008; WEAVER et al., 2016). The effect of physical activity on bone occurs due to mechanical impact generated by ground reaction forces and muscle contractions during practice, in addition of hormones activity (BONNET; FERRARI, 2010; KOHRT et al., 2004). Despite that, it is unclear whether only gravitational impact generates bone adaptations, or the intense muscle contractions performed in high-intensity exercises are enough to stimulate anabolic bone responses. In theory, the physical exercise combining high-intensity exercise and higher ground reaction force can boost bone formation, but that is an assumption to be confirmed in paediatric groups yet.

High-intensity interval exercise (HIIE) has proven to be a time-efficient and safe strategy with several health benefits for children and adolescents (CAO; QUAN; ZHUANG, 2019; EDDOLLS et al., 2017). Moreover, evidence from animal models indicates that HIIE appears to be more effective than continuous exercise, as short periods of rest between bouts are essential to restore the responsiveness of bone cells to the stimulus applied (ROBLING et al., 2002). However, research focused on bone health involving bone metabolic markers remains limited, especially studies comparing HIIE protocols with gravitational impact, e.g., high-intensity interval running (HIIR), and without gravitational impact, e.g., high-intensity interval cycling (HIIC). To date, there are two studies in the literature comparing the effect of HIIR and HIIC on bone turnover makers (BTM) and osteokine, being both studies carried out with active adult women (KOUVELIOTI et al., 2018, 2019b).

BTM and osteokines are considered dynamic variables of bone tissue, being considered relevant markers of anabolic or catabolic effect of an exercise intervention (DOLAN et al., 2020). The most important BTMs are procollagen type 1 N-terminal ropeptide (P1NP) and C-terminal telopeptide of type I collagen (CTX-I), which are fragments of collagen released in the blood stream by osteoblasts during formation and by osteoclasts during resorption, respectively (SZULC et al., 2017). Osteokines, on the other hand, are signalling proteins realized mainly by osteocytes, which influence osteoblasts and osteoclasts acitivation/differentiation (BURGERS; WILLIAMS, 2013). Sclerostin is a catabolic osteokine that acts inhibiting the canonical Wnt pathway, consequently decreasing bone formation (CHOI; ROBLING, 2021; GALEA; LANYON; PRICE, 2017). Another catabolic osteokine also produced by osteocytes is the Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), that, when bounded to its receptor RANK in the membrane of the pre-osteoclasts, induces its differentiation, increasing bone resorption (YOU et al., 2008). Osteoclastogenesis may also be inhibit by the over expression of osteoprotegerin (OPG), an anabolic protein that binds to RANKL and prevents it to induce osteoclasts differentiation (ROBLING et al., 2008; YOU et al., 2008). Although studies have explored these variables in paediatric populations, it is not clear what effect HIIR and HIIC has on these variables in adolescent girls not engaged in any exercise routine.

Despite the positive effects of HIIE on paediatrics' health, studies should also consider the impact the interventions have on affect (i.e., pleasure and displeasure) and enjoyment, as they might predict the adherence in physical exercise program (DISHMAN et al., 2005; DISHMAN; SALLIS; ORENSTEIN, 1985; EKKEKAKIS, 2009; RHODES; KATES, 2015). Evidence in the literature indicates that HIIE can generate unpleasant feelings, decreasing the adherence to the exercise program, due to a combination of cognitive processes and physical sensations (BIDDLE; BATTERHAM, 2015). Conversely, despite the unpleasant feelings generated by the exercise, the reward mechanism may also act, generating a positive feeling of pleasure as result of increased self-efficacy and excitement after an intense exercise session (MALIK et al., 2018; ROSE; PARFITT, 2007).

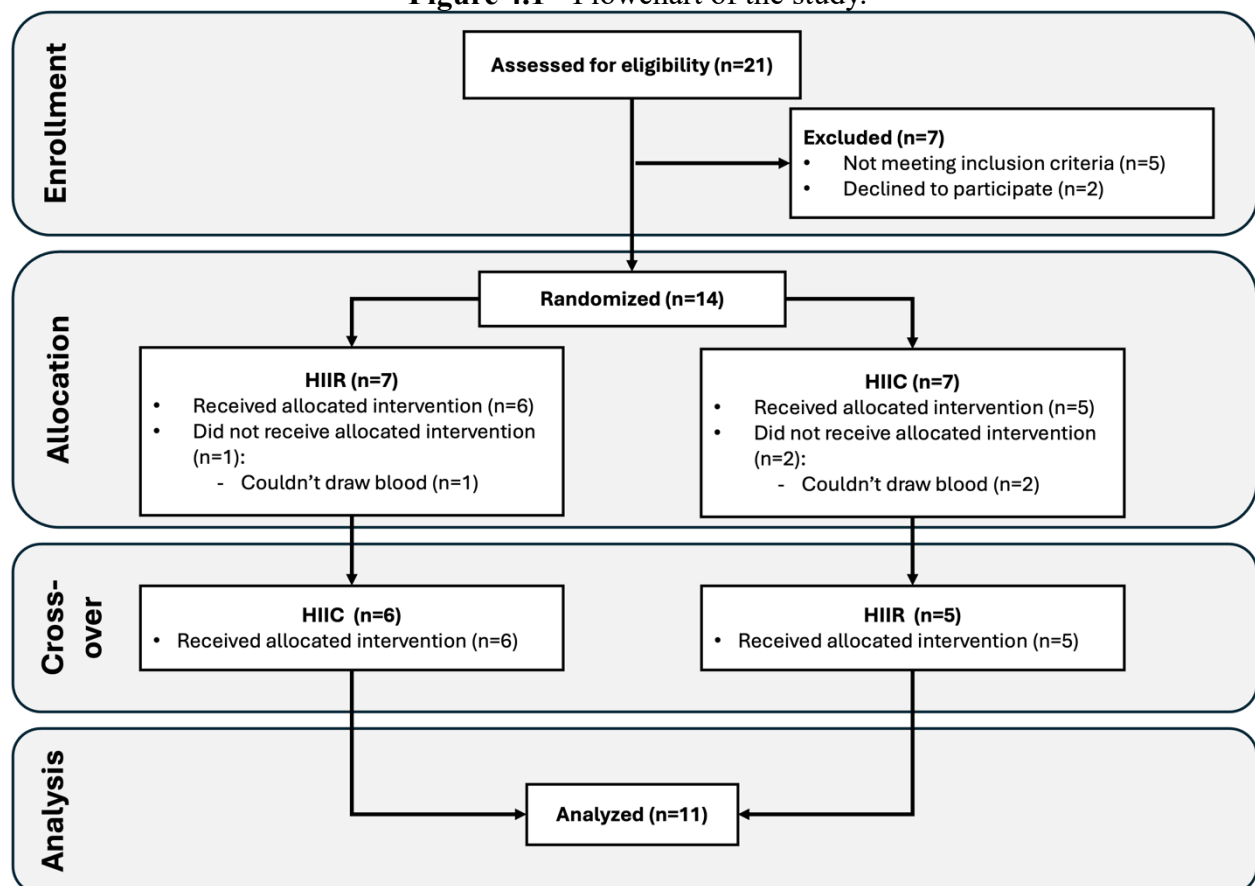
Thus, the aim of this study was to investigate the impact of HIIR and HIIC on bone turnover markers and osteokines in untrained adolescent females, while assessing the effect of HIIC and HIIR on their affective perception and enjoyment.

Methods

4.2.1. Participants

In the city of Presidente Prudente, Brazil, female adolescents were recruited from schools and via online publications to participate in this study. A total of 21 adolescents were assessed for eligibility, which inclusion criteria were: (i) be female; (ii) aged between 14 and 19 years; (iii) non-smokers; (iv) not enrolled in sports or exercise training for the previous 6 months; (v) be free of musculoskeletal injury that would impair exercise, and be able to perform running and cycling. Exclusion criteria were: (i) not passed 2 years from PHV, (ii) could not draw blood. After initial screening, 14 of them met the inclusion criteria. However, after being assigned, 3 adolescents were excluded from the study because they could not draw blood. Thus, 11 adolescents completed both trials (**FIGURE 4.1**).

Figure 4.1 - Flowchart of the study.



Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling. Source: from the author.

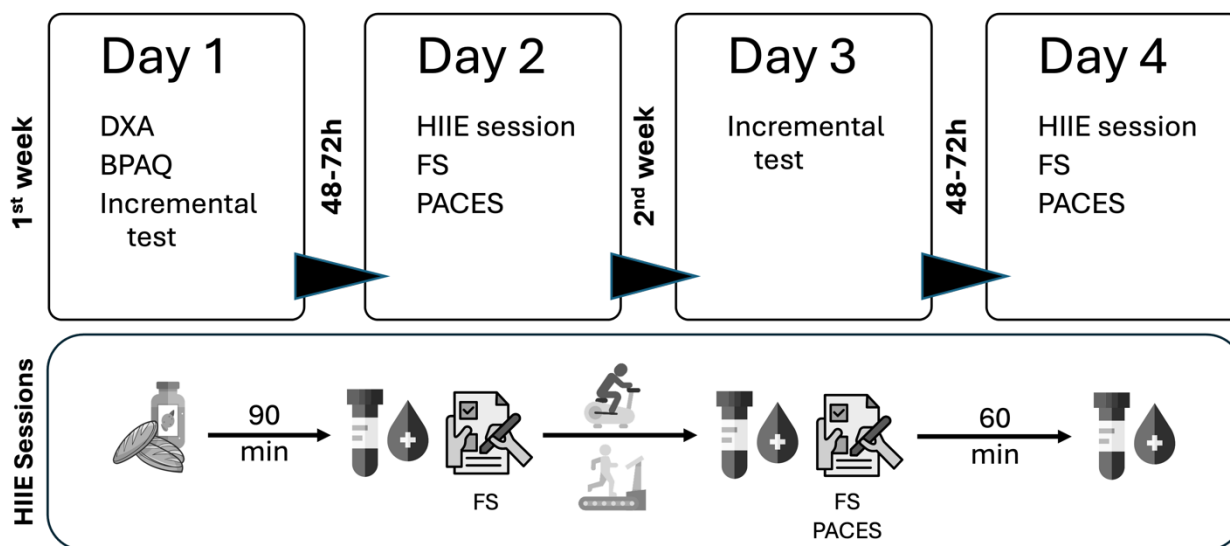
4.2.2. Ethical aspects

The study was conducted considering the Declaration of Helsinki and received ethics approval from the Research Ethics Boards of the Sao Paulo State University (CAAE 67621222.8.0000.5402 / Approval 5.951.255).

4.2.3. Study Design

This was a cross-over randomized designed study. Participants assigned to the study performed two high-intensity exercise trial in random order: a high-intensity interval running (HIIR) trial on the treadmill and a high-intensity interval cycling (HIIC) trial on a cycle ergometer. The order of trials was block randomized using the Excel software. Participants had to visit the laboratory at São Paulo State University twice over two weeks. During their first visit, participants completed baseline assessments such as body composition and anthropometrics, answered questionnaires, and performed an incremental test on either the bicycle or treadmill according to their assigned exercise mode. The second visit happened 48h to 72h later, when they performed the first high-intensity interval trial of the same exercise mode as the incremental test performed earlier in the week. The third and fourth visits followed the same procedures – with the exception of the baseline assessments – but alternating the exercise modes (cycling and running) (**FIGURE 4.2**).

Figure 4.2 - Study timeline and procedures.



Note: DXA, dual-energy X-ray absorptiometry; BPAQ, bone-specific physical activity questionnaire; FS, feeling scale; PACES, physical activity enjoyment scale; HIIR, high-intensity interval running; HIIC, high-intensity interval cycling. Source: from the author.

4.2.4. Intervention protocol

Before the incremental test performed during the first and third visits, the protocol was explained in detail, security information was given, and they were allowed to familiarize themselves with running or cycling at different intensities. In the treadmill, participants warmed up for 4-min at 5 km/h with 1% slope. Then, speed was increased to 6km/h and increments of 1km/h were done every minute until voluntary fatigue. Similarly, the incremental test on the bicycle started with a 4-min warm-up at 35 W, followed by an increase to 60 W and increments of 25 W every minute until voluntary fatigue.

After 48 to 72 hours of the incremental test, in the morning (between 07:30 and 10:30) the participants performed the high-intensity interval trial in the exercise mode (running or cycling) they were tested in that week. Both trial started with a 4-min warm-up followed by 8 bouts of 1-min effort at the maximal workload achieved in the incremental test, separated by 1-min passive recovery intervals. Heart rate (HR) was continuously monitored with an OH1 Sensor (Polar®), and Rate of Perceived Exertion (RPE) was evaluated using the Borg Scale, recording maximum values..

The morning before each trial, participants were given a standardized breakfast containing orange juice and whole wheat bread (butter was allowed upon request). On the morning of each trial, blood samples were collected pre-exercise (1.5 hours post-prandial), 5-min and 60-min post-exercise.

4.2.5. Baseline measurements

4.2.5.1. Body composition

The height (cm) of the adolescents were measured using a wall stadiometer (E120A, Tonelli, Brasil) with 0.1 cm accuracy, and body mass (kg) using a digital scale (Welmy, model W300A), with 0.1 kg accuracy. Based on the values obtained, the peak height velocity (PHV) of these adolescents, an important indicator of somatic maturation, was estimated based on the mathematical models (MOORE et al., 2015). The equation provides the time (in years) left (negative values) or past (positive values) for the PHV, then, subtracting the value of age, we can estimate the age at which PHV occurred (APHV).

The bone density (BMD [g/cm²]) and content (BMC [g]) were measured by the bone densitometry technique [dual-energy X-ray absorptiometry - DXA], (General Electrics brand,

model WH - Prodigy Primo). The device was calibrated before the beginning of the measurements, following the manufacturer's recommendations, and three examinations were performed: total body, lumbar spine, and femoral neck. The measurements were conducted by a single, previously trained evaluator and, during the exam, all participants were wearing light clothing, barefoot, with no metal objects attached to their bodies. The total body analysis, besides providing the variables of bone density and content, also provided the values of lean soft tissue and body fat, both in percentage and absolute values.

4.2.5.2. Questionnaires

The adolescents answered the Bone-specific Physical Activity Questionnaire (BPAQ) (WEEKS; BECK, 2008). It collects information regarding historical bone-relevant physical activity in two domains: exercise loading history from birth (past-BPAQ [pBPAQ]) and from the previous 12 months (current-BPAQ [cBPAQ]).

Over the two weeks of assessments and tests, a trained dietitian applied the 24-hour multiple-pass dietary recall with participants via online calls. Participants were previously instructed to follow their usual diet. The calls were made on Mondays, Wednesdays, and Saturdays, accounting for two weekdays and one weekend day, and they were asked to report all foods and beverages consumed on the day before the call. A photographic manual was used to reduce memory bias of portion sizes. The information collected was used to estimate their mean energy intake over the week and the quantities of micronutrients and macronutrients consumed in the week preceding each trial, using a food composition table (BARANOWSKI, 2012).

In addition, information about the date on which menarche occurred and whether the adolescents are on contraceptive methods were collected. If they did, information about the method used was collected.

4.2.6. Outcome variables

4.2.6.1. Blood analysis

Approximately 3 mL of whole blood was collected into pre-chilled Vacutainer SST tubes and EDTA from participants' median cubital vein in the antecubital fossa while lying in a supine position. Samples were collected using a standard venipuncture technique by a trained nurse during each session at pre-exercise, and 5 min and 60 min post-exercise. Blood samples were inverted 8-

10 times before sitting for 30 min at room temperature. The samples were then centrifuged at 3000 g for 15 min at 4° before the serum and plasma were aliquoted into 1.5ml microcentrifuge tubes (Eppendorfs®) and stored at -80°C until analysis.

The analyses of P1NP and CTX-I were carried out by a private laboratory that adheres to the Brazilian standards set by the Ministry of Health of the Brazilian government. P1NP was analyzed from serum and the CTX-I, from the plasma EDTA; both used the electrochemiluminescence technique. Additionally, RANKL, OPG and sclerostin were analysed from serum by the enzyme-linked immunosorbent assay (ELISA) with the following kits: #DSST00, P416285; #DY626, P412568; #DY805, P356096 (R&D System, Minneapolis, USA). The intra-assay coefficient of variation for the standard curve in each assay were for sclerostin: 3,7%; RANKL 1.1 and 0.9%; OPG: 0.7 and 1.0%.

4.2.6.2. Affective perception and enjoyment

Before and 5 min after each trial, the adolescents answered the Feeling-Scale (FS), a 11-point single-item bipolar measure ranging from +5 to -5 (ALVES et al., 2019). The difference between pre- and post-exercise was used to estimate exercise impact on self-reported affect and arousal from exercise. Then, 10 min after completing each trial, the adolescents answered the Physical Activity Enjoyment Scale (PACES), which quantifies enjoyment of physical activity through a 17-item bipolar scale that is separated by a 7-point scale (e.g., 1 = “I enjoy it”; 7 = “I hate it”, 4 = “neutral”) (ALVES et al., 2019).

4.2.7. Statistical Analysis

Normality and variability of the residuals were tested using graphic visualization (i.e., histograms and Q-Q plots). Variables that violated the normality of the residuals were log-transformed. Linear mixed models were used to assess the fixed effects of exercise mode and time (repeated measure) and the interaction between these factors on the outcomes, while accounting for the individual variability (random effect). Kenward-Roger's method was set to adjust the degrees of freedom in the model. Additionally, we tested the impact of TBLH-aBDM and pBAPQ in each model, but they were not significant nor improved the goodness-of-fit of the models; thus, only baseline level of the outcome was included as a covariate in the model. False Discovery Rate (FDR) was used to adjust the p-values for multiple comparisons when a significant effect was

found. An independent T-test was used to analyze differences in nutritional variables between weeks and differences between modes on PACES score. Additionally, we also tested the effect of each mode on individual items from PACES. Effect size was calculated using Cohen's-d: <0.50 small effect-size, 0.50-0.79 moderate effect-size, 0.80-1.29 large effect-size, and ≥ 1.30 very large effect-size (COHEN, 2013). Statistical significance was set at an alpha level of 0.05 and analysis were carried using "stats", "lme4", "emmeans" and "effectsizes" packages in R.

4.3. Results

The **Table 4.1** presents the characteristics of the sample. The adolescent females presented a mean age of 17.4 (0.9) years, 37.1% of FM and BMI of 24 (5.1). Considering the nutrient intake during the week of each test, there was no difference in relation to the intake of macronutrients, i.e., protein ($p = 0.634$), carbohydrate ($p = 0.885$) and lipids ($p = 0.958$), and micronutrients, i.e., calcium ($p = 0.652$), phosphorus ($p = 0.408$) and vitamin D ($p = 0.334$) (**TABLE 4.2**).

Table 4.1 - Samples' characteristics (n=11).

	Mean (SD)	95%CI
Age (years)	17.4 (0.9)	16.7 to 18.0
Body mass (kg)	65.1 (14.6)	55.3 to 75.0
Height (cm)	164.8 (8.0)	159.4 to 170.2
BMI	24.0 (5.1)	20.6 to 27.4
FM (%)	37.1 (6.1)	33.0 to 41.2
LST (kg)	35.2 (5.7)	31.4 to 39.1
Maturity Offset (years)	4.4 (0.8)	3.8 to 4.9
TBLH-aBMD (z-score)	0.8 (1.1)	0.1 to 1.5

Note: SD, standard deviation; BMI, body mass index; FM, fat mass; LST, lean soft tissue, TBLH-aBMD, total body less head areal bone mineral density.

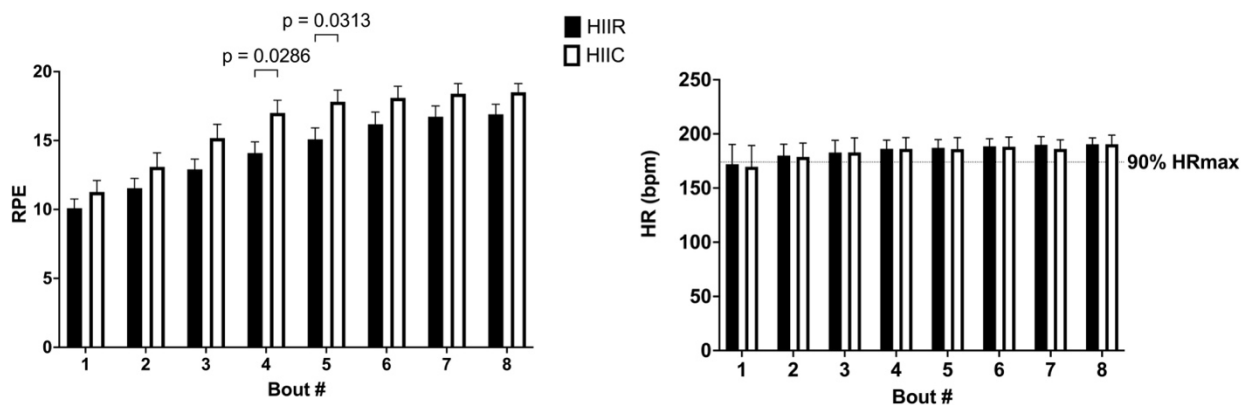
Table 4.2 - Macro and micronutrients intake over the week preceding HIIE trials (n=11).

Nutrients (g)/body mass (kg)	Past-HIIR Mean (SD)	Past-HIIC Mean (SD)	p-value
Protein (g/kg)	1.64 (0.59)	1.51 (0.61)	0.634
Carbohydrate (g/kg)	4.16 (1.71)	4.04 (2.22)	0.885
Lipids (g/kg)	1.05 (0.49)	1.07 (0.55)	0.958
Calcium (mg/d)	446.5 (217.0)	484.0 (163.5)	0.652
Phosphorus (mg/d)	1161.8 (360.2)	1039.2 (319.6)	0.408
Vitamin D ($\mu\text{g/d}$)	2.6 (2.5)	1.77 (1.0)	0.334

Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling; SD, standard-deviation.

The HR at the end of every bout was consistently greater than 90% of participant's maximum HR (HRmax), as determined by the incremental test. Although HR at the end of every effort stage was precisely similar between modes, the perceived exertion during these bouts differed between HIIC and HIIR. Interestingly, RPE increased faster during HIIC, becoming significantly greater than HIIR after the fourth ($p=0.029$) and fifth ($p=0.031$) bouts. However, from the sixth bout onwards, RPE became similar again between modes (**FIGURE 4.3**).

Figure 4.3 - RPE and HR (mean $\pm$ SEM) change during HIIR and HIIC (n=11).

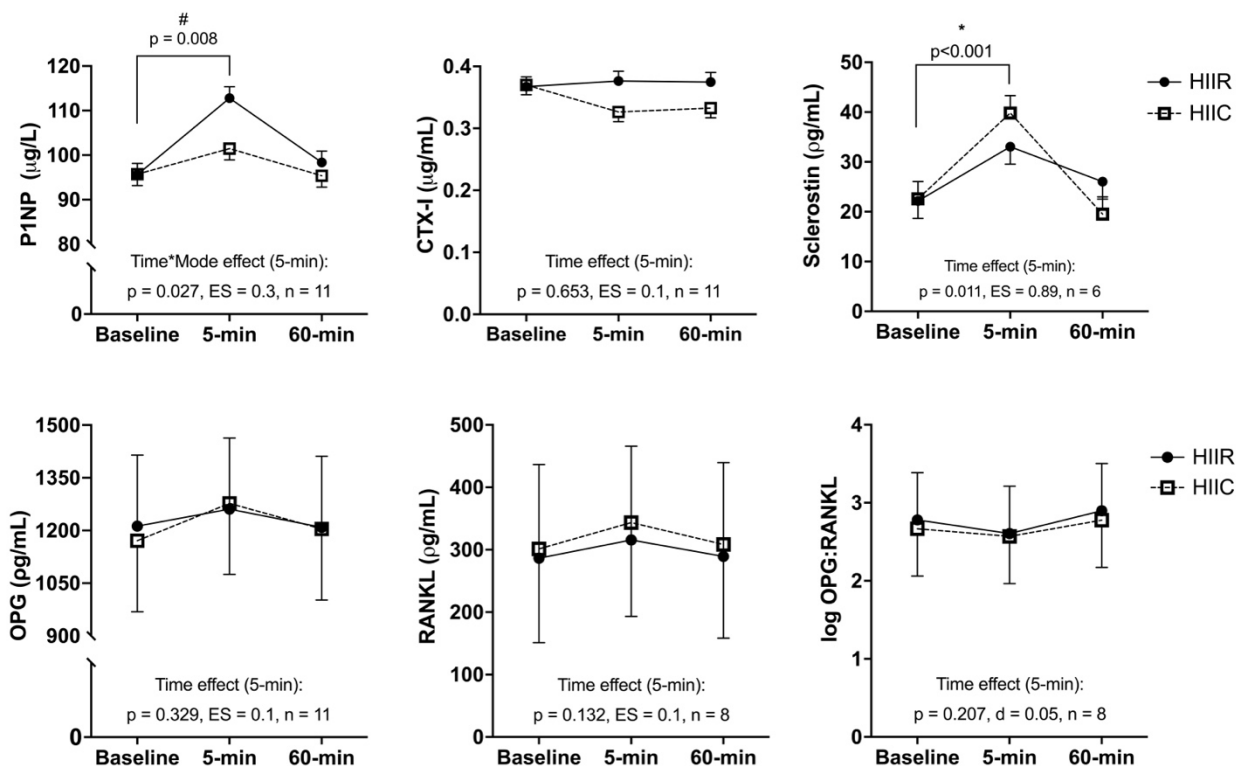


Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling; RPE, rate of perceived exertion; HR, heart rate. Source: from the author.

4.3.1. Bone Markers

A significant increase in serum P1NP was observed 5-min post HIIR (interaction effect: $\beta = 11.418$; $p = 0.027$; $d = 0.3$ [small effect]), but not following HIIC, with concentration returning to baseline levels 60-min post exercise. Neither HIIR nor HIIC changed serum concentration of CTX-I (time effect: $\beta = 0.009$; $p = 0.653$; $d = 0.1$ [trivial effect]). In contrast, both modes led to an increase in sclerostin 5-min post exercise (time effect: $\beta = 10.886$, $p = 0.011$; $d = 0.9$ [large effect]). Regarding osteokines, HIIR and HIIC did not affect OPG (time effect: $\beta = 48.164$; $p = 0.329$; $d = 0.07$ [trivial effect]), RANKL (time effect: $\beta = 29.316$; $p = 0.132$; $d = 0.1$ [trivial effect]) or OPG:RANKL ratio (time effect: $\beta = -0.174$; $p = 0.207$; $d = -0.1$ [trivial effect]) (**FIGURE 4.4**).

Figure 4.4 - Bone turnover makers and osteokines response (mean $\pm$ SEM) to HIIR and HIIC.



Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling; ES, effect size, #difference between baseline and 5-min post-exercise in HIIR; *difference between baseline and 5-min post-exercise; baseline measurements were inserted as a covariate. Source: from the author.

4.3.2. Affect and enjoyment

As shown in **Table 4.3**, HIIC negatively impacted the affective perception of the adolescents following exercise, while the score did not significantly change following HIIR (interaction effect: $\beta = -2.818$; $p = 0.035$; $d = -1.0$ [large effect]). Moreover, the enjoyment for the exercise was greater after HIIR compared to HIIC (75.6 vs 67.2; $\Delta = 8.5$ [95%CI 2.8 to 14.1], $d = 1.5$ [very large effect]). The individual analysis of the items from the PACES showed that HIIR was more exhilarating ($p = 0.014$, $d = 1.2$ [large effect]), invigorating ($p = 0.040$, $ES = 0.9$ [large effect]), energizing ($p = 0.037$, $d = 1.0$ [large effect]) and made the adolescents feel physically better ($p = 0.007$, $d = 1.3$ [very large effect]) compared to HIIC (**FIGURE 4.5**). Although the interaction effect was not significant, cortisol has slightly increased post-HIIC but not post-HIIR ($\Delta = 4.0$, $p = 0.080$; $d = 1.0$ [large effect]) (**TABLE 4.4**).

Table 4.3 - Effect of HIIR and HIIC on affective perception and enjoyment (n=11).

	FS		Fixed effects			PACES	
	Pre Mean (95%CI)	Post Mean (95%CI)	Time effect $\beta \pm SE$ (p-value)	Mode effect $\beta \pm SE$ (p-value)	Time-by-mode effect $\beta \pm SE$ (p-value)	Mean (95% CI)	Δ (95%CI)
HIIR	3.3 (2.0 to 4.6)	2.0* (0.7 to 3.3)	-2.7 $\pm$ 0.9 (p<0.001)	-1.15 $\pm$ 0.7 (p=0.104)	-2.8 $\pm$ 1.3 (p=0.035)	75.6* (71.8 to 79.5)	8.5 (3.1 to 13.8)
HIIC	3.5 (2.2 to 4.8)	-0.6 ^a (-1.9 to 0.8)				67.2 (62.9 to 71.4)	

Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling; FS, Feeling Scale; ^astatistical difference between pre- and post-exercise; *Statistical difference from HIIC.

Table 4.4 – Cortisol response (mean $\pm$ 95%CI) to HIIR and HIIC (n=6).

	FS		Fixed effects		
Cortisol (μ g/dL)	Pre Mean (95%CI)	Post Mean (95%CI)	Time effect $\beta \pm SE$ (p-value)	Mode effect $\beta \pm SE$ (p-value)	Time-by-mode effect $\beta \pm SE$ (p-value)
HIIR	7.5 (4.8 to 10.1)	9.5 (6.9 to 12.2)	-2.03 $\pm$ 1.14 (p=0.092)	2.03 $\pm$ 1.15 (p=0.092)	4.02 $\pm$ 2.18 (p=0.080)
HIIC	9.7 (7.0 to 12.3)	11.7 (9.1 to 14.4)			

Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling. ^astatistical difference between pre- and post-exercise; *Statistical difference from HIIC.

4.4. Discussion

This study aimed to investigate whether HIIE with and without impact-loading (HIIR and HIIC, respectively) leads to similar bone marker responses in female adolescent. Our results showed that the bone marker of formation, P1NP, significantly increased immediately after HIIR but not following HIIC; however, the effect size of this change was of small magnitude. Additionally, both modes of exercise were ineffective to alter serum concentrations of important osteokines, i.e., OPG and RANKL, involved in the signaling pathways of bone turnover cells, such as osteoblast and osteoclast. On the other hand, HIIR and HIIC increased the serum concentration of sclerostin immediately after exercise, with a large effect size for the change. Despite the overall effect on bone markers being similar between HIIR and HIIC, their impacts on affective responses and enjoyment differed, with HIIR eliciting more positive responses characterized by reduced affective arousal and greater enjoyment.

Our results are partially in line with Kouvelioti et al (2018), whose study also investigated the influence of HIIE involving impact on bone markers but with recreationally active women. Although the authors found a rising trend in P1NP after HIIR (12% increase 5-min post-exercise, and 19% increase 1h post-exercise), this increase was not sufficient to reach significant time-by-mode interecation effect (KOUVELIOTI et al., 2018). On the other hand, the significant effect of the time-by-mode interaction found in our study on this marker showed a small effect size, but a similar change (18%). The explanation for these differences might be related to the fact that we did not account for plasma volume changes, which could inflate the differences found in the markers; however, the adolescents were encouraged to keep hydrating throughout the whole trial, potentially attenuating the influnce of this factor on the results. Besides, it is also possible that the younger and untrained sample from our study presented greater responsiveness to impact (HAAPASALO et al., 1998; PEARSON; LIEBERMAN, 2004), resulting in greater secretion of P1NP following exercise, such as we hypothesized for the study. Studies investigating age-related differences in bone markers showed that younger individuals – either boys and girls – present higher concentrations of bone markers, such as sclerostin, amino-terminal cross-linking telopeptide (NTX), and bone-specific alkaline phosphatase (bone-ALP), and the response of these markers to plyometric exercise is greater in the younger sample (DEKKER et al., 2017; KISH et al., 2015).

Nevertheless, Dolan et al (2020) have shown in a systematic review and meta-analysis that bone formation markers, such as P1NP, indeed show small transient increases within 15-min post-exercise, although this would be related to exercise-induced damage of connective tissue or haemodynamic shifts (DOLAN et al., 2022).

Contrasting effects of HIIE without impact on bone resorption markers are found in literature. While our study showed that CTX-I serum concentration did not change following both HIIR and HIIC, Theocharidis et al (2020) observed an increase in CTX-I concentrations following high-intensity interval swimming among adolescents (THEOCHARIDIS et al., 2020), while plyometric exercise would downgrade CTX-I following plyometric exercise on adolescent females (KURGAN et al., 2020). Conversely, CTX-I concentrations increased significantly following HIIE, regardless of impact, in young men (KOUVELIOTI et al., 2019a) but not in young women (KOUVELIOTI et al., 2018). CTX-I is a resorption marker that is more likely to increase in response to longer exercise (e.g., endurance exercise) or in exercise trials that cause disruption in calcium homeostasis (GUILLEMANT et al., 2004; HAAKONSEN et al., 2015; SHEA et al., 2014; SHERK et al., 2017). Exercise-induced decrease in serum ionized calcium leads to increased secretion of parathyroid hormone (PTH), which is responsible for stimulating bone resorption to counteract the drop in calcium in the bloodstream. (WHERRY; SWANSON; KOHRT, 2022). Thus, exercise-induced increase in CTX-I may not necessarily predict a mechanical adaptation of the bone (e.g., an initial catabolic stimuli necessary to activate remodeling cycle), but be a consequence of hormonal and metabolic disturbances caused by the exercise, mainly related to the calcium homeostasis (DOLAN et al., 2022). In this sense, the lack of time and mode effect on CTX-I found in this study could be a positive sign that, different from previous findings on the effect of cycling on bone, HIIC may not induce a catabolic effect marked by the unchanged CTX-I concentrations in adolescent females.

The activity of osteoblast and osteoclast is highly influenced by the osteocytes, the mechanosensors within the bone and major responsible for bone homeostasis (HUGHES et al., 2020). They orchestrate the bone response to mechanical loading by secreting factors and proteins, such as sclerostin, RANKL, and OPG, that will act in pathways related to osteoblastic and osteoclastic differentiation (CHOI; ROBLING, 2021; GOODMAN; HORNBERGER; ROBLING, 2015; YOU et al., 2008). In our study, HIIR and HIIC increased sclerostin similarly 5 min post-

exercise, with values returning to baseline 60 min post-exercise. Increased sclerostin following exercise has also been found in other studies involving women and different modes of exercise (GUZMAN et al., 2022; KOUVELIOTI et al., 2019b; KURGAN et al., 2020; PICKERING et al., 2017). This glycoprotein is known to increase the resorption activity of osteoclasts while inhibiting osteoblasts' differentiation via inhibition of canonical Wnt signaling, thus increasing bone catabolism. Studies on animals have shown that mechanical loading downregulates sclerostin while it plays a crucial role in bone loss under unloading conditions (LIN et al., 2009; ROBLING et al., 2008). In this sense, one would expect that the increase in sclerostin after exercise observed in humans could be a signaling of osteocytes to initiate the targeted-remodeling process due to tissue microdamage due to high exercise intensity or repetitive impact (HUGHES et al., 2020); however, bone remodeling induced by sclerostin would also involve downregulation of OPG and increased RANKL, which were not found significant over the 60-min follow-up of the study. Interestingly, previous studies have shown that increased sclerostin following exercise might be associated with increased pro-inflammatory cytokine tumor necrosis factor alpha (TNF- α) (KOUVELIOTI et al., 2019b; OHORI et al., 2019), which was found augmented following both modes in our trial (data not shown).

Regarding OPG and RANKL, neither HIIR nor HIIC had an impact on these markers following exercise. The RANKL stimulates osteoclastogenesis by connecting to its receptor RANK on the surface of osteoclast precursors, while OPG, a decoy receptor, blocks this action by binding to RANKL, inhibiting the connection with its receptor (YOU et al., 2008). Thus, the balance between them is frequently used as an indicative of bone anabolism/catabolism. To the best of our knowledge, only one study investigated the effect of HIIR and HIIC on these cytokines in young female adults, and they found that both modes increased the concentration of RANKL, while OPG remained unchanged (BORZOOEIAN et al., 2023). However, a plyometric session performed by adolescent girls was effective to decrease RANKL concentrations post-exercise, while OPG was not affected (DEKKER et al., 2017; KLENTROU et al., 2018). Conversely, when studies were carried out in males, plyometric exercise had a positive impact on OPG in boys and men (KISH et al., 2015), while HIIC increased both OPG and RANKL in young male (MEZIL et al., 2015).

Considering the similar effect that HIIC and HIIR had on bone turnover markers and cytokines, with just a small effect on P1NP in favour of HIIR, the overall efficacy of the intervention

as a source for clinical application should be considered in light of the effect it has on affect and enjoyment of the adolescent, since it may predict future physical activity behavior (DISHMAN et al., 2005; RHODES; KATES, 2015). For this reason, we assessed the impact that each intervention had on their affective perception (e.g., pleasant and unpleasant feelings) as well as how much they enjoyed each trial. Affective arousal was nearly three times greater following HIIC compared to HIIR, which did not significantly change between pre and post exercise. Additionally, post-exercise enjoyment was significantly different between modes, with a large effect size in favor of HIIR. This findings can be explained by the Dual-Mode Theory (DMT), which proposes that affective response to exercise is a product of the interplay between cognition (e.g., self-efficacy) and interoceptive cues about physiological symptoms (e.g., HR, acidosis, etc.) (EKKEKAKIS, 2009). Although HR was similar between modes, RPE almost predicted ($p = 0.09$; data not shown) the differences in the FS found between HIIR and HIIC. This could have occurred because of sensations involved in each trial, such as “burning sensation” in the quadriceps, as widely reported by the participants, which they might not be used to feel based on their training experience background. During cycling, the work rate is distributed among fewer muscle fibers compared to running; consequently, the metabolic stress on each muscle fiber may be higher, as each fiber requires a greater amount of energy (ACHTEN; VENABLES; JEUKENDRUP, 2003). In this sense, Malik et al (2018) have also reported a negative correlation between RPE following HIIR. According to Rose and Parfitt (ROSE; PARFITT, 2007) an individual may experience a positive affective response when they perceive they can complete an exercise session while being comfortably challenged. Consequently, although HIIR was also highly-demanding from the adolescents, the lower negative perceptions might have influenced the enjoyment for the exercise, as shown by the PACES’s results.

The results should be interpreted in light of the limitations of the study. First, the sample size of the study is particularly low and might have underpowered our analysis, specially regarding the osteokines. Although each adolescent is their own control in a cross-over design, it would be useful to have a non-exercising group to confirm that the only differences found in the study were related to the exercise and not diurnal and post-prandial fluctuations. The latter could potentially influence the bone markers, mainly CTX-I, which is usually indicated to be measured in a fasted state (QVIST et al., 2002). However, Scott et al. (2012) showed that fasting did not substantially affect the bone metabolic response to exercise in men, despite a minor reduction of the duration of

the increase in CTX-I found in their study. Nonetheless, future studies should investigate whether this is replicable to women and pediatric population. Furthermore, each adolescent performed their trials in the same moment of the day, with all of them completing their trials between 08:00 and 11:00 in the morning, after having a standardized breakfast.

In conclusion, our study shows that HIIR had a positive but small effect on the formation marker P1NP in adolescent females, while HIIC did not affect this outcome. Despite that, both modes did not change the circulating level of the anabolic osteokine OPG nor the catabolic RANKL, while a transient increase in sclerostin was found significant 5 min post-HIIR and HIIC. Overall, HIIR seems to be the most appropriate intervention for adolescent females regarding its potential benefit for the bone, but more importantly, because it was shown to be more enjoyable and less unpleasant for them.

4.5. Acknowledgments

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5. CYTOKINE AND ADIPOKINE RESPONSE FOLLOWING HIGH-INTENSITY INTERVAL RUNNING AND CYCLING IN FEMALE ADOLESCENTS.

5.1. Abstract

The purpose of this study was to compare the response of cytokines, and adipokines to high-intensity interval running (HIIR) versus cycling (HIIC) in female adolescents. Eleven adolescent females at post-peak height velocity, aged 15 to 19 years, performed two trials (HIIR and HIIC) in random order. Each participant performed a progressive incremental test for each mode of exercise to determine the >90% max workload for the trials. The trials consisted of 8 bouts of 1 min of running or cycling at >90% maximal workload with 1 min of passive recovery in between bouts. Blood samples were collected pre-exercise, 5- and 60-min post-exercise. IL-6, IL-10, TNF- α , leptin and adiponectin were measured in serum. IL-6 increased at 60 min following exercise compared to pre-exercise (time-effect: $\beta=1.139$, $p=0.003$; $ES=1.29$) in both trials. IL-10 started to increase immediately after exercise (time effect: $\beta=0.155$, $p=0.031$; $ES=0.92$) and peaked 60 min after exercise compared to pre-exercise (time effect: $\beta=0.202$, $p=0.005$; $ES=1.20$). TNF- α concentrations peaked 5 min after exercise, returning to baseline levels after 60 min of exercise, with a higher increase in HIIR compared to HIIC (interaction effect: $\beta=1.575$, $p=0.025$; $ES=2.39$). No change was observed in the IL-6/IL-10 ratio, but a significant decrease was observed in the TNF- α /IL-10 ratio 60 min post-exercise (time effect: $\beta=-0.163$, $p=0.028$; $ES=0.93$). Adiponectin was elevated 60 min post-exercise compared to pre-exercise in both modes (time-effect: $\beta=-4.178$, $p=0.008$; $ES=1.13$). No change was observed in leptin and the adiponectin/leptin ratio. No significant correlations were found between changes in cytokines and adipokines. Thus, in female adolescents, HIIR and HIIC induced an anti-inflammatory effect, as observed by the increased expression of IL-6 and IL-10 within 60-min post-exercise, despite the transient increase of the pro-inflammatory TNF- α from baseline to 5-min post-exercise. Although no change in leptin was expected, adiponectin decreased significantly 60-min following HIIR and HIIC.

Keywords: Physical exercise, aerobic exercise, metabolism, inflammation, adipose tissue

5.2. Introduction

The decline in physical activity levels from childhood to adolescence is a well-documented trend, with research indicating that both boys and girls experience reduced activity levels throughout adolescence (COOPER et al., 2015). However, girls tend to have consistently lower overall physical activity and higher sedentary behavior compared to boys (COOPER et al., 2015). This swift towards a more sedentary lifestyle during adolescence can have significant long-term health consequences. Low physical activity and increased sedentary time are associated with various negative health outcomes including low-grade inflammation and cardiometabolic risk factors, such as metabolic syndrome and overweight/obesity, as well as future chronic diseases, such as cardiovascular disease and osteoporosis later in life (HENSON et al., 2013; JANSSEN; LEBLANC, 2010; PERRY et al., 2023; WERNECK et al., 2020).

In contrast, consistent physical activity and regular exercise can play a crucial role in disease prevention, largely through its effects on the immune system and its ability to manage chronic systemic inflammation (FURMAN et al., 2019; KLARLUND; HOFFMAN-GOETZ, 2000). This action is characterized by the secretion of anti-inflammatory cytokines such as interleukin 10 (IL-10) and the muscle-derived, anti-inflammatory acting interleukin 6 (IL-6), as well the inhibition of pro-inflammatory cytokines such as interleukin 1-beta (IL-1 β) and tumor necrosis factor-alpha (TNF- α) (FURMAN et al., 2019; PETERSEN; PEDERSEN, 2005). In adults, the therapeutic effect of regular exercise on chronic inflammation has been attributed to the transient spikes in cytokine concentrations following acute exercise bouts (ALLEN; SUN; WOODS, 2015). Indeed, cytokines such as IL-6, IL-10 and TNF- α typically peak immediately after exercise and return to baseline within 5 to 24 hours (CABRAL-SANTOS et al., 2019; CERQUEIRA et al., 2020). In female adolescents, a single bout of plyometric exercise can induce the secretion of muscle-derived IL-6, potentially leading to an increase in the secretion of IL-10 and the inhibition of TNF- α (KURGAN et al., 2020).

In adipose tissue, leptin and adiponectin regulate the inflammatory response, with their ratio often used as an indicator of adipose tissue dysfunction associated with inflammation (FRÜHBECK et al., 2017). While higher concentrations of leptin are associated with a pro-inflammatory action due to its role in stimulating TNF- α , adiponectin acts oppositely, reducing the expression of TNF- α and increasing that of IL-10, i.e., exerting an anti-inflammatory effect and

improvement on the insulin sensibility. (REN et al., 2022). Acute and chronic aerobic exercise seems to decrease leptin concentration and increase adiponectin levels, although available meta-analyses on the topic indicate the need for further investigation due to the significant heterogeneity in study results that arise from differences in study design, exercise protocols, participant characteristics, and measurement methods (BOUASSIDA et al., 2010; CERQUEIRA et al., 2020; RACIL et al., 2013; YU et al., 2017).

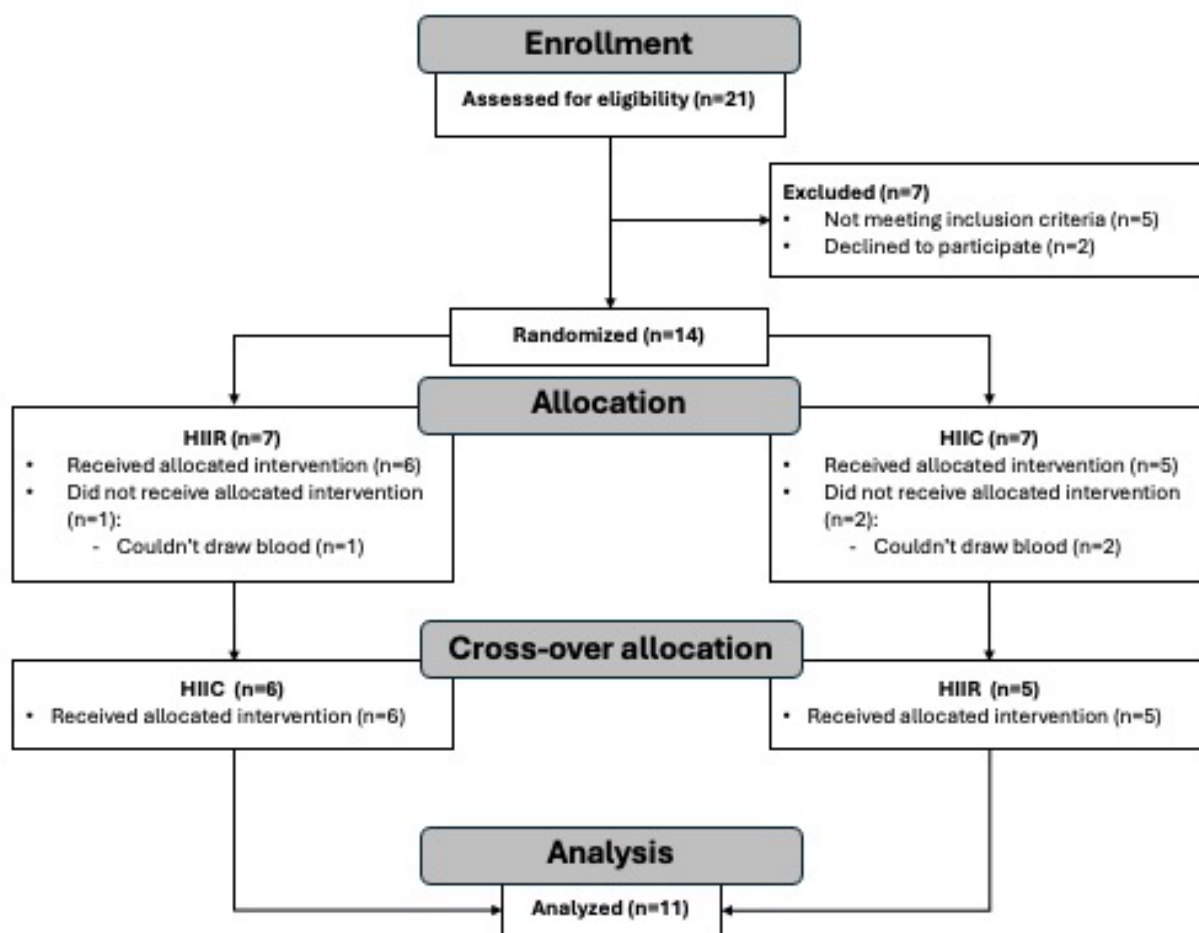
Importantly, the type, intensity, and duration of exercise can significantly influence cytokine and adipokine secretion and regulation (CERQUEIRA et al., 2020). For example, the effect of exercise on IL-10 can vary depending on the type of exercise performed with aerobic (cycling and running) and intermittent exercise leading to a more pronounced response compared to resistance exercise (CABRAL-SANTOS et al., 2019). Compared to moderate intensity, high-intensity exercise also significantly impacts adiponectin levels in young obese females (RACIL et al., 2013). Indeed, Kouvelioti et al. (2019) assessed the effect of high-intensity interval running (HIIR) and cycling (HIIC) in active young adults and observed that the response in IL-10 and TNF- α differed between the type of exercise (KOUVELIOTI et al., 2019b). This result is interesting because there are individuals who, due to some form of limitation from injury or compromised mobility, are advised to engage in impact-free exercise, eliciting the importance of investigating different types of exercise. However, to our knowledge, no such studies exist comparing the effects of different HIIT modes on the inflammatory response in adolescents. Thus, the purpose of this study was to compare the acute effect of HIIR and HIIC on inflammatory (i.e., IL-6, IL-10, TNF- α), and adipose-derived (adiponectin, leptin) markers, and examine potential associations among these exercise-induced factors, in a group of insufficiently active female adolescents.

5.3. Methods

5.3.1. Participants

A total of 21 female adolescents were recruited from schools across Presidente Prudente, Brazil, and through online media to participate in this study. To be included in the study, participants should be females, aged between 14 and 19 years, non-smokers, not enrolled in sports or exercise training for the previous 6 months, be free of musculoskeletal injury that would impair exercise, and be able to perform running and cycling. After the initial eligibility screening (n=21), 7 adolescents did not meet the inclusion criteria and were not enrolled in the study (e.g., participating in organized sports or resistance training programs [5] or declined to participate [2]), and 3 adolescents dropped out after being assigned to the study (e.g., afraid of taking blood [1], blood could not be drawn [2]), resulting in 11 adolescents completing the study. All adolescents and their legal guardians signed the consent and assent form before participating in the study (**FIGURE 5.1**).

Figure 5.1 - Flowchart of the study.



Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling. Source: from the author.

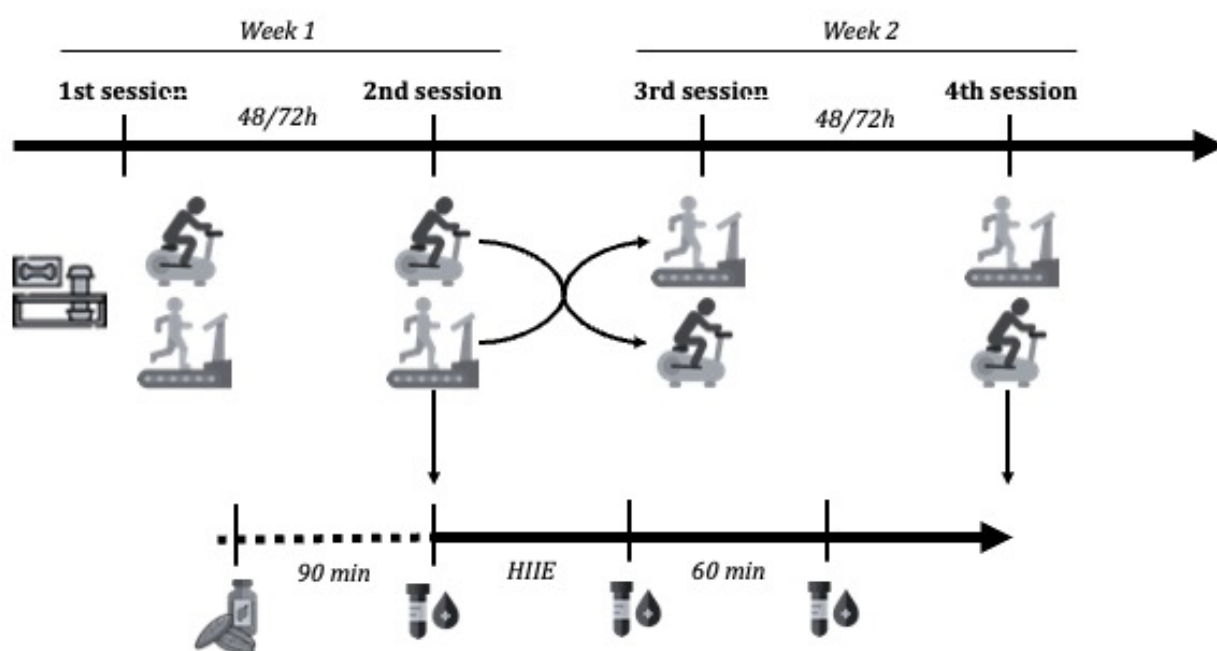
The study was conducted per the Declaration of Helsinki and received ethics approval from the Research Ethics Boards of the Sao Paulo State University, Brazil and Brock University, Canada. All participants agreed to participate in the study by signing informed consent.

5.3.2. Study design

This was a cross-over randomized controlled designed study, where each participant performed two high-intensity exercise trials in random order: a high-intensity interval running (HIIR) trial on the treadmill and a high-intensity interval cycling (HIIC) trial on a cycle ergometer. Block randomization of the order of trials was done using Excel. Participants were invited to the Sao Paulo State University laboratory four times over 2 weeks. In the first visit, they underwent baseline assessments, including body composition and anthropometrics, and completed a series of

questionnaires. During the same visit, participants performed an incremental test on either the bicycle or treadmill, depending on the exercise mode (cycling or running) they were assigned first. On the second visit, they performed the first high-intensity interval trial of the same exercise mode as the incremental test. On the third visit and fourth visits, participants performed the incremental test and second high-intensity interval trial, respectively, on the alternate exercise mode (cycling or running) (FIGURE 5.2).

Figure 5.2 - Study protocol and timeline.



Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling. Source: from the author.

5.3.3. Incremental test and exercise protocol

During the first and third visits to the laboratory, participants executed an incremental test on the bicycle or treadmill. Before each test, they received an explanation of the protocol and were allowed to familiarize themselves with running or cycling in different intensities. During the HIIR trial, participants warmed up for 4 min at 5 km/h and 1% slope in the treadmill. Following the warm-up, the speed increased to 6 km/h and continued with increments of 1 km/h every minute until voluntary fatigue. The HIIC trial started with a 4-min warm-up at 35 W, which then was increased to 60 W, continuing with increments of 25 W every minute until voluntary fatigue. Heart rate was monitored continuously during the test with an OH1 Sensor (Polar®) and perceived exertion was evaluated using the Borg Scale, and the maximum values were recorded.

Forty (48) to 72 hours after each incremental test, participants performed the high-intensity interval trial in the exercise mode (running or cycling) they were tested in that week. During each trial, participants performed 8 bouts of 1-min effort at >90% maximal workload separated by 1-min passive recovery intervals. The heart rate and perceived exertion were recorded at the end of every effort and recovery intervals.

All high-intensity interval trials were conducted in the morning between 07:30 and 10:30. The morning before each trial, participants were given a standardized breakfast containing orange juice and whole wheat bread (butter was allowed upon request. On the morning of each trial, blood samples were collected pre-exercise (1.5 hours post-prandial), 5 min and 60 min post-exercise.

5.3.4. Baseline measurements

Lean soft tissue mass (kg), fat mass (Kg) and relative body fat (%), total and regional, were measured by whole-body dual-energy X-ray absorptiometry (General Electrics, model WH – Prodigy Primo). Height and sitting height were assessed using a stadiometer (E120A, Tonelli, Brasil) and body mass was assessed using a standard scale (Welmy, model W300A). Maturation status was estimated using Moore’s equation for somatic maturation that provides the time (in years) from the peak of height velocity (PHV) (MOORE et al., 2015). After 10 min at rest in the sited position, heart rate (HR) at rest was assessed using an automatic Omron HEM-742 device. Three measurements were made with 1 min intervals between them.

5.3.5. Blood analysis

Approximately 3 mL of whole blood was collected into pre-chilled Vacutainer SST tubes and EDTA from participants’ median cubital vein in the antecubital fossa while lying in a supine position. Samples were collected using a standard venipuncture technique by a trained nurse during each session at pre-exercise, and 5 min and 60 min post-exercise. Blood samples were inverted 8-10 times before sitting for 30 min at room temperature. The samples were then centrifuged at 3000 g for 15 min at 4° before the serum and plasma were aliquoted into 1.5ml microcentrifuge tubes (Eppendorfs®) and stored at -80°C until analysis. In the present study, serum was used to measure circulating concentrations of IL-6, IL-10, TNF- α , while plasma was used to measure circulating concentrations of adiponectin, leptin, glucose and lactate dehydrogenase (LDH).

IL-6, IL-10 and TNF- α were measured in triplicate with ELLA single assay kits (cat.# SPCKBPS-003028, ST01B-PS-000276, ST01B-PS-002803, respectively; ProteinSimple, San Jose, CA). The inter-assay coefficients of variation were 9.9% for IL-6, 6.6% for IL-10 and 8.7% for TNF- α . The intra-assay coefficients of variation for IL-6, IL-10 and TNF- α were 4.6%, 6.69% and 2.54%, respectively. The concentrations of leptin and adiponectin were acquired by the enzyme-linked immunosorbent assay (ELISA) using commercial kits (R&D System, Minneapolis, USA). Finally, in order to control for potential differences in the metabolic demand between the exercise trials, blood glucose and LDH were measured using the colorimetric method with Labtest commercial kits (Lagoa Santa, Minas Gerais, Brazil).

5.3.6. Statistical analysis

The variability of the residuals was assessed using histograms and Q-Q plots. Variables that violated the normality of the residuals were log transformed. Linear mixed models were used to assess the main effects of exercise mode and time (repeated measures), as well as the interaction between these factors. Individuals were set as random effect. Models were adjusted for the baseline measure of the outcome being analyzed. The models where adipokines were the outcome, fat mass (%) was considered as a covariate. Effect size was calculated using Cohen's-d: <0.50 small effect-size, 0.50-0.79 moderate effect-size, 0.80-1.29 large effect-size and >1.30 very large effect-size (COHEN, 2013). Statistical significance was set at an alpha level of 0.05 and performed using Stata 18.5.

5.4. Results

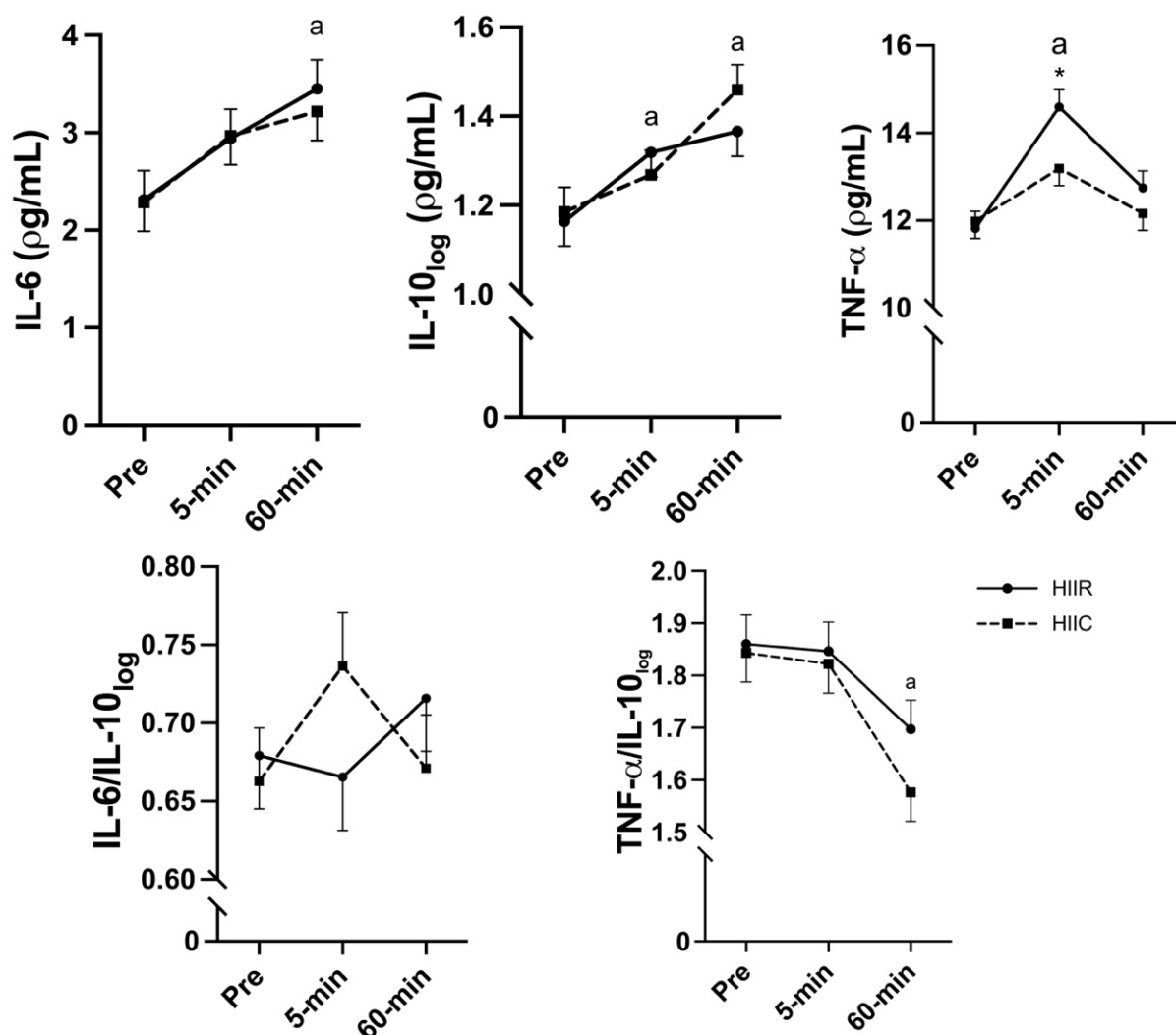
There were significant changes in all cytokines and adiponectin, but not leptin, during both trials. Without difference between modes of exercise, IL-6 concentrations increased over time, peaking at 60 min post exercise (time effect: $\beta=1.139$, 95%CI 0.400 to 1.877, $p=0.003$; ES=1.29 [large effect-size]). The same pattern was observed in IL-10, which presented a significant increase already at the end of the exercise (time effect: $\beta=0.155$, 95%CI 0.014 to 0.295, $p=0.031$; ES=0.92 [large effect-size]) and peaked 60 min post-exercise (time effect: $\beta=0.202$, 95%CI 0.062 to 0.342, $p=0.005$; ES=1.20 [large effect-size]). TNF- α peaked 5 min post-exercise with a greater increase in favor of HIIC (23.5% vs 10%; interaction effect: $\beta=1.575$, 95%CI 0.202 to 2.947, $p=0.025$; ES=2.39 [very large effect-size]), both modes returning to baseline levels after 60 min post-exercise. The log-IL-6/IL-10 and log-TNF- α /IL-10 ratios provide a better comprehension of the anti-inflammatory effect exerted by the HIIC and HIIR. Although no change was observed in IL-6/IL-10 ratio, TNF- α /IL-10 ratio decreased significantly 60 min post-exercise (time effect: $\beta=-0.163$, 95%CI -0.309 to -0.017, $p=0.028$; ES=0.93 [large effect-size]), resulting in a significant anti-inflammatory milieu in both modes (**FIGURE 5.3**).

Table 5.1 - Participant's characteristics (n=11).

	Mean (SD)	95%CI
Age (years)	17.4 (0.9)	16.7 to 18.0
Body mass (kg)	65.1 (14.6)	55.3 to 75.0
Height (cm)	164.8 (8.0)	159.4 to 170.2
BMI	24.0 (5.1)	20.6 to 27.4
FM (%)	37.1 (6.1)	33.0 to 41.2
LST (kg)	35.2 (5.7)	31.4 to 39.1
Maturity Offset (years)	4.4 (0.8)	3.8 to 4.9

Note: SD=standard deviation; BMI=body mass index; FM=fat mass; LST=lean soft tissue PHV=peak of height velocity.

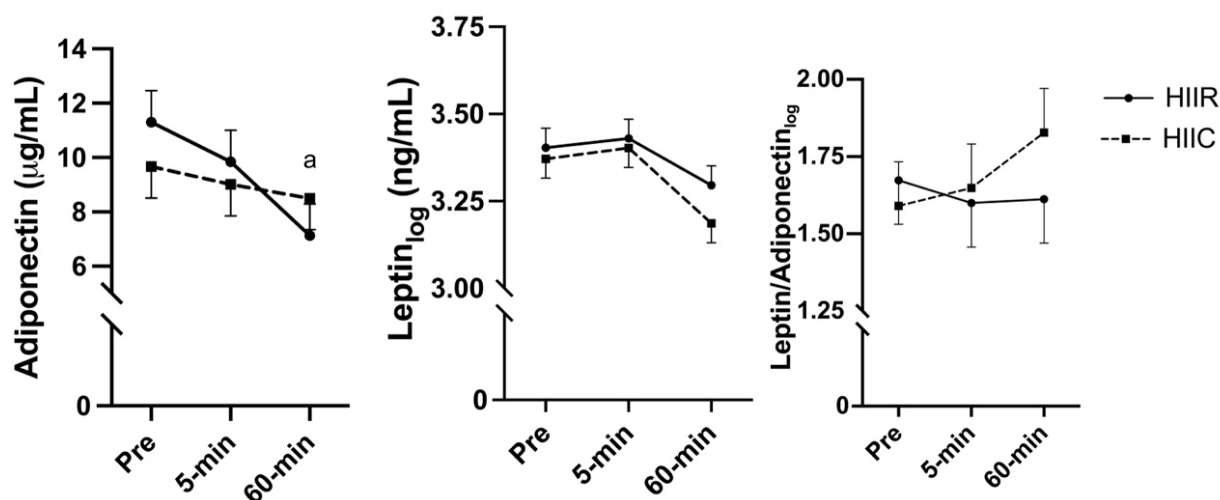
Figure 5.3 - Cytokines response to HIIR and HIIC (n=11).



Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling; a= difference from pre-exercise; * = difference between HIIR and HIIC; analysis adjusted to baseline measurements. Source: from the author.

Related to the adipokines, there were no significant changes in leptin from pre- to 5 min ($\beta=0.026$, 95%CI -0.103 to 0.156, $p=0.691$), although a marginally significant 10,2% decrease in leptin has been observed 60 min post-exercise (time effect: $\beta=-0.108$, 95%CI -0.237 to 0.021, $p=0.101$, ES=0.70 [moderate effect-size]). A significant reduction in adiponectin was observed from pre- to 60 min post-exercise irrespective of exercise mode (time-effect: $\beta=-4.178$ 95%CI -7.272 to -1.083, ES=1.13 [large effect-size]), with a greater – but not significant - decrease in HIIR compared to HIIC (-40% vs -12,1%, respectively). The leptin/adiponectin ratio, which provides a measure of fat mass disturbance, did not change over time in either mode (FIGURE 5.4).

Figure 5.4 - Adipokines ratio response to HIIR and HIIC.



Note: HIIR, high-intensity interval running; HIIC, high-intensity interval cycling; a= difference from pre-exercise; analysis adjusted to baseline measurements. Source: from the author.

In addition, the two trials have resulted in similar metabolic outcomes characterized by elevated LDH concentrations 5 min following exercise (time effect: $\beta = -0.188$, 95%CI 0.004 to 0.373, $p = 0.046$; ES=0.89 [large effect-size]), returning to baseline levels 60 min post-exercise. Blood glucose showed no significant main effects or interaction (interaction effect: $\beta = 5.546$, 95%CI -20.818 to 9.726, $p = 0.477$; ES=0.43 [small effect-size]); however, it was slightly elevated 5 min following HIIR but not following HIIC (7,8% vs 1,5% increase 5 min post HIIR and HIIC, respectively).

5.5. Discussion

This is the first study comparing the effects of different types of high-intensity interval exercise on inflammatory cytokines and adipokines in adolescent females. Our results show that, irrespective of the exercise mode, high-intensity interval exercise led to an increase in circulating levels of anti-inflammatory cytokines for at least one hour after exercise, favoring an anti-inflammatory milieu in this population. Additionally, there was a transient increase in TNF- α immediately after high-intensity interval exercise, with a higher increase observed after running compared to cycling. This finding suggests that running results in more pronounced transient muscle soreness due to damage, than does cycling. Furthermore, there is an association between this cytokine and energy metabolism (CABRAL-SANTOS et al., 2015), indicating that the mode of exercise may have an independent impact on energy metabolism. Lastly, both HIIR and HIIC led to a decrease in the adiponectin concentrations 60 min post-exercise, with a tendency of also decreasing leptin, thus maintaining the leptin:adiponectin ratio.

The only previous study that compared the effect of HIIC and HIIR on cytokines in adults (men and women) observed similar results as those found in the present study. Specifically, Kouvelioti et al (2019) observed a significant effect of high-intensity interval exercise in pro- and anti-inflammatory cytokines, with a few differences in timing and magnitude between cycling and running. For the anti-inflammatory cytokines, they observed an increase in IL-6 and IL-10 mainly 60 min after HIIR and HIIC, with IL-10 starting to increase significantly 5 min following HIIR. Similarly, our study shows a significant increase in both cytokines 60 min after the exercise. Partially in line with (KOUVELIOTI et al., 2019b), we observed an increase in TNF- α following HIIR that was two-fold higher than that observed following HIIC (24% vs 10%, respectively), although this increase was significant in both trials. (Mezil et al. (2015) also observed a significant increase in TNF- α immediately after HIIC, while in their study, IL-6 was elevated immediately post-exercise, decreasing to baseline levels by 60 min post-exercise, and with no effect on IL-10. In any case, since the greater increase in TNF- α 5 min after HIIR compared to HIIC was not seen in active adults (Kouvelioti et al 2019), we speculate that the TNF- α response might be dependent on the exercise mode in insufficiently active adolescents. In our previous study in a sample of adolescent swimmers, high intensity interval swimming did not change the concentrations of TNF- α , supporting our hypothesis (SANDERSON et al., 2020). On the other hand, the TNF- α

concentrations were similar 60 min post-exercise in our adolescents, demonstrating that this difference was transient.

Exercise-induced muscle damage has been shown to be the cause of increased TNF- α and IL-6 post running and cycling (STARKIE et al., 2001; ULLUM et al., 1994), with the former triggering the production of IL-6 and IL-10 and initiating a cytokine cascade (DE WAAL MALEFYT et al., 1991; SCHINDLER et al., 1990). IL-10 is an anti-inflammatory cytokine, whose classic role is precisely to negatively regulate the secretion of pro-inflammatory cytokines, such as IL-1, IL-1 β , and TNF- α (DE WAAL MALEFYT et al., 1991). In this sense, IL-10 may have increased to counter the increase in TNF- α post-exercise. A similar interaction between IL-6 and TNF- α is also possible, according to which, when acting as a myokine, IL-6 may inhibit the secretion of TNF- α (SCHINDLER et al., 1990). However, it is important to mention that the post-exercise increase in IL-6 may occur independently of TNF- α or muscle-damage, responding instead to other local factors such as increased heat shock proteins and reactive oxygen species, as well as low glucose availability and glycogen content (FISCHER, 2006; ULLUM et al., 1994). Considering that regular physical exercise is associated with the prevention and treatment of diseases related to low-grade chronic inflammation (WÄRNBERG et al., 2010), our findings involving an acute exercise bout suggest that these benefits may occur in female adolescents practicing HIIT regularly, regardless of the chosen training model.

The adipokines, such as adiponectin and leptin may act as regulators of the inflammatory response and have an important role in glucose metabolism. We found a significant reduction in adiponectin 60 min post-exercise irrespective of exercise mode. Contrary to our results, Vardar et al. (2018) found an acute increase in plasma adiponectin 5 min following a single session of HIIE, which was accompanied by a decrease in leptin (VARDAR et al., 2018). In our study, although leptin was slightly below the baseline levels at 60 min post-exercise in both modes, this decrease was not significant. A previous study in adolescent females did not report an effect of plyometric exercise on leptin (KURGAN et al., 2020), which corroborates the lack of leptin response following both high intensity trials in the present study. Ferguson et al (2004), who assessed the effect of continuous aerobic exercise on adiponectin in healthy adult participants, did not find exercise-induced changes (FERGUSON et al., 2004). As previously shown (FASSHAUER et al., 2002), TNF- α , which increased post-exercise, downregulates adiponectin, therefore explaining our

results. Furthermore, changes in adiponectin may be more sensible after long interventions that cause reductions in body fat (HOTTA et al., 2001; RACIL et al., 2013). Meta-analyses on this topic suggest that either leptin or adiponectin present a more consistent response to chronic exercise, independently of the training mode, with changes in fat mass being the most important predictor of those changes (BOUASSIDA et al., 2010; FEDEWA et al., 2018; YU et al., 2017). In any case, despite the adiponectin decrease, the small, non-significant decrease in leptin 60 min after exercise led to the stabilization of the adiponectin:leptin ratio. The adiponectin:leptin ratio has been widely used as an adipose tissue dysfunction and insulin resistance marker (FRÜHBECK et al., 2017).

Another important aspect is the energy demand, and the metabolic disturbance that may have been different between the exercise modes, with a greater stress generated by HIIR. In this context, the biomechanical differences between the two exercise modes, particularly regarding the number of muscles recruited (greater in running) and the types of muscle fibers involved (greater recruitment of type II fibers), can lead to varying levels of fat oxidation, distinctly activating counter-regulatory hormones and cytokines related to blood glucose control (ACHTEN; VENABLES; JEUKENDRUP, 2003; CAPOSTAGNO; BOSCH, 2010). This activation results in an increase in blood glucose and fatty acids. Based on the review by Pedersen et al (PEDERSEN et al., 2007), we speculate that cytokines production during HIIR led to greater fat oxidation compared to HIIC, which could also be linked to the higher TNF- α levels after running as TNF- α plays a role in enhancing lipolysis in adipose tissue (CABRAL-SANTOS et al., 2015). Although not statistically significant, the trend towards increased glucose concentrations immediately post-exercise in favor of HIIR was observed, supporting this perspective. According to Lira et al. (2015), the late increase in IL-6 and IL-10 may also be a counter response to the catabolic effect of exercise (i.e., glycogenolysis and lipolysis) (LIRA et al., 2015); however, further studies are needed to confirm this hypothesis and the mechanisms involved in the exercise-induced expression of cytokines and its effect on energy metabolism in sedentary girls.

Since this study represents the first to test the acute effect of different high-intensity interval exercise protocols on the immunological and hormonal response of female adolescents, the results need to be interpreted with caution considering its limitations. Although the cross-over design of the study represents an important strength, with every participant being their control, there is a high heterogeneity in terms of body composition between the participants. On the other

hand, adding percent body fat in the statistical models did not improve the models nor did it explain the changes in the regression analysis, except for the adipokines. In addition, longer periods of tracking these cytokines (e.g., 24 and 48 hours after exercise) would be important to fully understand inflammatory and hormonal behavior after high-intensity interval exercise. Finally, our study did not control for menstrual cycle phase. However, although a recent meta-analysis shows that women present a greater leptin concentration during luteal phase, there are no menstrual phase related fluctuations previously reported for adiponectin and the other cytokines investigated in our study (NOTBOHM et al., 2023).

In summary, our results indicate that following both high intensity interval cycling and running trials, female adolescents presented an increase in anti-inflammatory cytokines for at least one hour after exercise. On the other hand, these exercise trials only generated significant changes in the circulated adiponectin but not leptin. These results suggest that the cytokine and adipokine response to exercise seems to be mainly related to intensity and duration rather than the exercise mode. Therefore, impact-free exercise models can be an interesting alternative, especially for adolescents with injuries or limitations in running. Future research should consider other types and modes (e.g., rowing, swimming, etc.) of high-intensity exercise for their effect on regulatory factors in adolescents.

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6. THESIS CONCLUSION

To the best of our knowledge, this is the first thesis that proposes to investigate the effect of different modes of HIIE, with (HIIR) and without gravitational impact (HIIC) on bone turnover markers, osteokines, cytokines, and adipokines of untrained adolescent females, while accounting for how each mode impacts their affective perception and enjoyment. The results of this thesis show that, although HIIR and HIIC differed significantly regarding outcomes such as the bone marker of formation P1NP and pro-inflammatory $\text{TNF-}\alpha$, with greater increases observed following HIIR, such differences were small.

The overall impact of HIIR and HIIC on the observed outcomes seems to be similar. However, based on the negative impact that HIIC had on the adolescents' affective perception and the fact that they reported HIIR to be more enjoyable, we believe that HIIR would be the best strategy to improve health outcomes related to bone and inflammation of adolescent females not involved in any exercise routine.

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8. DESCRIPTION OF ACADEMIC ACTIVITIES (COURSES, PUBLICATIONS, CONFERENCES) DURING MASTER’S COURSE

Official date of enrollement in the graduate program: April 4, 2022.

8.1. Courses (n=8)

A total of 26 credits were completed, referring to the courses:

- I. Training in Teaching, Innovation and Entrepreneurship (4 credits): taken in the 1st semester/2022 with grade A;
- II. Research training (methodology, statistics and bioethics, philosophy of science) (4 credits): attended in the 1st semester/2022 with grade A;
- III. Exercise prescription and recovery strategies (4 credits): taken in the 1st semester/2022 with grade A;
- IV. Research seminars (2 credits): attended in the 1st semester/2022 with grade A;
- V. Physical activity and metabolism (4 credits): taken in the 2nd semester/2022 with grade B;
- VI. Health and physical activity, conceptions and practices (4 credits): attended in the 2nd semester/2022 with grade A;
- VII. New technologies applied to the monitoring and prescription of physical training (4 credits): taken in the 2nd semester/2022 with grade A;
- VIII. Metabolic, morphological and molecular adaptations induced by physical exercise (4 credits): taken in the 2nd semester/2024 with grade A;

8.2. Complementary activities (n=5)

- I. **Emerging Leaders in the Americas Program (ELAP)/Canadian Bureau for International Education:** from November/2023 to February/2024, at Brock University, under supervision of Dr. Panagiota Klentrou;
- II. **Research Internship through Research Internship Scholarships Abroad (BEPE)/São Paulo Research Foundation (FAPESP):** from March/2024 to August/2024, at Brock University, under supervision of Dr. Panagiota Klentrou;
- III. **Lecture:** course “Basketball” at the Physical Education Department (UNESP), from October/2022 to February/2023.

- IV. **Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans:** course (4h) by National Research Council Canada;
- V. **Data Analysis with R Programming:** course (33h) by Google Inc.

8.3. Participation in scientific conferences/publications on books of abstracts (n=6)

- I. **Narciso, P. H.;** Agostinete, R. R. ; Maillane-Vanegas, Santiago ; Silva, J. C. M. ; Polidoro, V. A. ; Faustino-Da-Silva, Y. S. V. ; Luiz-De-Marco, R. ; Fernandes, R. A. **CORRELAÇÃO ENTRE OS DOMÍNIOS DE ATIVIDADE FÍSICA, BEM-ESTAR MENTAL E ANSIEDADE ESPORTIVA DE ADOLESCENTES ATLETAS - ABCD GROWTH STUDY.** In: IX Congresso Brasileiro de Metabolismo, Nutrição e Exercício (CONBRAMENE), 2023, Londrina - PR. IX Congresso Brasileiro de Metabolismo, Nutrição e Exercício (CONBRAMENE), 2023.
- II. **Narciso, P. H.;** Morano, A. E. V. A. ; Agostinete, R. R. ; Giannopolous, A. ; Bell, M. ; Coish, J. ; Antunes, B. M. M. ; Lira, F. S. ; Fernandes, R. A. ; Klentrou, Panagiota . **Inflammatory cytokines and regulators following high-intensity interval running and cycling in adolescents..** In: 2024 Ontario Exercise Physiology Conference, 2024, Hamilton. 2024 Ontario Exercise Physiology Conference, 2024.
- III. **Narciso, P. H.;** Morano, A. E. V. A. ; Agostinete, R. R. ; Giannopolous, A. ; Bell, M. ; Coish, J. ; Antunes, B. M. M. ; Lira, F. S. ; Fernandes, R. A. ; Klentrou, Panagiota . **Inflammatory Cytokines and Regulators Following High-Intensity Interval Running and Cycling in Adolescents.** In: 2024 North American Society for Pediatric Exercise Medicine Conference: Giving Kids a Chance Through Exercise, 2024, Louisville. Abstracts From the 2024 North American Society for Pediatric Exercise Medicine Conference: Giving Kids a Chance Through Exercise (August 2024, Louisville, Kentucky, USA), 2024. v. 36. p. 1-17.
- IV. **Narciso, P. H.;** Morano, A. E. V. A. ; Gomes, R. L. ; Agostinete, R. R. ; Maillane-Vanegas, S. ; Lira, F. S. ; Fernandes, R. A. ; Klentrou, Panagiota . **Effect Of Impact During High-intensity Interval Exercise On Bone Markers In Female Adolescents.** In: American College of Spors Medicine Annual Conference, 2024, Boston. ACSM 2024 Annual Meeting, 2024. ACSM.

- V. **Narciso, P. H.**; Gil-Cosano, Jose J. ; Luiz-De-Marco, Rafael ; Agostinete, R. R. ; Klentrou, Panagiota ; Fernandes, R. A. . ANÁLISE DOS DETERMINANTES DE MARCADORES DE REMODELAÇÃO ÓSSEA ENTRE DIFERENTES TIPOS DE MODALIDADES ESPORTIVAS E NÃO-ATLETAS JOVENS: ABCD GROWTH STUDY. In: I Simpósio de Atividade Física e Exercício em Populações Pediátricas (SAFE-PED), 2024, Londrina. IMPOSIO SOBRE ACTIVIDAD FÍSICA Y EJERCICIO EN POBLACIÓN PEDIÁTRICA, 2024. v. 11. p. Suplemento.
- VI. Bell, M. ; Retsidou, I. ; Saabni, S. ; **Narciso, P. H.** ; Stears, H. ; McGraw, J. ; Josse, A. ; Klentrou, Panagiota . Inflammatory and Bone Responses to a 16-week Dietary Intervention in Youth Athletes: Greek Yogurt or Whey Protein?. In: ONTARIO EXERCISE PHYSIOLOGY CONFERENCE, 2024, Hamilton. 2024 ONTARIO EXERCISE PHYSIOLOGY CONFERENCE, 2024.

8.4. Published and submitted papers (n=9)

8.4.1. First author

- I. **Narciso, Pedro H.**; von-Ah Morano, Ana E.; Agostinete, Ricardo R., Werneck, André O.; Giannopoulos, Anthony; Bell, Madison; Antunes, Bárbara M.; Lira, Fabio S.; Fernandes, Rômulo A.; Klentrou, Panagiota. Cytokine and adipokine response following high-intensity interval running and cycling in female adolescents. *[Under review at European Journal of Applied Physiology]*
- II. **Narciso, Pedro H.**; Agostinete, Ricardo R.; von-Ah Morano, Ana E.; Werneck, André O.; Antunes, Bárbara M.; Vlachopoulos, Dimitris; Lira, Fabio S.; Fernandes, Rômulo A.; Klentrou, Panagiota. Acute effect of high-intensity interval cycling and running on bone turnover markers and osteokines in adolescent females. *[Under construction]*
- III. **Narciso, Pedro H.**; Gil-Cosano, Jose J.; Luiz-De-Marco, Rafael; Werneck, André O.; Agostinete, Ricardo R.; Klentrou, P.; Fernandes, Rômulo A. Differences in bone turnover markers among athletes of different types of sports: ABCD-Growth Study. *[Under construction]*
- IV. **Narciso, Pedro H.**; Agostinete, Ricardo A.; Tebar, William R.; Saraiva, Bruna T. C.; Fernandes, Rômulo A.; Beretta, Victor S.; Ferrari, Gerson; Christofaro, Diego G. D.

Relationship of the 24-Hour Movement Guidelines with bone health in Brazilian low-income children and adolescents: a cross-sectional study. *[Under construction]*

8.4.2. Coauthor

- V. Agostinete, Ricardo R.; Werneck, André O.; **Narciso, Pedro H.**; Ubago-Guisado, Esther; Coelho-E-Silva, Manuel J.; Bielemann, Renata M.; Gobbo, Luis Alberto; Lynch, Bruna Turi; Fernandes, Romulo Araújo; Vlachopoulos, Dimitris. Resistance training presents beneficial effects on bone development of adolescents engaged in swimming but not in impact sports: ABCD Growth Study. BMC Pediatrics, , v. 24, p. 247, 2024.
- VI. Agostinete, Ricardo R.; **Narciso, Pedro H.**; Coelho-e-Silva, .; Manuel J.; Bielemann, Renata M.; Gobbo, Luis A.; Lynch, Bruna; Fernandes, Romulo A.; Vlachopoulos, Dimitris. Possible mediating role of inflammatory markers on the negative association between training load and bone mineral density in adolescent swimmers: ABCD-Growth Study. *[Under review at Frontiers in Endocrinology]*
- VII. Gil-Cosano, Jose J.; Ubago-Guisado, Esther; Llorente-Cantarero, Francisco J.; Marmol-Perez, Andres; Rodriguez-Solana, Andrea; Pascual-Gazquez, Juan F.; Mateos, Maria E.; Molina-Hurtado, Jose R.; Garcia-Fontana, Beatriz; **Narciso, Pedro Henrique**; Klentrou, Panagiota; Gracia-Marco, Luis. Movement Behaviors and Bone Biomarkers in Young Pediatric Cancer Survivors: A Cross-Sectional Analysis of the iBoneFIT Project. Nutrients, v. 16, p. 3914, 2024.
- VIII. Bell, Madison; **Narciso, Pedro H.**; Josse, Andrea; Roy, Brian D.; Falk, Bareket; Klentrou, Panagiota. Effect of Dairy Protein Supplementation on Bone Turnover and Inflammation in Varsity Athletes: A 16-Week Study. *[Under construction]*
- IX. Luiz-de-Marco, Rafael; **Narciso, Pedro H.**; Maillane-Vanegas, Santiago; Faustino-da-Silva, Yuri da S.; Agostinete, Ricardo A.; Gomes, Rayana L.; Fernandes, Rômulo A. Pathways linking sports participation to bone density and geometry among adolescents: ABCD Growth Study. *[Under review at Archives of Osteoporosis]*

9. APPENDICES

9.1. Ethical Approval

FACULDADE DE CIÊNCIAS E
TECNOLOGIA DA
UNIVERSIDADE ESTADUAL
PAULISTA - UNESP/CAMPUS
PRESIDENTE PRUDENTE



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Impacto do HIIT na saúde óssea de adolescentes do sexo feminino: ensaio clínico sobre a influência do impacto mecânico

Pesquisador: Pedro Henrique Narciso Picchi

Área Temática:

Versão: 1

CAAE: 67621222.8.0000.5402

Instituição Proponente: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.951.255

Apresentação do Projeto:

Projeto bem apresentado, redigido e estruturado, com introdução detalhada sobre o estado da arte sobre o tema e bem referenciada (atualizada). Projeto com jovens adolescentes do sexo feminino (idade entre 14 e 17 anos, com primeira menstruação já registrada e há mais de uma ano do pico de velocidade de crescimento, o que elimina a possibilidade de se trabalhar com adolescentes que ainda não entraram no estágio maturacional. Por essa razão, o programa de treinamento de alta intensidade não oferece maiores riscos à saúde da amostra a ser analisada. Objetivos claros e bem apresentados entre primários e secundários, com metodologia e desfechos apresentados detalhadamente. Projeto está dentro do cronograma.

Objetivo da Pesquisa:

O objetivo principal do estudo será investigar o efeito da prática de 12 semanas de treino intervalado de alta intensidade (HIIT) ou em esteira ou em cicloergômetro sobre a saúde óssea de adolescentes do sexo feminino comparando, sendo considerado um terceiro grupo controle.

Avaliação dos Riscos e Benefícios:

O risco relatado foi a possibilidade de sintomas musculoesqueléticos (dor e/ou microlesões) em virtude do exercício, porém controlados. Tendo em vista o treinamento ser realizado em esteira ou cicloergômetro com acompanhamento personalizado, os riscos são bastante reduzidos.

Endereço: Rua Roberto Simonsen, 305 º Faculdade de Ciência e Tecnologia da Unesp º Prédio da Administração º SI 04
Bairro: Centro Educacional **CEP:** 19.060-900
UF: SP **Município:** PRESIDENTE PRUDENTE
Telefone: (18)3229-5315 **Fax:** (18)3229-5300 **E-mail:** cep.fct@unesp.br

FACULDADE DE CIÊNCIAS E
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PAULISTA - UNESP/CAMPUS
PRESIDENTE PRUDENTE



Continuação do Parecer: 5.951.255

Os benefícios citados são as melhora da composição corporal, da aptidão cardiorespiratória, do perfil lipídico e inflamatório, além da possível melhora da saúde óssea.

Comentários e Considerações sobre a Pesquisa:

Pesquisa original e importante para a área do exercício e saúde para a área pediátrica, com poucos riscos ou riscos controlados sobre a saúde das adolescentes voluntárias.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos apresentados adequadamente, em especial, o termo de assentimento (amostra menor de idade) e TCLE.

Recomendações:

Nenhuma.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Considerações Finais a critério do CEP:

Em reunião realizada no dia 17.03.2023, o Comitê de Ética em Pesquisa da Faculdade de Ciências e Tecnologia - Unesp - Presidente Prudente, em concordância com o parecerista, considerou o projeto APROVADO.

Obs: Lembramos que pesquisas que se enquadram na resolução 466/12 devem apresentar relatório parcial e final, e pesquisas que se enquadram na resolução 510/16 devem apresentar relatório final.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1985645.pdf	12/01/2023 11:17:42		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termo_de_assentimento.doc	12/01/2023 11:17:20	Pedro Henrique Narciso Picchi	Aceito
Declaração de Pesquisadores	Termo_de_compromisso.pdf	12/01/2023 11:17:02	Pedro Henrique Narciso Picchi	Aceito

Endereço: Rua Roberto Simonsen, 305 º Faculdade de Ciência e Tecnologia da Unesp º Prédio da Administração º SI 04
Bairro: Centro Educacional **CEP:** 19.060-900
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**FACULDADE DE CIÊNCIAS E
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PRESIDENTE PRUDENTE**



Continuação do Parecer: 5.951.255

TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	12/01/2023 11:15:59	Pedro Henrique Narciso Picchi	Aceito
Declaração de Instituição e Infraestrutura	Autorizacao_do_responsavel_para_utilizar_espacos_da_FCT.doc	08/11/2022 14:08:18	Pedro Henrique Narciso Picchi	Aceito
Outros	Questionario.pdf	08/11/2022 14:06:33	Pedro Henrique Narciso Picchi	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_mestrado_detalhado.docx	12/09/2022 10:41:09	Pedro Henrique Narciso Picchi	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Declaracao_Unilab.pdf	12/09/2022 10:40:21	Pedro Henrique Narciso Picchi	Aceito
Folha de Rosto	pbrasil.pdf	12/09/2022 10:36:25	Pedro Henrique Narciso Picchi	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

PRESIDENTE PRUDENTE, 17 de Março de 2023

Assinado por:
Edna Maria do Carmo
(Coordenador(a))

Endereço: Rua Roberto Simonsen, 305 º Faculdade de Ciência e Tecnologia da Unesp º Prédio da Administração º SI 04
Bairro: Centro Educacional **CEP:** 19.060-900
UF: SP **Município:** PRESIDENTE PRUDENTE
Telefone: (18)3229-5315 **Fax:** (18)3229-5300 **E-mail:** cep.fct@unesp.br

9.2. Consent and assent terms

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título da Pesquisa: “impacto do HIIT na saúde óssea de adolescentes do sexo feminino: ensaio clínico sobre a influência do impacto mecânico”

Nome do Pesquisador: Prof. Dr. Rômulo Araújo Fernandes

A sua filha está sendo convidada a participar desta pesquisa que tem como finalidade **identificar o efeito do exercício intervalado de alta intensidade (HIIT) em indicadores do metabolismo ósseo de adolescentes meninas**. Participação da pesquisa, adolescentes do sexo feminino e idade entre 14 e 19 anos. Ao participar deste estudo, o sr(a) permitirá que o seu filho (a): seja entrevistado (preencha questionários), tenha a pressão arterial aferida, peso / estatura medidos, participe de uma coleta sanguínea, uma espécie de radiografia de todos os ossos do corpo e dois protocolos de avaliação e exercício em esteira e bicicleta realizados na UNESP. Além disso, o jovem utilizará um relógio por um período de sete dias, o qual contará o número de passos dados ao longo do dia. Todas estas avaliações e entrevistas serão realizadas em 4 dias: a primeira avaliação terá duração aproximada de 1 hora; a segunda avaliação com duração estimada de 1:30h; a terceira avaliação com duração estimada de 30 minutos e a última avaliação com duração estimada de 1:30h. O jovem sob sua responsabilidade tem total liberdade de se recusar a participar do estudo, sem sofrer qualquer tipo de prejuízo. Além disso, sempre que quiser, poderá pedir mais informações sobre a pesquisa através do telefone do pesquisador do projeto e, se necessário, através do telefone do Comitê de Ética em Pesquisa.

O (a) sr(a) não terá nenhum tipo de despesa para participar desta pesquisa, bem como, nada será pago pela participação do jovem. Os únicos riscos envolvidos na participação deste estudo estão relacionados à possibilidade de desconforto durante a coleta de sangue e desconfortos musculares oriundos da prática de exercício físico. Os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em Pesquisa com Seres Humanos conforme Resolução no. 196/96 do Conselho Nacional de Saúde. Nenhum dos procedimentos usados oferece riscos à sua dignidade.

Todas as informações coletadas neste estudo são estritamente confidenciais. Somente o pesquisador e seu orientador terão conhecimento da identidade do jovem e nos comprometemos a mantê-la em sigilo ao publicar os resultados dessa pesquisa. O sr(a) receberá posteriormente os resultados de todos os exames feitos pelo jovem. O pesquisador se compromete a divulgar os resultados obtidos, respeitando-se o sigilo das informações coletadas, conforme previsto no item anterior.

Após estes esclarecimentos, solicitamos o seu consentimento de forma livre permitindo a participação do jovem sob sua responsabilidade na pesquisa. Portanto preencha, por favor, os itens que se seguem: Confirmando que recebi cópia deste termo de consentimento, e autorizo a execução do trabalho de pesquisa e a divulgação dos dados obtidos neste

estudo. Por favor, **não assine esse termo se ainda tiver dúvida a respeito**

Consentimento Livre e Esclarecido

Tendo em vista os itens acima apresentados, eu, de forma livre e esclarecida, manifesto meu consentimento em participar da pesquisa:

Adolescente: _____ DATA: ____ / ____ / ____

TELEFONE RESIDENCIAL: _____ CELULAR: _____ E-MAIL: _____

Assinaturas:

Responsável pelo Participante

Pesquisador

Orientador

Pesquisador: Prof. Dr. Rômulo Araújo Fernandes **Telefone:** 3229-5712

Coordenadora do Comitê de Ética em Pesquisa: Profa. Dra. Edna Maria do Carmo

Vice-Coordenadora: Profa. Dra. Andréia Cristiane Silva Wiezzel

Telefone do Comitê: 3229-5315 ou 3229-5526

E-mail cep@fct.unesp.br

TERMO DE ASSENTIMENTO

(No caso do menor entre 12 a 18 anos)

Você está sendo convidado (a) como voluntário (a) a participar da pesquisa **“IMPACTO DO HIIT NA SAÚDE ÓSSEA DE ADOLESCENTES DO SEXO FEMININO: ENSAIO CLÍNICO SOBRE A INFLUÊNCIA DO IMPACTO MECÂNICO”**. Nesta pesquisa pretendemos **identificar o efeito do exercício intervalado de alta intensidade (HIIT) em indicadores do metabolismo ósseo de adolescentes meninas**. O motivo que nos leva a estudar esse assunto são decorrentes do fato de que mulheres apresentam maior risco de desenvolver osteoporose (doença na qual o osso encontra-se fragilizado) na velhice, risco esse que pode ser mitigado durante a adolescência, bem como o exercício é, junto com a nutrição, o principal potencializados do crescimento ósseo. Para esta pesquisa adotaremos o(s) seguinte(s) procedimento(s): entrevista (preenchimento de questionários), aferição da pressão arterial, avaliação do peso / estatura, coleta sanguínea, uma espécie de radiografia de todos os ossos do corpo e dois protocolos de avaliação e exercício em esteira e bicicleta realizados na UNESP. Além disso, o jovem utilizará um relógio por um período de sete dias, o qual contará o número de passos dados ao longo do dia. Além disso, o jovem utilizará um relógio por um período de sete dias, o qual contará o número de passos dados ao longo do dia. Todas estas avaliações e entrevistas serão realizadas em 4 dias: a primeira avaliação terá duração aproximada de 1 hora; a segunda avaliação com duração estimada de 1:30h; a terceira avaliação com duração estimada de 30 minutos e a última avaliação com duração estimada de 1:30h. A segunda e quarta avaliação deverão ser realizadas no período da manhã.

Para participar desta pesquisa, o responsável por você deverá autorizar e assinar o termo de consentimento livre e esclarecido. Você não terá nenhum custo, nem receberá qualquer vantagem financeira. Você será esclarecido (a) em qualquer aspecto que desejar e estará livre para participar ou recusar-se. O responsável por você poderá retirar o consentimento ou interromper a sua participação a qualquer momento. A sua participação é voluntária e a recusa em participar não acarretará qualquer penalidade ou modificação na forma em que é atendido (a) pelo pesquisador que irá tratar a sua identidade com padrões profissionais de sigilo. Os únicos riscos envolvidos na participação deste estudo estão relacionados à possibilidade de desconforto durante a

coleta de sangue e desconfortos musculares oriundos da prática de exercício físico. Os resultados estarão à sua disposição quando finalizada. Os dados e instrumentos utilizados na pesquisa ficarão arquivados com o pesquisador responsável por um período de 5 anos, e após esse tempo serão destruídos. Este termo de consentimento encontra-se impresso em duas vias: uma via será arquivada pelo pesquisador responsável, e a outra será fornecida a você. Os pesquisadores tratarão a sua identidade com padrões profissionais de sigilo, atendendo a legislação brasileira (Resolução Nº 466/12 do Conselho Nacional de Saúde), utilizando as informações somente para os fins acadêmicos e científicos.

Eu, _____, portador (a) do documento de Identidade _____ (se já tiver documento), fui informado (a) dos objetivos da presente pesquisa, de maneira clara e detalhada e esclareci minhas dúvidas. Sei que a qualquer momento o meu responsável poderá modificar a decisão de participar se assim o desejar. Tendo o consentimento do meu responsável já assinado, declaro que concordo em participar dessa pesquisa. Recebi uma cópia deste termo de assentimento e me foi dada a oportunidade de ler e esclarecer as minhas *dúvidas*.

Presidente Prudente, ____ de _____ de 20____.

Assinatura do (a) menor

Assinatura do (a) pesquisador (a)

Em caso de dúvidas com respeito aos aspectos éticos desta pesquisa, você poderá consultar:

Pesquisador Responsável: Pedro Henrique Narciso Picchi

Fone: (18) 99614-608

E-mail: pedro.narciso@unesp.br

Coordenadora do Comitê de Ética em Pesquisa: Profa. Dra. Edna Maria do Carmo

Vice-Coordenadora: Profa. Dra. Andreia Cristiane Silva Wiezzel

Telefone do Comitê: 3229-5315 ou 3229-5526

E-mail cep@fct.unesp.br

9.3. Questionnaires

Confidential

ABCD-HIIT
Page 1

FS

Study ID

Como você esta se sentindo agora?

- ☐ Muito bem
- ☐ Bem
- ☐ Razoavelmente bem
- ☐ Neutro
- ☐ Razoavelmente mal
- ☐ Mal
- ☐ Muito mal

Confidential

ABCD-HIIT
Page 1

PACES

Study ID

Por favor, avalie como você se sentiu durante o exercício que acabou de realizar

Eu odiei

☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Eu adorei

Eu me senti interessada

☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Eu me senti entendiada

Eu gostei

☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Eu não gostei

Eu não achei prazeroso

☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Eu achei prazeroso

Eu não estava nem um pouco engajada nessa atividade

☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Eu estava engajada nessa atividade

Confidential

Page 2

Isso foi muito divertido

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Isso não foi nem um pouco divertido

Eu achei cansativo

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Eu achei energizante

Isso me deixou feliz

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Isso me deixou deprimida

Foi muito desagradável

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Foi muito agradável

Eu me senti mal fisicamente enquanto fazia essa
atividade

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Eu me senti bem fisicamente enquanto fazia essa
atividade

Confidential

Page 3

Não foi nem um pouco revigorante

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Foi muito revigorante

Eu não estou nem um pouco frustrada por isso

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Eu estou muito frustrada por isso

Isso não foi nem um pouco gratificante

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Isso foi muito gratificante

Isso não foi nem um pouco excitante

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Isso foi muito excitante

Isso foi muito estimulante

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Isso não foi nem um pouco estimulante

Confidential

Page 4

Isso não me deu uma forte sensação de realização

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Isso me deu uma forte sensação de realização

Isso não foi nem um pouco revitalizante

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Isso foi muito revitalizante

Eu me senti como se não houvesse nada mais que eu
preferisse estar fazendo

- ☐ 7
☐ 6
☐ 5
☐ 4
☐ 3
☐ 2
☐ 1

Eu me senti como se houvesse outras coisas em que eu
preferisse estar fazendo

PACES Score

Confidential

ABCD-HIIT
Page 1

BPAQ

Study ID

1. Por favor, liste qualquer atividade física que você tenha participado de forma regular. Marque as caixa que indicam quanto anos você tinha quando você praticou esse esporte/atividade física e por quanto tempo praticou.

Atividade 1

Atividade 2

Atividade 3

Atividade 4

Atividade 5

Atividade 6

Atividade 7

Atividade 8

Atividade 9

Atividade 10

Marque as caixa que indicam quanto anos você tinha quando você praticou esse esporte/atividade física e por quanto tempo praticou.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	N/
Atividade 1	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☒
Atividade 2	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Atividade 3	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Atividade 4	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Atividade 5	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Page 2

Atividade 1	
Atividade 2	
Atividade 3	
Atividade 4	
Atividade 5	
Atividade 6	
Atividade 7	
Atividade 8	
Atividade 9	
Atividade 10	
Frequência (por semana) 1	
Frequência (por semana) 2	
Frequência (por semana) 3	
Frequência (por semana) 4	

Confidential

Page 3

Frequência (por semana) 5

Frequência (por semana) 6

Frequência (por semana) 7

Frequência (por semana) 8

Frequência (por semana) 9

Frequência (por semana) 10

9.4. FAPESP Scholarships (MSc and BEPE)

DECLARAÇÃO

Declaramos para os devidos fins que **Pedro Henrique Narciso Picchi**, CPF 425.903.898-25, recebe/recebeu auxílio da **FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO**, CNPJ 43.828.151/0001-45, na(s) modalidade(s):

Programas Regulares / Bolsas / No País / Mestrado

Processo: 2022/08875-5
Título: Impacto do HIIT na saúde óssea de adolescentes do sexo feminino: ensaio clínico sobre a influência do impacto mecânico
Instituição: Faculdade de Ciências e Tecnologia de Presidente Prudente/FCT/UNESP
Orientador Prof(a). Dr(a): Romulo Araujo Fernandes
Período: 01/05/2023 a 31/10/2023 e 01/09/2024 a 30/04/2025
Valor Mensal: R\$ 3.300,00

Programas Regulares / Bolsas / No Exterior / Bolsa Estágio de Pesquisa no Exterior / BEPE - Mestrado

Processo: 2023/10153-0
Título: COMPREENDENDO O IMPACTO DO EXERCÍCIO FÍSICO, ALÉM DE DIFERENTES MODELOS DE SUPLEMENTAÇÃO, SOBRE AS OSTEOCINAS E A FORÇA ÓSSEA AVALIADA POR ULTRASSOM QUANTITATIVO (QUS) EM ADOLESCENTES E ADULTOS JOVENS: UM ESTUDO CONTROLADO RANDOMIZADO
Instituição: Faculdade de Ciências e Tecnologia de Presidente Prudente/FCT/UNESP
Orientador Prof(a). Dr(a): Romulo Araujo Fernandes
Instituição no Exterior: Brock University / St. Catharines / Canadá
Supervisor de Bolsa no Exterior: Panagiota Klentrou
Período: 01/03/2024 a 31/08/2024



As bolsas de pesquisa outorgadas pela FAPESP caracterizam-se como doação, sendo isentas do Imposto de Renda, não configuram vínculo empregatício e não objetivam pagamento de salários.

São Paulo, 15 de abril de 2025.

A handwritten signature in black ink, appearing to read "Flávio Cardoso da Silva".

Flávio Cardoso da Silva
Assessor

FLÁVIO CARDOSO DA SILVA
Assessor



Documento emitido em: 15 de abril de 2025 09:07:43 Horário Padrão de Brasília.
Código para verificação: 1707-5764-9481-D95E
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9.5. ELAP Scholarship

INTERNATIONAL SCHOLARSHIPS PROGRAM

SCHOLARSHIP CONFIRMATION LETTER

June 8, 2023

Immigration, Refugees and Citizenship Canada (IRCC)
Embassy of Canada or High Commission
Immigration Section for **Brazil**

To: Visa Officer

Re: **Confirmation of Global Affairs Canada Scholarship Recipient**

This letter is to confirm that the individual listed below is a recipient of a scholarship through the Global Affairs Canada International Scholarships Program (<http://www.educanada.ca>) which is under administration by the Canadian Bureau for International Education. Further details are provided below:

Scholarship recipient:	Pedro Henrique Narciso Picchi
Scholarship program:	Emerging Leaders in the Americas Program
Country of citizenship:	Brazil
Home institution:	São Paulo State University
Canadian host institution:	Brock University
Purpose of visit:	A full-time academic exchange in Canada related to the recipient's program of study currently undertaken at their home institution
Level of study and duration:	Graduate - four months or one academic term
Scholarship amount disbursed to recipient:	\$ 8,200 CAD

If applicable, consult the following website for information on the work permit LMIA exemption C44:
www.canada.ca/en/immigration-refugees-citizenship/corporate/publications-manuals/operational-bulletins-manuals/temporary-residents/foreign-workers/exemption-codes/public-policy-competitiveness-economy.html

Scholarship funds are intended to cover transportation, visas and permits, living and other expenses in Canada. Under the terms of this program, the tuition fees from the Canadian host institution are waived and recipients remain registered at their home institution during this exchange. All recipients of the 2023-2024 program must arrive in Canada no later than February 1, 2024. Please do not hesitate to contact us by email at admin-scholarships-bourses@cbie.ca or by phone at 613-237-4820 if your office should require any further information.

Sincerely,



Alice Danguy, Program Manager
Canadian Bureau for International Education

Administered by:

Canada



Canadian Bureau for
International Education
Bureau canadien de
l'éducation internationale



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Author: Julie M. Hughes, Colleen M. Castellani, Kristin L. Popp, et al

Publication: Exercise and Sport Sciences Reviews

Publisher: Wolters Kluwer Health, Inc.

Date: Jun 12, 2020

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Author: YASMEEN MEZIL, DAVID ALLISON, KIMBERLY KISH, et al

Publication: Medicine & Science in Sports & Exercise

Publisher: Wolters Kluwer Health, Inc.

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The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations

Author: C. M. Weaver et al

Publication: Osteoporosis International

Publisher: Springer Nature

Date: Feb 8, 2016

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