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DOCTORAL DISSERTATION

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Department	: Sport Sciences And Technology
Program	: Sport Sciences And Technology - PhD
Dissertation Title	: The Effects of High Intensity Interval Training on Inflammatory Cytokines and Bone Turnover Markers in Obese Women
Name of the Student	: Masoumeh ALIZADEH OSALOU
Date of Dissertation Defence	: July 31, 2015

This study has been accepted and approved as a Master / PhD dissertation by the examining committee, whose members are listed below.

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APPROVAL

This dissertation has been approved by the committee above in conformity to the regulations and bylaws of Hacettepe University Graduate Programs and has been accepted by the Board of Directors of the Institute of Health Sciences.

Prof. Ersin FADILLIOĞLU, MD
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ACKNOWLEDGEMENTS

I would like to express my special appreciation and thanks to my supervisor Professor Dr. Şükran Nazan Koşar, you have been a tremendous mentor for me. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. Your advice on my research has been priceless.

My special thanks to Dr. Hüsrev Turnagöl for his grate courage and support throughout my academic life at the Faculty and valuable contributions as a committee member. I would also like to express my special appreciation to Dr. Tahir Hazır for his valuable suggestions and support in collecting heart rate data and statistical analysis, and valuable contributions as a committee member. I would also like to thank my committee members Dr. Nevin Atalay Güzel, and Dr. Gülriz Erişgen for their contributions and valuable suggestions as my committee members even at hardship. I also want to thank to all committee members for letting my defense be an enjoyable moment, and for your brilliant comments and suggestions.

I would also like to thank all of my friends Yasemin Güzel and Muhammed Mustafa Atakan who supported me in writing, and incented me to strive towards my goal. I would especially like to thank physician and nurses of Medical Center. All of you have been there to support me when I recruited subjects and collected data for my PhD thesis. More and special thanks to Dr. Bakhtyar Tartibiyan and Dr. Malek Ahmadi and Dr. Mahsa Behnemoon, Cardiologist, to your valuable efforts during the study period.

I would like to thank all the participants in this study. You were all amazing! Thank you for all the smiles and good moments during exercise sessions and testing. I'm really grateful for you joining the study. Finally I would like to thank my amazing family for all their unwavering support and encouragement. Without you I wouldn't have been able to start studying again.

Ankara, July 2015

Masoumeh Alizadeh Osalou

ABSTRACT

Alizadeh Osalou, M. The effects of high intensity interval training on inflammatory cytokines and bone turnover markers in obese women. Graduate School of Health Sciences Sport Sciences and Technology Doctoral Dissertation, Ankara, 2015. Obesity induced chronic low grade inflammation is associated with increased circulating pro-inflammatory markers including Interleukin-1 alpha (IL-1α), tumor necrosis factor alpha (TNF-α), leptin, and C-reactive protein (CRP), and decreased anti-inflammatory markers. Increased pro-inflammatory cytokines may lead to bone resorption by increasing osteoclastogenesis and/or decreasing osteoblastogenesis. Exercise training known to decrease body fat percent, decrease pro-inflammatory cytokines and increase anti-inflammatory cytokines. However, data on the relation among obesity, inflammatory cytokines and bone health is very limited. Thus the purpose of this study was to evaluate the effect of high intensity interval training (HIIT) on inflammatory cytokines and bone turnover markers in obese women. Twenty four healthy, sedentary, obese women (Body mass index (BMI): 30-34.99 kg/m^2) and (age: 35.76±3.32 yrs.) engaged in a 12-week HIIT program (3 times/week, 40 min/session). Blood was drawn at baseline and after 12-week and assayed for IL-1α, TNF-α, leptin, and CRP, osteocalcin (OC), bone alkaline phosphatase (BALP), C-terminal telopeptide cross-links of Type 1 collagen (CTX). Body weight, BMI, body fat percent, maximum heart rate, IL-1α, TNF-α, leptin, CRP, and CTX decreased ($p<0.05$); VO_2max, time to exhaustion, adiponectin, and OC increased ($p<0.05$) while BALP didn't change after 12 weeks of HIIT intervention. In addition, changes in pro-inflammatory cytokines (TNF-α, IL-1α, leptin and CRP) over 12 weeks positively associated with the changes in bone resorption marker of CTX and negatively associated with the changes in bone formation markers (osteocalcin and BALP). All these associations were statistically significant except the correlations between CRP and OC, and between TNF-α, and BALP and CTX. In conclusion, findings of this study indicated that 12 weeks of HIIT program may improve bone health, as indicated by improvements in bone turnover markers, by decreasing body fat percent, increasing anti-inflammatory cytokines and decreasing pro-inflammatory cytokines in obese women.

Key words: Obesity, Inflammatory cytokines, Bone turnover markers, High intensity interval training

ÖZET

Alizadeh Osalou, M. Yüksek şiddetli aralıklı egzersizin obez kadınlarda inflamatuar sitokinler ve kemik dönüşüm belirteçleri üzerine etkisi. Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Spor Bilimleri ve Teknolojisi Programı Doktora Tezi, Ankara, 2015. Obez bireylerde düşük düzeyli kronik inflamasyon gözlenmektedir. Obez bireylerde interlökin-1 alfa (IL-1α), tümör nekrozu faktörü alfa (TNF-α), leptin ve C-reaktif protein (CRP) gibi pro-inflomatuar sitokinler artarken, adiponektin gibi anti-inflamatuar sitokinler azalmaktadır. İnflamasyon artışı kemik yıkımını artırırken kemik yapımını azaltmaktadır. Egzersiz ise vücut yağ oranını azaltmakta, obesiteye bağlı inflamasyonu düşürmekte ve kemik sağlığını geliştirmektedir. Ancak, düzenli egzersizin obezite, inflamasyon ve kemik sağlığı üzerine etkilerini birlikte inceleyen çalışmalar oldukça sınırlıdır. Bu çalışmanın amacı yüksek şiddetli aralıklı egzersizin (HIIT) obez kadınlarda inflamatuar sitokinler ve kemik dönüşüm belirteçleri üzerine etkilerini incelemektir. 24 sağlıklı, sedanter obez kadın (vücut kitle indeksi (VKİ): 30-34.99 kg/m^2, yaş: 35.76±3.32 yıl) rastgele yöntemle HIIT (n=14) ve Kontol (n=10) gruplarına ayrıldı. HIIT grubu 12 hafta süresince (3 gün/hafta, 40 dk/gün) yüksek şiddetli aralıklı egzersiz yapmıştır. Egzersiz programı öncesi ve sonrası alınan kan örneklerinde IL-1α, TNF-α, leptin ve CRP, osteokalsin (OC), kemik alkalen fosfataz (BALP), tip 1 kollajen çapraz bağlı C telopeptit (CTX) analiz edilmiştir. HIIT uygulaması, vücut ağırlığı, VKİ, maksimum kalp hızı, IL-1α, TNF-α, leptin, CRP ve CTX değerlerini düşürürken ($p<0.05$), VO_2max, tükenme süresi, adiponektin ve OC değerlerini artırmıştır ($p<0.05$). BALP değeri değişmemiştir ($p>0.05$). Ayrıca, pro-inflamatuar sitokinlerdeki değişimler (TNF-α, IL-1α, leptin and CRP) ile kemik yıkım belirteci CTX'deki değişim arasında pozitif, kemik yapım belirteçlerindeki (OC ve BALP) değişim arasında ise negatif korelasyon saptanmıştır. CRP ve OC, TNF-α ile BALP ve CTX arasındaki korelasyonlar dışında tüm korelasyonlar anlamlıdır ($p<0.05$). Sonuç olarak; bu çalışmanın bulguları uygulanan HIIT protokolünün obez kadınlarda vücut yağ oranını azalma, kemik dönüşüm belirteçleri ve inflamatuar sitokinlerde iyileşme sağlayarak kemik sağlığı için yararlı olabileceğini göstermiştir.

Anahtar kelimeler: Obezite, İnflamatuar sitokinler, Kemik dönüşüm belirteçleri, Yüksek şiddetli aralıklı egzersiz.

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ABBREVIATIONS

AGRP	Activity and agouti-related protein
AMA	American medical association
ANCOVA	Analysis of covariance
AT	Adipose tissue
AVF	Abdominal visceral fat
BALP	Bone alkaline phosphatase
BAT	Brown adipose tissue
BIA	Bioelectric impedance analyzer
BMD	Bone mineral density
BMI	Body mass index
CAD	Coronary artery disease
CHO	Carbohydrate
CIA	Collagen-induced arthritis
CK	Creatine kinase
CO_2	Carbon dioxide
COA	Coenzyme A
Cox II	Cyclooxygenase II
CRP	C-reactive protein
CSF	Colony-stimulating components
CTX	C-terminal telopeptide cross-links of Type 1 collagen
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DM2	Type 2 Diabetes Mellitus
ELISA	Enzyme-linked Immunosorbent Assay
EPOC	Excess post exercise oxygen consumption
ET	Exercise training
FFM	Fat free mass

HIIT	High intensity interval training
HMW	High molecular weight
ILs	Interleukins
IL-1	Interleukin-1
IL-1α	Interleukin-1 alpha
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-1ra	Interleukin 1 receptor antagonist
KD	Kilo Dalton
KO	Knocked out
LDL	Low-density lipoprotein
LMW	Low molecular weight
LPS	Lipopolysaccharides
MANCOVA	Multivariate analysis of covariance
MCP- 1	Monocyte chemoattractant protein 1
MHR	Maximum heart rate
mRNA	Messenger Ribonucleic acid
NF-kB	Nuclear factor kappa beta
NTX	N-telopeptides
OAFs	Osteoclast activating factor
OC	Osteocalcin
OPG	Osteoprotogrein
OR	Odds ratio
PICP	Type I pro-collagen carboxy-terminal propeptide
PPAR-y	Peroxisome proliferator-activated receptor- gamma
POMC	Pro- Opiomelanocortin
RANKL	Receptor activator of nuclear factor kappa beta ligand
SBP	Systolic blood pressure
TAC	Triglycerides

TEI	Total energy intake
TNF-α	Tumor necrosis factor alpha
TNFRs	Tumor necrosis factor receptors
TRACP	Tartrate resistant acid phosphatase
VO_2max	Maximum volume of oxygen uptake
WAT	White adipose tissue
WC	Waist circumference
WHR	Waist to hip ratio
Y NPY	Neuropeptide Y
1,25-(OH)2D	1,25-Dihydroxyvitamin D

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1. INTRODUCTION

Obesity is an epidemic problem affecting both low and high income countries in all over the world (1). Studies have well established that obesity is accompanied with chronic low-grade inflammation (2) associated with several diseases such as type 2 diabetes, metabolic disorders, cardiovascular diseases, cancer and bone diseases (3-9). Indeed, abnormal circulating levels of cytokines have been observed in obese individuals. Studies have reported that the levels of pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukine-1 alpha (IL-1α), C-reactive protein (CRP), and leptin increase and the level of anti-inflammatory cytokines such as adiponectin decrease in obese individuals (10-14). Furthermore, TNF-α, IL-1α, CRP, and leptin levels were found to be positively associated with the degree of adiposity (15, 16) and negatively associated with adiponectin (16, 17).

Obesity has traditionally been considered a protective factor for bone health due to its mechanical loading effect (18). However, recent studies showed that the relations between obesity and bone health is rather complex and other factors are also involved in the effects of obesity on bone health (19). In this regard, it has been shown that obesity-induced chronic low grade inflammation is associated with negative alterations in bone turnover (19-21). Furthermore, many chronic inflammatory diseases such as periodontitis, pancreatitis, inflammatory bowel syndrome and rheumatoid arthritis are associated with bone mass loss (19). These findings have increased interest in the relations among obesity, inflammation and bone metabolism.

Studies revealed that several adipokines, which are also related to obesity as mentioned above, are involved in osteoblastogenesis such as adiponectin, and osteoclastogenesis. Indeed, increased levels of TNF-α, IL-1α and leptin (in case of leptin resistance), and decreased adiponectin levels have been linked to increased bone resorption (16, 19, 20).

In summary, although obesity is considered as a protective factor for bone health due to mechanical loading, current literature suggests that it may have

deleterious effects on bone metabolism through several mechanisms (19). These mechanisms can be summarized as follows: increased marrow adipogenesis, decreased osteoblastogenesis, and/or increased osteoclastogenesis due to increased production of pro-inflammatory cytokines, and/or excessive leptin secretion, or decreased adiponectin production, and/or decreased calcium absorption. Therefore, countermeasures targeted to reduce body fat percent and pro-inflammatory cytokine levels, and increase anti-inflammatory cytokine levels may improve bone health in obese individuals.

In this regard, exercise training as a non-pharmacological approach deserves attention. It has been well established that aerobic exercise training reduces body fat percentage and improves inflammatory state (22-25). Indeed, it has been shown that exercise training reduces both fat mass and obesity-induced dysregulated expression of inflammatory adipokines in plasma (26-28) and white adipose tissue (29). Furthermore, there is clear proof that exercise, particularly weight bearing exercise, improves bone health due to mechanical loading (30).

Recent studies revealed higher or similar improvements in fitness and cardiovascular health with high intensity interval training (HIIT) compared to continues moderate exercise training (31-33). HIIT is composed of short periods of high intensity exercise separated by recovery periods at lower intensity or rest (31). This type of exercise enables people with varying fitness levels, even healthy untrained, obese, and clinical populations to perform brief periods of high intensity exercise training due to recovery intervals between training bouts (27, 34, 35). Other advantages of HIIT are its time saving nature and that some people find HIIT more enjoyable as it includes low and high intensity exercise intervals (36). Another advantage of HIIT protocol is that it may increase the resting metabolic rate following up to 24 h after the last exercise session due to the increased excess post exercise consumption EPOC, therefore it may enhance maximal oxygen consumption (VO_2max) more than other kind of exercises (37).

Recently it has been shown that only two weeks of HIIT reduced inflammation both in the circulation and adipose tissue in overweight and obese males (27) and in patients with post percutaneous coronary intervention (38).

Furthermore, HIIT results in fat loss particularly in overweight and obese individuals who possess larger fat mass initially (39). Twenty four weeks of HIIT in obese adults resulted in greater decrease in subcutaneous fat compared to aerobic training (39). (38) Has reported that HIIT may decrease CRP level in angina patients.

Considering the relations among obesity, inflammation, bone, and exercise it is logical to hypothesize that reducing body fat percent and inflammation in obese individuals may improve bone metabolism either increasing osteoblastagenosis or attenuating osteoclastogenosis. Although there are numerous studies reporting the beneficial effects of exercise training on either inflammatory and anti- inflammatory cytokines or bone health the interest in the interconnections among obesity, inflammation, bone health and exercise is relatively new. Therefore, studies on these interactions are very limited. Even up to now only three studies (30, 40-42) have reported the effects of exercise training (either aerobic interval, aerobic continues, resistance or combination of aerobic and resistance exercise training) on both bone metabolism and inflammatory state in obese individuals. Two of these studies conducted on obese adolescents (39, 42), and one on overweight men and women (30). Regarding the effects of HIIT program on inflammatory and anti- inflammatory cytokines there is limited studies in the literature (27, 43, 44) and only two (27, 44) were conducted on overweight or obese individuals. There is no study in the literature investigating the effects of HIIT on inflammatory and bone turnover markers at the same time either in lean or obese individuals. Thus, the purpose of this study was to determine the effects of HIIT on inflammatory cytokines and bone turnover markers in obese women.

1.1. Aim of the Study

The aim of this study was to determine the effects of high intensity interval training (HIIT) on inflammatory cytokines (TNF-α, IL-1α, leptin, CRP, and adiponectin) and bone turnover markers of osteocalcin, bone specific alkaline phosphatase (BALP), and carboxy terminal crosslinking telopeptides type 1 Collagen (CTX) in obese women.

1.2. Research Problem

Whether high intensity interval training HIIT improves inflammatory cytokines (TNF-α, IL-1α, leptin, CRP, and adiponectin) and bone turnover markers (osteocalcin, BALP, and CTX) in obese women?

1.2.1. Sub-problems

Whether high intensity interval training (HIIT);

1. Will decrease TNF-α, IL-1α, leptin, and CRP in obese women.
2. Will increase adiponectin level in obese women.
3. Will increase osteocalcin and BALP in obese women.
4. Will decrease CTX in obese women.
5. Whether inflammatory cytokines (TNF-α, IL-1α, leptin, CRP, and adiponectin) and bone turnover markers (osteocalcin, BALP, and CTX) are associated with each other in obese women.

1.3. Research Hypotheses

High intensity interval training (HIIT) will improve inflammatory cytokine levels (TNF-α, IL-1α, leptin, CRP, and adiponectin) and bone turnover markers (osteocalcin, BALP, and CTX) in obese women.

1.3.1. Sub-hypotheses

High intensity interval training HIIT;

1. Will decrease obesity-induced pro-inflammatory cytokines (TNF-α, IL-1α, leptin, and CRP) in obese women.
2. Will increase adiponectin levels in obese women.

3. Will increase bone turnover markers of osteocalcin and BALP in obese women.

4. Will decrease bone turnover marker of CTX in obese women.

5. There will be an association between inflammatory cytokines (TNF-α, IL-1α, leptin, CRP, and adiponectin) and bone turnover markers (osteocalcin, BALP, and CTX) in obese women.

1.4. Definitions

Obesity: Obesity is an abnormal increase of body fat mass, and defined as a body mass index of greater than or equal to 30 kg/m^2 (45).

Inflammation: Low-grade systemic inflammation has been defined by a 2- to 3-fold increase in the level of pro and anti-inflammatory cytokines, C - reactive protein, and normal occurring cytokines antagonists (41, 46).

Tumor necrosis factor alpha (TNF-α): Is a pro-inflammatory cytokine which is originally produced by monocytes and macrophages, and plays main role in inflammatory and diseases conditions (47).

Interleukin I (IL-1α): IL-1α is a type of glycoproteins which is synthesized by macrophages. It increases body temperature, stimulates of disease promotion, and it stimulates the early phase progenitor cells of bone marrow (48).

Leptin: Is a kind of peptide hormone generated originally by white adipose tissue. It may also be produced in low levels by other cells like brown adipose tissue. Leptin regulates body weight and energy intake (49).

Adiponectin: It is almost completely produced by adipocytes (visceral adipose tissue) and it has been seen in high levels in serum. Adiponectin regulates energy homeostasis, carbohydrates, and fat oxidation (50).

C- reactive protein (CRP): It is an acute phase protein and originally produced by the liver in response to substrates that released from macrophages and

fat cells. CRP in general promotes inflammations, infections, and tissue damages (51).

Carboxy -terminal crosslinking telopeptide type one collagen (CTX): It is a biochemical measure of bone resorption (52). Its normal range for 30-39 years is 60-650 pg/ml and for 40 years is 40-465 pg/ml (52).

Osteocalcin (OC): A biochemical measure of bone formation. Osteocalcin is a protein thought to be secreted by the osteoblasts, responsible for bone formation (53). Its normal range for premenopausal women is 9-42 ng/ml (54).

Bone-specific Alkaline Phosphatase (BALP): BALP is a bone-specific iso-enzyme of alkaline phosphatase produced by the osteoblast cells (55). It reflects the cellular activity of osteoblasts (56). Its normal range for premenopausal women is 14 µg/l (57).

High intensity interval training (HIIT): HIIT is a type of exercise training strategy with repeated periods of high intensity exercise immediately followed by varied recovery periods between the bouts (36, 58, 59).

1.5. Significance of the Study

Obesity has been associated with increased morbidity and risk of chronic diseases (60). It is also associated with abnormal bone structure and function, with the severity of the defects being linked to the severity and duration of the obesity (61). In the mid-to-late 2000's, studies showed that the risk of bone disease in obese persons with a BMI of greater than 30 kg/m^2 to be 2 to 3 times more than of lean persons (BMI 18.5 to 24.9 kg/m^2) (7, 62).

Obesity leads to altered levels of numerous hormones (63) some of them may affect bone health. For example, adipocyte-derived hormones, like leptin or adiponectin, play a role in bone's response to obesity by different mechanisms (64). Increased body weight due to lean mass strengthens bone, in contrast increased body weight due to fat mass weakens bones (65).

Recent reports, also, have suggested that the accretion of abdominal fat mass, irrespective of body mass index (BMI), can increase the risk of osteoporosis via diminishing bone mineral density (BMD) (66, 67). Consequently, while increased body weight due to increased lean body mass may have a supportive effect on bone health (mechanical burden), weight increase due to increased body fat mass (largely abdominal) may harmfully impact bone formation (physiological effect) (68).

Exercise training has anti-inflammatory effects (69). Anti-inflammatory effects of regular exercise are probably due to the decrease in visceral fat mass, which results in reduced release of adipokines, reduced hypoxia due to decrease in adipocyte size, increased angiogenesis and associated increase in blood flow, increased release of anti-inflammatory cytokines with each exercise session (70).

Findings of the present study indicated that HIIT program is effective in reducing inflammatory state, improving anti-inflammatory adiponectin, and improving bone formation and reducing bone resorption markers in obese women. Additionaly, changes in inflammatory cytokines (TNF-α, IL-1α, leptin and CRP) over 12 weeks positively correlated with the changes in bone resorption marker of CTX and negatively correlated with the changes in bone formation markers (osteocalcin and BALP).

This is the first study comparing the effects of HIIT on both inflammatory cytokines and bone turnover markers in healthy, obese women. Most of the studies on the effects of exercise training on inflammatory cytokines relatively few inflammatory cytokines were investigated while in this study TNF-α, IL-1α, leptin, and CRP, as well as an anti-inflammatory cytokine adiponectin were investigated together. Furthermore, this study is the first study in reporting the chronic effect of HIIT on IL-1α and leptin. Therefore, the results of this study contributes the understanding of the relationship among obesity, inflammation, and bone health in obese women as well as the effect of HIIT on these variables and the relations among them.

2. REVIEW OF LITERATURE

Recent literature has shown that obesity induced low grade chronic inflammation may deteriorate bone health (71). Fortunately, exercise training is effective both to reduce body fat percent and inflammation and to improve bone health (72). High intensity interval training (HIIT) has been found to be more effective compared to aerobic training induced metabolic adaptations (73). Thus, the purpose of this study is to determine the effects of HIIT on inflammatory and bone turnover markers in obese women. This chapter presents a review of the literature on obesity, inflammation, bone health, the interactions of the three conditions, and the effects of exercise interventions on these conditions, particularly the effect of HIIT.

2.1. Obesity

Obesity is defined as irregular or extreme fat index that may damage health. When BMI in adults is greater than 30 kg/m^2, they are categorized as obese (74). However, as BMI doesn't show fat mass and lean mass, it can be misleading to assess obesity.

During the last 35 years worldwide obesity has increased two times. According to the World health organization (WHO), percentages of overweight and obese adults in 2014 were 39% and 13%, respectively (75, 76). Obesity increases the risk of morbidity and mortality all over the world (76, 77). In this regard, obesity is a global epidemic and public health problem (60). Almost 3.4 million adults die every year as a result of being overweight or obese (75). Furthermore, 44% of the diabetes, 23% of the ischemic heart diseases and 7- 41% of cancer problems are attributed to overweight and obesity (78).

Etiology of obesity is multi-factorial including genetic, metabolism, and environmental factors (excess energy intake and/or low energy expenditure or low physical activity level i.e.) and the interactions between these factors.

Recently research findings have showed that adipose tissue plays a critical role, as it produces many bioactive molecules known as adipokines which regulate carbohydrate and lipid metabolism, immune function, bone turnover and blood

coagulability. Additionally, many of these molecules may serve as blood markers of cardio-metabolic risk. Thus, adipose tissue is now thought as an endocrine organ and obesity is considered as a chronic and systemic inflammatory disease. Moreover, the American Medical Association (AMA) took the stance that obesity is a disease (45).

2.1.1. Pathophysiology of Obesity Associated Diseases

Studies have well established that obesity is associated with several diseases such as type 2 diabetes, metabolic disorders, cardiovascular diseases, cancer and bone diseases (3-9, 79-82). This association between obesity and chronic diseases has led to researchers to study on the pathophysiological mechanisms of obesity related diseases and the consequences of excess adipose tissue.

In this regard, findings of the studies on the association between obesity and insulin resistance by Hotamişligil et al (75, 77, 83) shown that there were a connection between inflammatory and metabolic responses. They revealed that compared to lean adipose tissue, obese adipose tissue secretes inflammatory cytokines which can inhibit insulin signaling (75, 78, 83). This is the first study to demonstrate that there is an interaction between immune and metabolic pathways and how any discomfort in this interaction effects cellular and systemic metabolism.

One of the theory relations between obesity and its comorbidities is an inflammatory mechanism (81). Several studies has revealed that the interaction between chronic low grade inflammation and metabolic disorders (4). Indeed, abnormal circulating level of cytokines has been observed in obese individuals. Increased levels of pro-inflammatory cytokines (TNF-α, IL-1α, CRP, and leptin) and decreased levels of anti-inflammatory cytokines (such as adiponectin) have been observed in obese individuals (10, 14, 21, 24, 84-86). Additionally, TNF-α, IL-1α, CRP, and leptin levels were positively related to the degree of adiposity (16, 74, 76, 87) and negatively correlated with adiponectin (16, 17, 50, 76).

Recent evidences have indicated that obesity and inflammation are associated with each other and lead to many metabolic disorders (82). Obesity with

inflammation may cause further dysregulation of white adipose tissues which consequently results in increased release of adipokines and lipolysis (88, 89).

Relationship between metabolism and immunity plays a key role in increased prevalence of obesity concomitant with chronic co-morbidities. Obesity deteriorates immune function in turn leads to increased risk of infections as well as chronic low-grade inflammation (4, 89).

2.1.2. Interaction between Obesity and Inflammation

There are two different forms of adipose tissue (AT), brown adipose tissue (BAT) and white adipose tissue (WAT). BAT is associated with thermogenesis. Recent studies revealed that BAT is functional in adult individuals (90, 91) while WAT is associated with mainly lipid storage in the form triacylglycerides (TAG). Thus, the amount of WAT increases in obesity. In turn, obesity is associated with increased lipolysis resulting in excess release of free fatty acid (FFA) which may trigger inflammatory pathways and impair insulin signaling (Figure. 2.1). Furthermore, WAT functions as an endocrine organ as it secrets adipokines (91).

Hypertrophic adipocytes release chemo-attractants such as macrophage chemoattractant protein (MCP-1) which draws immune cells into the tissue (Fig. 2.1). Release of pro-inflammatory adipokines by adipocytes, pre-adipocytes, and infiltrating immune cells results in polarization of macrophages to a pro-inflammatory M1 phenotype, and drive an inflammatory T cell population (91).

Obesity induced low–grade chronic inflammation is characterized by increased local and circulating levels of pro-inflammatory adipokines released mostly by hypertrophied visceral white adipocytes VWA) involving TNF-α, interleukins (ILs), leptin, and adiponectin (Fig. 2.1) (4, 92). Figure 2.2 summarizes the major cell types involved in obesity-induced inflammation.

Firstly, metabolic signals due to excess positive energy balance initiate the inflammatory response and in turn, it impairs the metabolic homeostasis. Although the adipose tissue is the predominant tissue expressing cytokines in obesity, other

tissues such as liver, pancreas, brain as well as muscle experience increased inflammatory state in obesity (2).

Another characteristic of obesity induced inflammation is the increased infiltration of immune cells into the metabolic tissues. For instance, studies shown that there is an increase in the macrophage population in the adipose tissue of obese compared to lean counterparts (93). As mentioned previously, macrophages further contribute to increase to tissue cytokine expression (2).

Gregor and Hotamişligil (2) in a review on "Inflammatory mechanisms in obesity" summarizes the features of obesity induced inflammation as follows: It is metabolic in nature, develops gradually over time, it is moderate to low grade inflammation and chronic in nature.

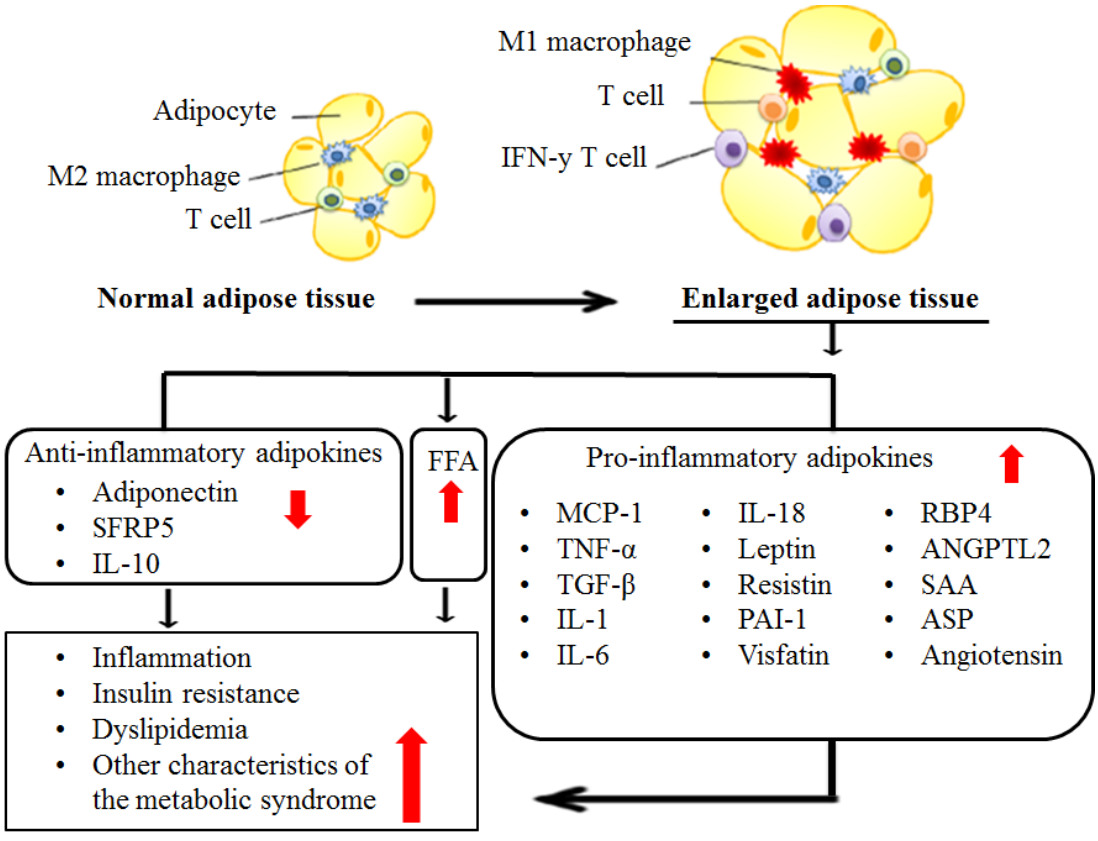

Figure 2.1. Mechanisms of obesity induced inflammation. Adapted from (90)

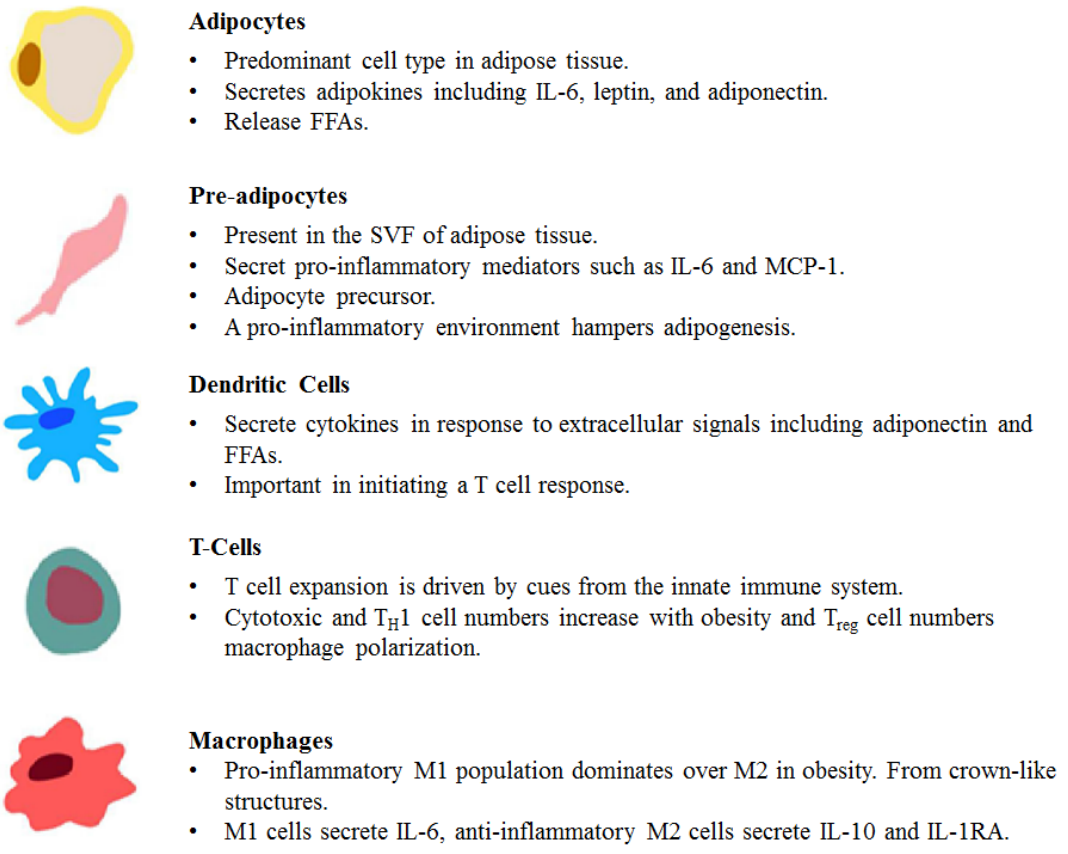

Figure 2.2. Major cell types involved in obesity-induced inflammation. Adapted from (90).

2.1.3. Interaction between Obesity and Bone Health

Although obesity has traditionally been considered a protective factor for bone health due to its mechanical loading effect (18, 60), but recent studies have showed that obesity-induced chronic low grade inflammation is associated with negative alterations in bone turnover (19, 21, 94, 95). Furthermore, there is accumulating evidence that obesity is a risk factor for osteoporosis and bone fractures (96, 97). It has also been shown that adipose tissue own, an endocrine organ, produces and releases bioactive molecules, influencing metabolism, bone remodeling, insulin resistance and inflammation (2, 98-100). These findings have increased the interest in the relations among obesity, inflammation and bone metabolism.

2.2. Bone

Bones keep structural maintenance for the rest of the body, let movement, motion by providing levers for the muscles, keep vital inner organs and structures, provide maintenance of mineral homeostasis, help acid-base balance, assist as a pool of growth factors and cytokines (101). The adult human skeleton is comprised of 80% cortical bone and 20% trabecular bone in general (98). This proportion varies in different parts of the body. For example, the vertebra is combined of cortical to trabecular bone in a ratio of 25 to75. This proportion is 50 to 50 in the femoral head and neck and 95 to 5 in the radial diaphysis (98).

Several factors affect bone health including age, gender and size, tobacco and alcohol use, chronic inflammatory disorders, long-term use of corticosteroid medications, genes, physical activity and mechanical loading, hormones, obesity, and nutrition. On the other site eating foods with rich in calcium and vitamin D, getting sufficient exercise, and having good health behaviors help to keep bones in strong condition (10). Healthy dietary habits and participating in regular exercise improves bone health and prevents bone loss. Bone fractures due to fragile bones can be painful and sometimes need operation to restore. They may also results in long-lasting health problems (10). This in turn increases the economic costs due to both work absenteeism and expenses for treatment. Therefore, improving bone health particularly in childhood and young adulthood and reducing age-related bone loss has a great significance for a healthy, high quality life as well as reducing the health-costs.

2.2.1. Bone Growth, Modeling, and Remodeling

Bone is a dynamic tissue where regeneration (osteoblastogenesis) and destruction (osteoclastogenesis) occur consistently throughout lifetime. During the early years bone growth and development mainly occur longitudinally at growth plates. Cartilage proliferates in the epiphyseal and metaphysical parts of long bones and successively undergoes mineralization to produce new bone (102). Bone modeling is developed by which bones changes their general form in reaction to physiologic effects or mechanical forces and leading to regular change of the

skeleton to the forces that it encounters. Bone may enlarge or alter their structures by elimination or adding of new bone cells to appropriate surface by independent activity of osteoblasts and osteoclasts in response to biomechanical loads (102).

This physiological cycle helps keep a constant for homeostatic functions, and preserving the biomechanical properties of bone. During bone resorption, calcium and new matrix components are released into the blood flow where they are absorbed or excreted (103). As bone formation continues, skeletal-specific proteins (enzymes or matrix components) can leach into the circulation in relatively high concentrations (104, 105). Bone modeling and remodeling occurs by which bone is renovated to maintain their strength, power, and mineral hemostasis (106, 107). Bone formation and resorption rates are not the same throughout lifetime as bone formation is higher during childhood and early adulthood while bone resorption is higher in adults and increases with aging (107).

Bone resorption mediated by osteoclast takes nearly 2 to 4 weeks and bone formation takes nearly 4 to 6 month to be completed at normal conditions (108). Osteoclast growth, activation, and resorption are controlled by the relativity of receptor activator of nuclear factor Kappa B (NF-kB) ligand (RANKL) to osteoprotegerin, IL-1, and interleukine- 6 (IL-6) (109, 110). After accomplishment of bone resorption, the bone formation begins by monocytes, osteocytes which circulate from bone matrix and pre-osteoblasts (111, 112). Several markers are involved in bone turnover which divide into two parts: bone formation and bone resorption markers.

2.2.2. Bone Formation Markers

Bone formation markers consisted and released to circulatory system by osteoblasts are:

1. Osteocalcin (OC)
2. Bone alkaline phosphatase (BALP)
3. Type I pro-collagen carboxy-terminal propeptide (PICP) (113).

Bone loss increases when bone resorption occurs more rapidly than it does in bone growth. Bone resorption markers are naturally synthesized by osteoclast cells. Bone resorption markers are:

4. C- Terminal crosslinking telopeptide type 1collagen (CTX).
5. Pyridinoline (Pyr), deoxypyridinoline (Dpyr).
6. N-telopeptides (NTx) (114).

Type 1 bone collagen is the major product of osteoblasts. It comprises 95% of the extracellular none mineral bone matrix. Other proteins such as osteopontin, osteonectin and osteocalcin are also secreted to form the osteoid or organic substrate in which mineralization occurs (115) . Osteoblasts can be identified by staining for the enzyme alkaline phosphatase; attaching to their cell membranes. This alkaline phosphatase is functionally similar but antigenically is different in hepatic, intestinal, liver or placental alkaline phosphatase (115).

2.2.3. Bone Alkaline Phosphatase

Bone alkaline phosphatase is an enzyme which removes the phosphate group from molecules (116, 117). It is one of the bone turnover markers which is located on the outer cell surface of the osteoblasts. Therefore, it is specific to bones (118). It is driving in high level only from juvenile and active osteoblast cell into extracellular fluid so it can be measured in serum easily (118). The serum amount of BALP is too sensitive for changes in bones (116, 117). It is significantly higher in healthy individuals. An increased level of BALP is associated with increased adiponectine and osteocalcin levels in patients with osteoporosis (116, 117). Serum concentration of bone-specific alkaline phosphatase (BALP or bone ALP) reflects the cellular activity of osteoblasts (115, 119).

2.2.4. Bone Resorption Markers

Bone resorption is initiated by osteoclasts, which contain acid phosphatase (119, 120). Although acid phosphatase activity is present in other tissues, such as prostate gland and blood cells, the type of osteoclast enzyme can be recognized by its insensitivity to inhibition by tartrate acid tartrate resistance acid phosphatase

(TRACP). Osteoclasts attaches to the bone surface and secrete acid and hydrolytic enzymes that resorb bone, releasing bone minerals and collagen fragments (119, 120).

2.2.5. C-Terminal Crosslinking Telopeptide of Type I Collagen (CTX)

CTX has been known as a bone resorption marker which is derived from osteoclast during bone resorption, and it is used as a serum biomarker to measure the rate of bone resorption. This test measures the CTX concentration in the blood to determine osteoclast activity during bone resorption (117).

2.2.6. Osteocalcin

Osteocalcin is a calcium-binding protein which is considered as a bone turnover marker (116, 117). It constitutes 15% of the non-collagenous protein fraction in bones (116, 117). Until recently, it was believed to be produced only by osteoblasts, odontoblasts and hypertrophic chondrocytes, and its functions were limited to the skeletal system (116, 117). However, studies from rodents have reported that there is a close association between osteocalcin and fat mass (121). Human studies, further, confirmed the inverse associations between osteocalcin and BMI, fat percentage, waist circumference, blood glucose and triglyceride levels, insulin resistance, metabolic syndrome and even coronary heart disease (122, 123). Recently, Foresta et al. (123) have shown that osteocalcin can be produced in adipose tissue. This finding suggests that the relationship between osteocalcin and fat mass is not merely dependent on the bone tissue itself and the adipose tissue also contributes the relationship by secreting osteocalcin itself.

The serum concentration of osteocalcin indicates the rate of osteocalcin synthesis by osteoblasts. However, only 50% of newly synthesized osteocalcin is released into the circulation, while the remaining 50% is incorporated into hydroxyapatite. It has been proposed that another form of osteocalcin, undercarboxylated osteocalcin, may be an independent risk factor for hip fracture (124).

The association between osteocalcin and BMI has not been sufficiently studied in youth. The first study in the pediatric population has found that osteocalcin levels were lower in obese children compared to non-obese controls (125). During weight loss, osteocalcin levels correlate negatively with changes in BMI and leptin; substantial weight loss leads to a significant increase in osteocalcin levels, with a concomitant decrease in leptin (126). Another study in children found inverse correlation between osteocalcin and fat mass, fat percentage, and BMI (127). However, in order to verify this relationship, adjustment for additional factors that influence bone metabolism should be done.

2.2.7. Relationship between Inflammatory Cytokines and Bone Health

A link between inflammation and bone disease is well established in a variety of clinical conditions (128). It is obvious that even a low level of inflammation influence bone remodeling and increase fracture possibility (129). Although inflammation can influence approximately every organ of the body, the most interest has been concentrated on the common chronic inflammatory diseases; these are inflammatory joint disease such as rheumatoid arthritis, inflammatory bowel disease, lung inflammation (asthma or chronic obstructive pulmonary disease), renal disease (nephritis), vasculitis, and disease affecting nervous system and all muscles (130). These diseases results in bone loss by similar mechanisms, however, each disease has unique features. Treatments of these diseases are also different, which in turn affect bone metabolism differently (131). Figure 2.3 summarizes the role of inflammatory cytokine network in inflammatory bone loss.

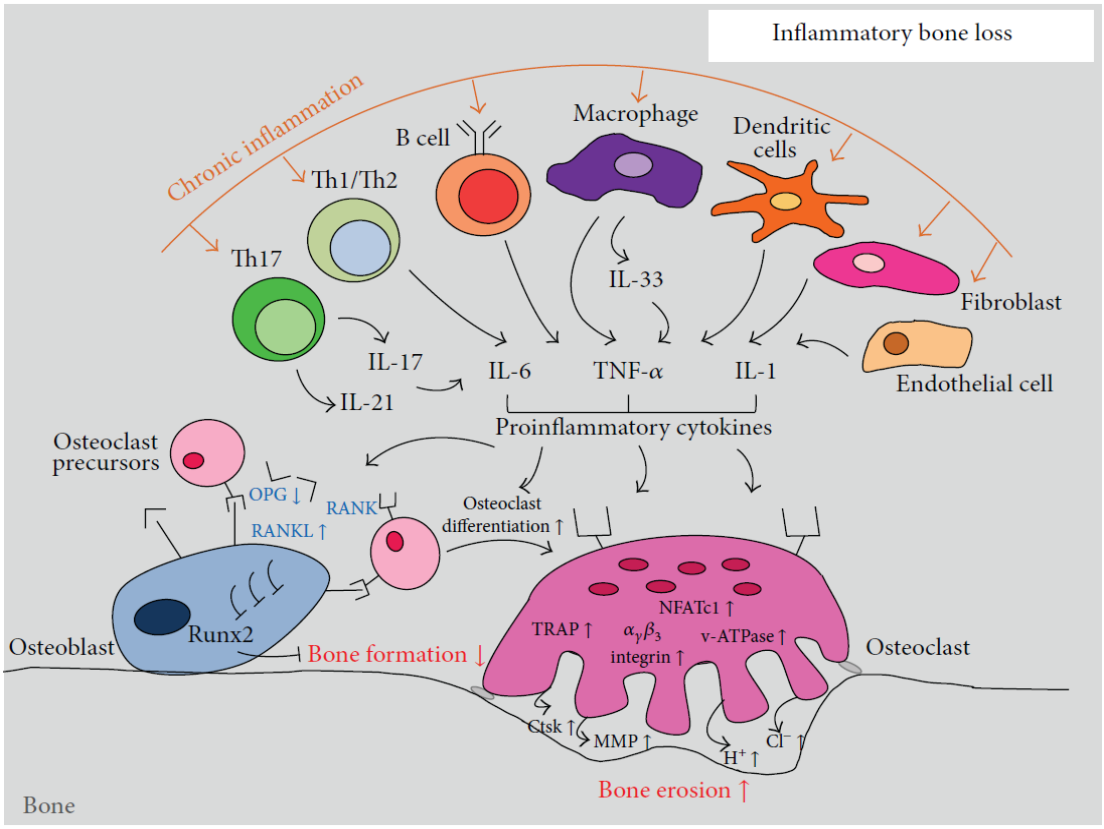

Figure 2.3. The role of inflammatory cytokine network in inflammatory bone loss. Adapted from (132).

Plasma leptin levels are positively associated with BMD and bone mass (133-136). Iwamoto et al (133) reported a positive relationship between serum leptin and calcaneal BMD in men and women (137). Furthermore plasma leptin levels were significantly lower in women with vertebral fractures than in those without fractures (138). Cauley et al. (136) revealed that individuals with normal leptin level had fewer bone loss after a longitudinal study conducted on 3075 men and women who has been monitored for 5 years.

Studies shown that leptin prevents osteoclast generation, probably by augmenting osteoprotegerin mRNA (131, 139, 140). Increased expression of pro-inflammatory cytokines in obese subjects may increase osteoclast differentiation and bone resorption by triggering receptor activator of nuclear factor kB ligand (RANKL)/RANK and osteoprotogrein pathway (77). Furthermore, increased leptin

and decreased adiponectin release adipose tissue in obese individuals may directly or indirectly affect bone formation and resorption (1).

2.2.8. Relationship between Bone and Adiposity

Obesity has long been considered protective against bone loss and osteoporosis due to mechanical loading of increased body weight (18, 141). Mechanical loading stimulates bone formation by increasing proliferation and differentiation of osteoblasts and osteocytes, and decreasing apoptosis (19, 142). Recent advances in measurement of body composition enabled researchers both to precisely measure the fat and lean mass and to determine the associations between these components and bone health. Results of these studies revealed that excessive fat mass may not be protective against osteoporosis, even it is related to low total bone mineral density and total bone mineral content (143, 144). In humans increased adiposity has been linked to suboptimal attainment of peak bone mass (19), increased risk of bone fracture (145, 146) and osteopenia (19).

The association between adiposity and bone health has been reviewed in detail in several papers (19, 21, 147, 148). Current literature suggests three mechanisms by which adiposity affects bone metabolism:

1. Adipocyte derived endocrine cytokines and growth factors affect osteoblasts and osteoclasts. In this respect visceral fat depots release cytokines including resistine, TNF-α, IL-1 and IL-6 that interfere with bone remodeling by enhancing bone resorption or suppressing bone formation.
2. Adipokines (leptin, adiponectin i.e.) influence bone remodeling by endocrine actions or through their effect on hypothalamic centers regulating sympathetic tone. Discharges of sympathetic impulses interfere with bone remodeling by inhibiting osteoblast differentiation and augmenting osteoclast activity. Obesity is associated with increased leptin levels and decreased adiponectin levels both of which adversely affect bone remodeling.

3. Paracrine factors elaborated by adipocytes within the bone marrow milieu influence nearby cells on the trabecular bone surface. Bone marrow adipocytes are fundamental to both skeletal function and hematopoiesis. Furthermore, both osteoblasts and adipocytes are differentiated from the same progenitor, the mesenchymal stem cells (MSC). Therefore, increased adipogenesis may decrease osteoblastogenesis. In this regard, it has been shown that mechanical loading stimulates osteoblast differentiation and inhibits adipogenesis by down-regulating peroxisome proliferator-activated receptor gamma (PPARg) or by stimulating a durable beta-catemin signal (149).

Furthermore, another consideration with the link between obesity and bone health is high fat diet which inhibit calcium absorption from intestinal wall and thus decrease calcium accessibility for bone formation (150). Feeding mice with a high-fat diet for 14 weeks reduced trabecular bone volume in the tibia despite of substantial increase in body weight and bone formation markers (19). Furthermore, obesity induced by high fat intake displayed increased bone marrow adiposity associated with reduced BMD in some bones in mice (151). In postmenopausal obese women increased bone resorption and decrease bone formation occur due to up-regulation of pro-inflammatory cytokines and bone resorption markers such as (CTX), and down regulation of bone formation markers such as (OC) and (BALP) (152).

Fat accumulation is closely linked to bone formation and resorption. Osteoblasts and adipocytes are derived from a common multi-potential mesenchymal stem cell (Figure 2.4) (19). Osteoclasts are differentiated from monocyte/macrophage precursors of hematopoietic stem cells origin (19). Adipocytes secrete several cytokines such as TNF- α, IL-1β, IL-6, adiponectin, and leptin which are capable of modulating osteoclastogenesis through RANKL/RANK/ osteoprotegerin (OPG) pathway (19).

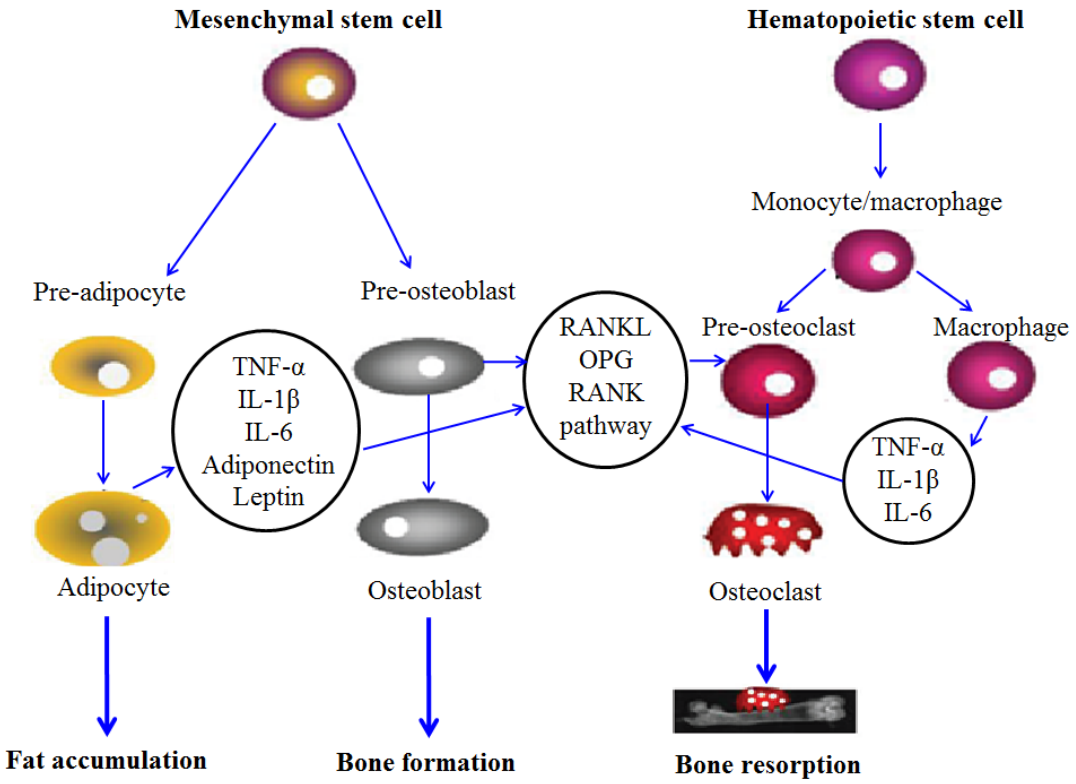

Figure 2.4. Bone metabolism regulated by adipocytes, osteoblasts, and osteoclasts. Adapted from (19).

In bones, stromal cell divides into adipocytes, osteoblasts, and chondrocytes (153). They are balance between adipose and bone tissue. Low-density lipoprotein (LDL) and oxidation also may increase bone loss by conducting marrow stromal cell to convert into adipogenic instead of osteogenic differentiation (154). There is evidence that higher amount of adipose tissue in obesity is accompanied by a low activity of bone formation and high activity of bone resorption markers (155).

One of the most important factors that play a main role in initiation of adipogenesis and suppressing of osteoblastogenesis is peroxisome proliferator-activated receptor- gamma (PPAR-y) (156). It has been shown to increase adiposity and reduce bone mass (157, 158).

2.3. Inflammation

Inflammation is a regular and essential physiological response to recondition homeostasis in response to a variety of stimulus (89) and is a process by which the body's white blood cells and substances produce substrates to protect body from infection against foreign organisms, such as bacteria or viruses (89). Due to normal immune response characteristic of inflammation, initially it is beneficial. However, if the stimulus triggering the inflammation is chronic in nature, inflammation itself creates additional inflammation. However, excess and chronic inflammation may result in further damage in different types of tissues (159).

In this regard, there are two types of inflammation: acute and chronic inflammation. Acute inflammation starts very fast and becomes strict. Its symptoms can be observed for in a few days. However, in specific cases, it may continue for a few weeks (35). Chronic inflammation or long-term inflammation develops gradually and remains in time for several months, even for many years (160).

As mentioned previously, adipose tissue is now considered an endocrine organ which releases bioactive molecules collectively known as adipokines (74). Hypertrophied adipocytes of the obese produces and releases critical inflammatory cytokines that are responsible for chronic inflammation in obesity and influence bone metabolism.

2.3.1. Tumor Necrosis Factor Alpha (TNF-α)

Tumor necrosis factor alpha is a pro-inflammatory cytokine originally produced by monocytes and macrophages (47, 161, 162). While macrophages and T-cells are the predominant (163), there are several extra cells that can equally produce TNF-α, such as B lymphocytes (B cells), neutrophils, mast cells, endothelial cells, osteoclasts and osteoblast cells, cardiac myocytes units, lymphoid cells, astrocytes, and adipocytes (164, 165). TNF-α in human was cloned in 1985 (166) and it has been association with different inflammatory, infectious and malignant situations. TNF-α is also a main cytokine implicated in the control of cell proliferation and differentiation, and lipid breakdown (167, 168).

TNF-α is not typically visible in healthy persons, but its serum and tissue levels rising are observed in inflammatory and infectious circumstances (169, 170). Serum levels are associated with the severity of infection (162, 171). One of the main biological features of TNF- α is in the host protection against to bacterial, viral, and parasitic infections. Physiologically, TNF- α is vital for the common answer to infection, but it can be harmful when it is produced inappropriate or dangerous way (172).

Relationship between TNF- α and Obesity

TNF- α has a strong positive association with body mass index (BMI) and fat mass (173). Circulating levels of TNF-α increases in obese individuals and decreases with weight loss (19, 163).

TNF- α exerts numerous effects on adipose tissue including lipid metabolism and insulin signaling. Thus, excess levels of TNF-α stimulates adipocytes apoptosis (174) and insulin resistance (158). Accumulating evidence shows that TNF- α not only reduces adipocyte differentiation by stimulating leptin expression (174, 175), but also increases lipolysis (176), decreases lipogenesis, and thus, leads to lipid enlargement (177). An increase in TNF-α promotes the secretion of other pro-inflammatory cytokines IL-1, IL-6, CRP from liver during injuries (47) and reduces anti-inflammatory cytokines like adiponectin (79, 80, 168).

Lately, studies have revealed that adipose TNF- α plays a key role in adipocyte differentiation and lipid concentration (88, 178). TNF- α deteriorates adiponectin action in parts like skeletal muscle and diminishes adiponectin level in adipose tissue (4, 179). Findings have revealed that the increased expression of TNF-α in adipose tissue stimulates the progression of obesity. It also increases the risk of diabetes inducing insulin resistance (180) by suppressing the insulin receptor substrate 1 signaling type (181).

Relationship between TNF- α and Bone

There are three key factors involved in osteoclast differentiation and function (182, 183). These factors are:

1. Receptor activator of nuclear factor-kB (NF-kB/RANKL) which is an osteoclast differentiation factor
2. Receptor activator of NF-kB ligand (RANKL) (also called osteoclast differentiation factor, osteoprotegerin ligand and tumor necrosis factor-related activation-induced cytokine)
3. Osteoprotegerin (OPG) which is an osteoclast inhibitory factor

TNF-α has three role in bone metabolism. First, it promotes the activation of osteoclast activity by stimulating the secretion of RANKL on the surface of osteoblast cells (163). Increased expression of RANKL contributes bone resorption through inducing osteoclast differentiation and activity. Second, TNF-α contributes bone resorption by stimulating osteoblast apoptosis and inhibiting osteoclast apoptosis. Third, it is involved in the activation of IL-1 in osteoblasts and indirectly causes bone loss (184). TNF-α and IL-1 can synergies with RANKL to directly potentiate bone resorption by osteoclasts (130). Treating the patients with anti-TNF agents showed dissociation of inflammation and new bone formation in ankylosing spondylitis (185).

2.3.2. Interleukin I (IL-1)

Interleukin I (IL-1) is a glycoprotein with molecular weight of 17 kDa. As a pro-inflammatory cytokine, IL-1 plays a crucial role in cell growth, tissue repair, and chronic inflammatory diseases. Systemic effects of IL-1 involves in regulation of basic metabolic rate, blood glucose levels, blood pressure, iron metabolism and bone remodeling (186, 187). In order to make large group of high proliferative latent energy, it stimulates early phase bone marrow originator cells.

The IL-1 family comprises of two pro-inflammatory cytokines including IL-1α, IL-1β, and an anti-inflammatory agent, the IL-1 receptor antagonist (IL-1Ra) (186-188). Although IL-1α and IL-1β have similar biological activities, their functional roles in the body are different (80, 189). Both IL-1α, IL-1β transduce signals by binding to IL-1 type I receptor (IL-1RI) with an equal affinity. IL-1Ra serves as a natural competitive inhibitor of IL-1α, IL-1β by binding but not activating IL-1RI (16, 190, 191).

Both IL-1α and IL-1β are derived by complex proteolytic cleavage form larger precursors. IL-1 can be produced in large amounts by monocytes and macrophages and is a major mediator of inflammatory responses. Its many actions include the induction of pyrogenic responses, and the stimulation of an array of other cytokines. IL-2 and IL-6 stimulate the synthesis of prostaglandins (192), particularly via the inducible form of cyclooxygenase (CoxII), which in turn is suppressible by gluco-corticosteroids (193). IL-1Ra acts as a naturally occurring inhibitor of IL-1 by blocking binding of IL-1 to its receptor. IL-1ra can inhibit the bone loss in ovariectometized rats, suggesting that IL-1 mediates the bone loss seen after estrogen deprivation (194). 30% of systemic IL-1 is produced by adipose tissue in individuals (195).

IL-1α is first synthesized as a 31 kDa precursor protein, ProIL-1α. Removal of N-terminal amino acids from ProIL-1α leads to 18 kDa mature form of IL-1α. Both ProIL-1α and mature IL-1α are biologically active. Mature IL-1α is released into the extracellular milieu (196).

IL-1β is synthesized as an immature 31 kDa protein, Pro-IL-1β. This Pro IL-1β remains in the cytosol until converted into a mature 17.5 kDa IL-1β (196).

Relationship between IL-1 and Bone

Bone is highly sensitive to IL-1, which regulates both bone formation (197) and bone resorption (198, 199). IL-1 indirectly raises osteoclast formation by stimulating prostaglandin E synthesis in osteoblasts and stromal cells (200). Collectively, these enzymes can contribute to the breakdown of connective tissue matrix. IL-1 is one of the most potent known inducers of bone resorption and was one of the first osteoclast- activating, factors (OAFs). IL-1 causes bone resorption and is associated with monocytes leukemia's and inflammatory erosive conditions in bone, e.g. osteomyelitis, rheumatoid arthritis, and periodontal disease (198, 199). IL-1 also has complex effects, often inhibitory, on the production of matrix components, including collagen, osteocalcin and proteoglycans (201).

IL-1 enhances RANKL expression in osteoblasts/stromal cells (202) while OPG can inhibit IL-1 mediated osteoclast formation (203). In addition, IL-1 contributes the fusion of mononuclear osteoclasts resulting in multi nucleation (204); IL-1 is also directly involved in the survival of osteoclast cells (205, 206).

Osteoporotic fractures and bone loss in patients with post-menopausal osteoporosis are associated with a polymorphism in the IL-1Ra but not in the IL-1b genetic factor (207). It has been proposed that a higher Relativity of IL-1/IL-1Ra alters post-menopausal osteoporosis (208). The ratio of IL-1 in the serum to IL-1Ra in monocytes of women with post-menopausal osteoporosis is increased from the normal levels was seen in females (208). Consequently, these explanations obviously indicate that excess IL-1 signaling under pathological conditions enhances bone resorption. Lee et al (16, 190) demonstrated that IL-1 has plays a significant role in physiological bone metabolism in mice. Further, osteoblast-derived IL-1 is important for RANKL expression in the osteoblasts, while bone marrow cell-derived IL-1 has its specific functions in the differentiation of osteoclast progenitors to osteoclasts. Since IL-1α, IL-1b, and IL-1Ra are produced in joints under physiological conditions, they concluded that the physiological levels of IL-1 and IL-1Ra could involve in bone metabolic syndromes.

2.3.3. Leptin

Leptin, a 16 kD protein, has been discovered in 1994 (209). It is a kind of peptide hormone which is generated originally by white adipose tissue; it may be also produced in low level by other cells like brown adipose tissue, skeletal muscles, and liver (210). It regulates body weight and energy intake and it may balance them by increasing a sensation of satiety in hypothalamus (211). Leptin is an adipokine (cytokine primarily produced by adipocytes) which regulates appetite and stimulates energy expenditure (212, 213). This hormone plays a major role in the control of body fat stores through coordinating and regulation the behavior of feeding, metabolism, nervous system, and body energy balance (213). In vitro studies, leptin has been shown to act as a mitogen on many cell types and appears to play an essential role in malignant cell growth (214).

During obesity, leptin is overexpressed and so resistance of leptin may be seen (210, 211). Enhancing amounts of adipose tissue is accompanied with more leptin concentration which in turn affects immune system and expression of cytokine (215). Leptin is activated by many factors such as insulin, glucocorticoids, TNF-α, and estrogens (215). Investigations have been informed that leptin can improve metabolic dysfunction in some patient with lipodystrophy or in person with leptin lack. More leptin amounts in the serum meaningfully correlate with adipose tissue has been shown the occurrence of the leptin resistance. Studies have revealed that obese individual have more leptin concentration in their blood (216). Leptin functions in the body is interesting; as fat mass reduces, leptin level decreases in order to stimulate of appetite and inhibited energy expenditure up to fat mass restored, when body fat mass increases, the leptin level increases and it suppresses appetite until fat mass starts to decrease (216). Leptin functions as pro-inflammatory character in over weight and obese body, and its concentration rises in obesity because of the resistance of leptin (217). It is a hormone that performs on leptin receptors in the hypothalamus to powerfully control appetite and advance augmented energy expenditure (218). Conversely, it is also identified that leptin has inflammatory consequences, like expanding the extraction of inflammatory cytokines including TNF- α by working on monocytes (219).

Leptin seems to assist with central nervous system and inhibits neuropeptide Y (NPY) activity and agouti-related protein (AGRP) (220) which effects and stimulates pro- opiomelanocortin (POMC) which prevents more eating and revises energy consumption (134). Studies about the role of leptin on the skeletal system are contradictory. Mundy stated that regulation of leptin is responsible for augmented of body weight and bone density. Mice with absence of leptin (ob/ob) are obese and have more bone density. He claimed that leptin leads to lose bone and fat accumulation. Once leptin inserted into the brain of the mice it inhibits the bone formation and also reduces body mass (20).

Relationship between Leptin and Bone

The role of leptin on bone total mass remained controversial, most of the vitro investigations addressed that leptin leads to increase of osteoblast proliferation and

differentiation and also suppress adipogenic differentiation of bone marrow cells. Srinivasan has stated a unwanted relationship between leptin and CTX level in patient with type 2 diabetes (221). However in vivo studies researchers have shown the negative relationship between leptin and osteoblast cells. Therefore, the effects of leptin bone total mass (BTM) are not clear and also Srinivasa has found no relationship between leptin and bone formation markers (221). Thus, the impact of leptin on bone seems to be complex; it has both positive and negative effects on bone (222). It has been documented that the adipocyte-derived hormone leptin regulates bone metabolism, but it is not known how leptin influences bone formation and resorption in obesity. Jensen established that 4.2% decrease in total body bone mineral density and 4.0% decrease in hip in obese women after 6 months of diet leading to 5.5% weight loss (223). Calcium supplementation decreases bone loss in people. In an analysis of 130 young female showed an abnormal prevalence of fractures and very low bone density, they also observed that bone density did not change by uses of estrogen but they have found that consumption of calcium protects bone density loss in young women (224).

2.3.4. Adiponectin

Adiponectin almost completely produced by adipocytes (visceral adipose tissue) and it has been seen at high level in serum and it regulates energy homeostasis, carbohydrates, and fat oxidation (225). This hormone is in serum as two types: “low molecular weight (LMW) and high molecular weight (HMW)” (216, 226). The HMW types of adiponectin are prevail in the blood of health person and are usually reduced in obesity (227). Also high degree of adiponectin has been concomitant with low body fat (228). There are numerous clinical descriptions that support the relationship between adiponectin amounts and obesity for example serum adiponectin levels are inversely associated with visceral fat accumulation (229) and in type 2 diabetes patients’ serum of adiponectin is decreased, furthermore higher amount of adiponectin is accompanied with a low risk of improving type 2 diabetes and fat accumulation (230).

Relationship Adiponectin and Bone

A lot of studies have demonstrated that adiponectin has some functional roles in bones. Adiponectin is also produced by osteoblasts and stimulus proliferation of osteoblast cells in autocrine and paracrine passageways (231). Oshima have shown that adiponectin can inhibit the osteoclastogenesis and osteoclast activity (232). Adiponectin augments fatty acid oxidation and glucose consumption in skeletal muscle and prevents gluconeogenesis in the liver (138). Adiponectin also prevents the exudation and excretion of TNF-α in macrophages and augments the substrate of anti-inflammatory cytokines like interleukin IL-10 (233). Consequently, adiponectin is supposed to have anti-inflammatory outcomes. In agreement with that, the extraction of messenger RNA (mRNA) for adiponectin is decreased in the WAT of genetically in obese mice and obese individuals, and both obese individuals and diabetic patients have a lesser blood level of adiponectin in compared with healthy persons (234).

2.3.5. C- Reactive Protein (CRP)

C- reactive protein is an acute phase protein and originally produced by the liver. CRP usually promotes inflammations, infections, and tissue damages (235, 236). During injuries, adipose tissue can produce pro-inflammatory mediators. So it is unexpected that increase the level of CRP is associated with increase of fat free mass (236).Experiments have stated that CRP quantity was increased about 35% and 60% in obese men and women respectively with BMI more than $30kgm^2$ (237). Furthermore produces of CRP was found more than two fold in obese individuals than lean controls (229).

It is extremely sensitive systemic marker of inflammation. CRP expression is controlled by TNF-α (189). This serum amount enhances rapidly in response to different type of stimulus (238). Numerous investigations have shown that abdominal adiposity is accompanied with increase in CRP. In individuals with high BMI, CRP was notably higher in those people with more abdominal adiposity than control group (239). Now it is obvious that the level of TNF-α is increased via inflammation during bone loss, which causes to leak CRP from liver in more amounts. In additional, the

level of distributing inflammatory cytokines such as C-reactive protein were found to reveal the risk of upcoming cardiovascular disease in the overall people (51, 69, 184, 240).

Several large-scale population-based on studies in children and adolescents have shown higher levels of CRP in overweight subjects than in those of normal weight (241) . An AVENA study was found higher CRP levels in the overweight subjects than in their leaner peers (146). Under physiologic conditions (not during acute phase), CRP is synthesized at low rates. In chronic low-grad inflammatory states, such as obesity, the CRP level is increased and may be a measure of both acute exogenous inflammation and endogenous inflammation (242). This major acute phase plasma protein rises in response to infectious or tissue injury. In addition, CRP is the first line of innate host defense. Its main function is to recognize pathogens and damaged cells of the host reference. Studies have shown that it appears to play a main role in the clearance of apoptotic and necrotic host cells and contributes to restoration normal structure and function of injured tissues. Furthermore, CRP has harmful effects on cells and it is associated with atherogenesis and is involved in tissue damage during acute heart attack. In addition, it seems to have an active role in the inflammatory cascade, which promotes carcinogenesis by injuring DNA, inspiring angiogenesis and cell proliferation, while inhibiting apoptosis (243).

Relationship between CRP and Bone

CRP may affect bone health ultimately by making change on BTM in obesity. Many reasons, containing pro-inflammatory cytokines have been concerned in the pathogenesis of osteoporosis. C- reactive protein is an acute phase reactant that enhances in answer to tissue injury, inflammation, and disease (244). Amounts of CRP are linked exactly to interleukin- 6, a poliotropic cytokine that works directly on hepatic task and making of CRP (245). IL-6 is a strong moderator of the inflammatory activity and has been exposed both to augment with estrogen deficit and to associate with previous life illnesses and circumstances, such as osteoporosis, cancers, and weakness. Numerous investigations have discovered relationship between IL-6 and CRP via advanced bone loss and osteoporosis (244, 245). CRP was accompanied with bone mineral density between community dwelling elderly women

(244). Studies have been shown that CRP was inversely associated with BMD in large sample of men and women (246). Higher amounts of C-reactive protein (CRP) are concomitant with improved fracture possibility, although preceding findings on CRP and bone mineral density (BMD) have produced contradictory outcomes (247).

2.3.6. Interaction between Inflammation and Bone

Pro-inflammatory cytokines including TNF-α, IL-1, and IL-6 are key mediators in the process of osteoclast differentiation and bone resorption. Chronic inflammation and increased pro-inflammatory cytokines induce bone resorption and bone loss in patients with periodontitis (248), pancreatitis (249), inflammatory bowel disease (250), and rheumatoid arthritis (251). It has also been established that upregulated pro-inflammatory cytokines are primary mediators of osteopenia or osteoporosis. The accelerated bone loss at menopause is linked to increased production of pro-inflammatory cytokines including TNF-α, IL-1, and IL-6 (152). These pro-inflammatory cytokines are capable of stimulating osteoclast activity through the regulation of the RANKL/RANK/ OPG pathway (152).

Blocking the activity of IL-1 with an IL-1 receptor antagonist or the signaling of TNF-α with a TNF-binding protein led to decrease osteoclast formation and increases bone resorption in ovariectomized mice (252). The significant increase in the development of osteoarthritis in obese human subjects is another evidence that chronic inflammation influences bone metabolism (19).

2.4. Exercise

Specific health benefits and the role of regular exercise training on obesity, inflammatory and bone turnover markers are presented with more details at this part. Regular exercise can help us protect from obesity and osteoporosis and it improves health (40, 253) . For the greatest overall health benefits, experts have suggested that doing 20 to 30 minutes of regular exercise three or more times per week (254, 255). Regular physical activity that is performed on most days of the week reduces the risk of developing diabetes, helps to control weight, builds and maintains healthy bones, muscles, and joints. It also helps adults become stronger and better and promotes

psychological health (256). Physical activity helps to reduce body fat by building or preserving muscle mass and improving the body's ability to use calories. Once physical activity is conjoined with right nutrition, it can be benefit and control weight and inhibit obesity (257).

Attenuated of osteoporosis is another advantage of exercise training. Regular weight-loss exercise promotes bone formation and may prevent many forms of bone loss associated with aging. Studies have demonstrated the role of exercise training has a key role to prevent obesity and decrease the negative effects of inflammatory cytokines and bone turnover markers on body (258, 259). Epidemiological surveys have exposed that exercise is useful for stopping and recovering obesity. Exercise training (TR) not only leads to loss of WAT mass, but also affects the secretory reaction and extraction of adipokines in WAT (29). Exercise augments both energy expenditure and fat oxidation (260). Physical activity is known to have a positive effect on the skeleton by increasing BMD and BMC and it also expands bone health. Exercise has also anti-inflammatory effects on body (28). These effects are responsible for inhibiting the causes which make individuals more prone to injuries or infections (261).

According to the studies, lose muscle mass, strength and bone density occur in individual during lifetime. These disorders may be meaningfully reduced by accomplishment exercise on a regular foundation (262). The immediate effects of exercise on muscular system include more frequent muscle contraction; improve circulation of blood to muscles and a decrease of muscle disease. Modifications occur both in muscular system and skeletal system with regular exercise over time. (263). One of the most important training adaptations is an increase in the width and density of bones, which reduce likelihood of bone- linked damages, such as a break. Regular training increases the strength of muscles, tendons and ligaments, which in turn helps stabilize joints. It also improves and helps maintain joint flexibility and contribute to muscles hypertrophy. Muscle endurance improves with training, which means that it takes longer for muscles to fatigue when performing natural work, mainly repetitive jobs (264).

2.4.1. Effects of Exercise on Obesity

The modifications in health and body composition which be associated with physical exercise in individuals of standard weight would be very important in the behavior of obesity (265). On the other hand, one of the greatest evident incapacities rising from obesity is a decreased exercise tolerance, so harshly obese persons are incapable to do the exercise which would bring these profits (266). It has been recommended that training may present advantages on the obese individual by decreasing voluntary food consumption. The best way to prevent obesity is to restrict dietary and do exercise (267). You et al. has been reported a significant decreases of body weight, fat mass, fat percent, waist –hip circumference in thirty obese women (BMI=33±0,7kg/m2, age 58±1 years) after 20 weeks of moderate-intensity exercise intervention (268). Brian et al. has been established that high intensity exercise training significantly decreased total abdominal fat, abdominal subcutaneous fat percent and aria visceral fat in twenty seven middle age obese women with BMI (269). Whyte et al. has also been revealed that after high intensity interval training in obese men, VO_2max significantly increased and waist and hip circumferences decreased (270).

2.4.2. Effects of Exercise on Inflammatory cytokines

Exercise training has anti-inflammatory aspects. Thus, regular exercise training or physical activity can prevent the improvement of chronic disorders (70, 271). Moreover, training can be used as a medication to enhance the signs of several of these illnesses. Therefore, the hypothesis that exercise is assumed as medication is true (272). On the other hand, the protecting influence of a physically active lifestyle in versus to chronic inflammation- related disorders may also be recognized as an anti-inflammatory result of exercise (273). This may be attribute both to decrease in visceral fat mass and size to stimulation of an anti-inflammatory situation with each session of exercise (70). Study conducted by Hamer found a decrease the level of pro-inflammatory cytokines and increase the level of anti- inflammatory cytokines over 10 years follow up survey in 4289 men and women following regular moderate exercise training and high physical activity (274).

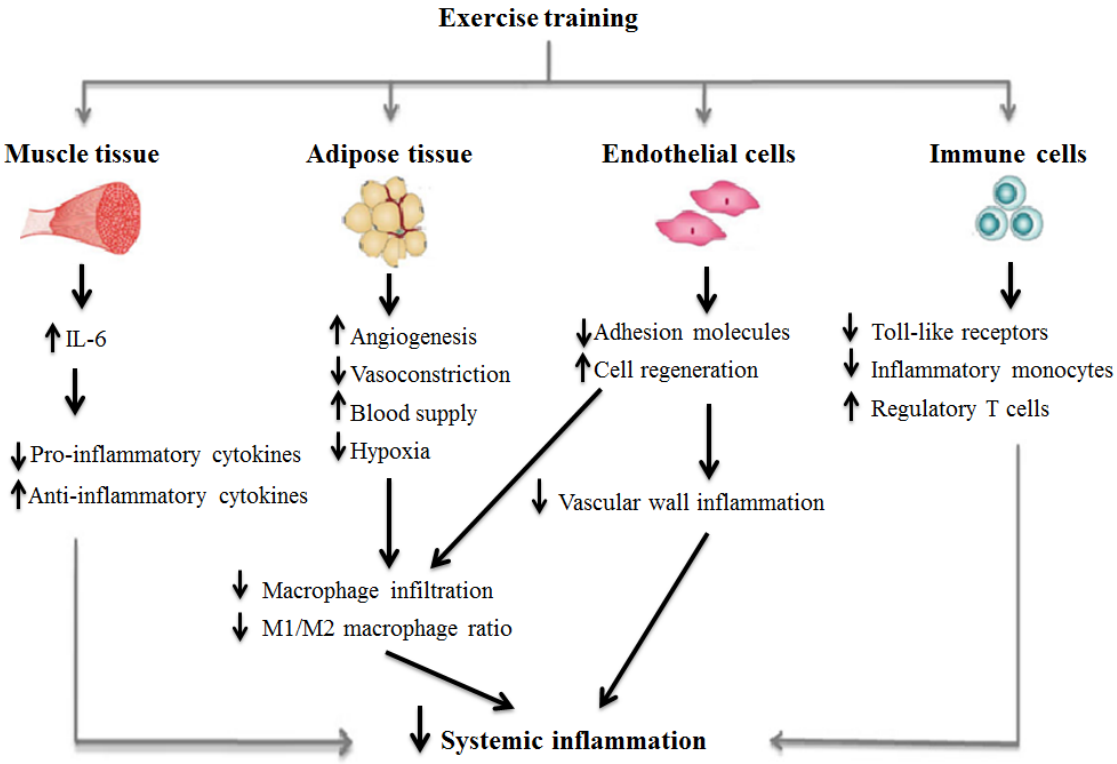

Figure 2.5. Potential mechanisms through which exercise training reduces chronic inflammation in obesity. Adapted from (268)

Acute exercise stimulates skeletal muscle release of IL-6, which further inhibits actions of pro-inflammatory (268). And increases levels of anti-inflammatory cytokines in other tissue/cells and systemically. Exercise training may increase angiogenesis, alleviate vaso-constriction, and increase blood flow, thereby reducing hypoxia and the associated chronic inflammation in adipose tissue. Exercise also reduces adhesion molecule production by endothelial cells and stimulates the regeneration these cells, and lowers vascular wall inflammation. Furthermore, exercise training may reduce the expression of toll-like receptors and production of pro-inflammatory cytokines in monocytes, lower the number of pro-inflammatory cells, and increase the number of regulatory T cells. All these mechanisms may contribute to the anti-inflammatory effects of exercise training, independent of its effects on weight loss.

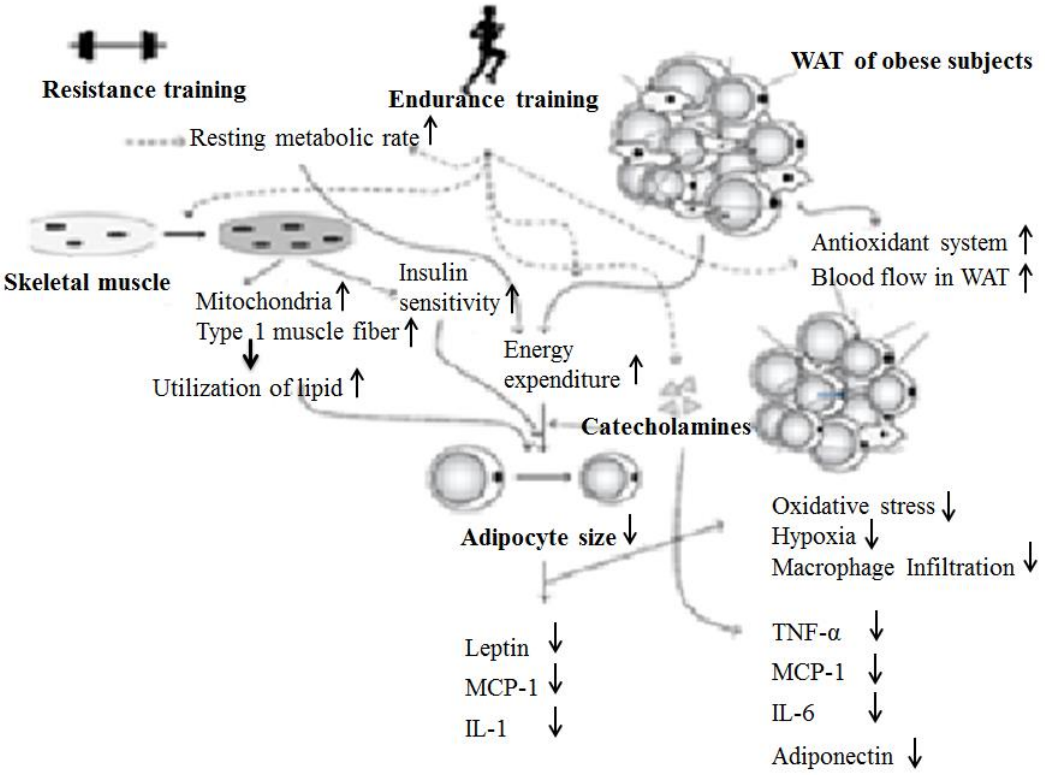

Figure 2.6. Schematic model for the effects of exercise training on expression of adipokines in WAT. Adapted from (29)

During endurance training, type 1 muscle fibers in skeletal muscle are selectively used for the execution of exercises, and therefore, energy expenditure using lipid increases. Triglycerides within the adipocytes are broken down due to the secretion of catecholamine's, and the resultant fatty acids are transported to tissues such as skeletal muscle. When exercise is repeated, adipocyte size is lessened. Decreases in adipocyte size are considered to result in the attenuation of dysregulated expression of adipocyte size-sensitive adipokines, such as leptin and oxidative stress in WAT. Moreover, catecholamine itself seems to correct disarray of adiponectin and TNF-α in WAT of obese subjects. In addition, endurance training might suppress oxidative stress and a hypoxic state of WAT due to an enhanced anti-oxidative system and increases in blood flow, respectively, which lead to the attenuation of the dysregulated expression of inflammatory-related adipokines involving TNF-α and MCP-1. In skeletal muscle, endurance training produces transition to type I muscle

fiber following the increase in mitochondria biogenesis and enhances insulin sensitivity. Consequently, enhanced glucose/lipid metabolism in skeletal muscle decreases adipocyte size. On the other hand, resistance and endurance training enhance resting metabolic rate, which is likely to cause the alteration of adipokine expression following WAT mass reduction due to increased energy expenditure in the resting state (29).

2.4.3. Effects of Exercise on TNF-α

Studies on the effects of exercise on circulating levels of TNF- α in humans showed that acute exercise increases (275-277) and exercise training either decreases (278), doesn't change (279), or even increases (280), circulating levels of TNF- α. This inconsistences in the literature might be due to the study design, intensity, duration and type of the exercise training, and subject characteristics (healthy-with chronic disease, age, obese-lean, gender, pre or post-menopausal etc.).

With respect to acute effects of exercise on TNF- α Scott et al (276) showed that 60 minutes of treadmill exercise bout at an intensities of 55% and 75% VO_2max increased the level of TNF- α by 18-22% in physically active men (276). However, TNF- α response was not different between the two exercise intensities (276). Similarly a significant decrease the plasma concentration of TNF- α level was observed immediately after incremental exercise test protocol on cycle ergometer in highly trained athletes (20-30 years) (275). Zwetsloot e al showed that an acute bout of HIIT significantly increased TNF- α level in young recreationally active men (277). They also showed that two weeks of HIIT program was not successful in inducing anti-inflammatory adaptations (277). Similarly Leggate et al reported that 2 weeks of HIIT didn't decrease plasma TNF- α level in overweight and obese males (27).

With respect to effects of exercise training on TNF- α, 6 weeks exercise training at an intensity of 50-80% VO_2 peak decreased the level of TNF- α from a baseline value of 13 ± 15.2 pg/mL by 1.9 ± 8.6 pg/mL in 89 men in their sixties (240). Another study by Ho et al showed that aerobic exercise training, resistance training and the combination of both were effective in reducing TNF-α level after 12 weeks

compared to baseline by 20.8 %, 26.9 %, and 32.6 %, respectively, in overweight and obese individuals. TNF-α levels in the combination group were significantly lower (−22.6 %) compared to the control when adjusting for baseline levels (316). Eight weeks of aerobic exercise was effective in reducing TNF- α levels (281, 282).

In another study Nikseresht et al 12 weeks of aerobic interval training consisted of running on a treadmill (4x4 minutes at 80–90% maximal heart rate, with 3-minute recovery intervals at 55–65% maximal heart rate) resulted in no change in plasma TNF- α levels in obese middle aged men (44). Similarly, a 10-week of treadmill exercise training program (30 to 40 min at exercise intensity of 55-65% of peak heart rate) training decreased TNF- α levels in obese young women with Down syndrome (283). On the other hand, Fisher et al investigated the effect of diet with and without exercise training on markers of inflammation and fat distribution in overweight women and found that decreased TNF- α level was associated to weight loss, particularly intra-abdominal fat loss, and exercise didn't result in further decrease in TNF- α level (284) . Schultz et al showed that 16 week of resistance training in obese adolescents decreased TNF-α concentrations (285).

A study on diabetic patients presented that even there was no alteration in blood TNF- α level after four weeks of dietary limitations and walking in lean diabetic patients, the concentration was diminished in obese patients (286, 287). Additionally, 12 weeks exercise training on a bicycle ergometer for 30 minutes a day, five days per week at 70% $V\dot{O}_2max$ reduced the circulating levels of both TNF- α and soluble TNF receptor 2 in obese adult women with and without insulin resistance (287). Conversely, a combination of diet therapy and exercise training for 15 weeks resulted in no change in TNF- α level in obese persons (288) Similarly 3 moths of aerobic exercise training didn't change plasma levels of TNF- α in moderately obese premenopausal women (289) In middle aged women 14 weeks of exercise training at either 50% or 70% maximal oxygen consumption didn't alter TNF- α levels although body fat percent decreased (290). In contrast to general findings, 12 weeks of endurance exercise training increased the blood level of TNF-α in adult women (291).

2.4.4. Effects of Exercise on IL-1α

Study on healthy trained men conducted by Ullum et al. have shown significant decrease of IL-1α, IL-1b, and TNF- α following bicycle exercise for 1 h at 75% of maximal oxygen uptake (292). He has observed that the plasma concentration of IL-1 didn't change immediately after a bout of exercise, however its level increased up to 150% after 3-4 hours after the end of exercise and then it came back to initial level after 9 hours of exercise ending (293). Li et al has been reported that the level of IL-1 increased immediately for all trained groups following high intensity exercise training in the certain degree of exercise intensity (293, 294). Evant has observed high concentration of IL-1 in men and women following 45 minutes cycling exercise training (295).

Willoughby found that the ability to secrete IL-1 for enterocoelia macrophage increased notably after high acute intensity exercise (296). Li et al discovered that in the certain condition, IL-1 increased for all training group after high intensity exercise. Both Ben and Espersen reported that IL-1 activity increased after long range training (297, 298). A lot of studies have reported that cytokine changes after 1 h of 75%VO_2max running training (193, 197, 297, 298). They found that the level of IL-1α and IL-1ß formed by separated cell out of body increased notably after exercise. Cannon's experiment was requested subjects moving 1 hour on the bicycle dynamometer with 60% VO_2max intensity. He reached a conclusion that activity of blood plasma IL-1 didn't increase immediately after exercise, but raised to 150% of rest amount 3 – 6 hours after exercise and restored to basis value 9 hours after exercise. Another report said the peak value of IL-1 was reached in 2 – 3 hours after exercise. But later, other studies found that the level of IL-1 and IL-6 are immediately increased after a high intensity exercise (299, 300). Current literature shows that exercise training decrease expression of IL-1 b and IL-12 in visceral adipose tissue of obese rats or mice (93).

2.4.5. Effects of Exercise on Leptin

The effects of both acute and chronic exercise training on leptin level of men and women revealed that the plasma concentration of leptin has not been changed

significantly after both type of exercise training. Then they concluded that the level of leptin was not affected by exercise (301, 302). The effect of 12 month long term endurance exercise on plasma level of leptin was studied on 186 men (44.9±2.5 year) and BMI with (28.6±3.4kg/m^2) overweight individuals, with metabolic syndrome in order to determine plasma concentration of leptin on subjects, results of the study shown significant decrease of leptin concentration in men following the exercise intervention at the end of the exercise (303).

Studies demonstrate that seven weeks of natural running decreases the countenance of mRNA for leptin in the visceral and subcutaneous WAT of obese rats (304). Moreover, additional study shown that stage of a short interval (four weeks) of free activity lessens leptin mRNA appearance in rat model of WAT (305). Conversely, one study found that 12 weeks of one-hour aerobic exercise sessions had no impact on the appearance of mRNA for leptin in subcutaneous WAT in obese individuals (306). There have been numerous investigations on the effects of ET on the human blood amount of leptin. Several paradigms have revealed that concentrations of leptin reduced in WAT (307, 308). By contrast, when no significant differences were detected in blood leptin concentration after ET, body fat decreased (309). Consequently, a decreased of plasma level of leptin after ET is due to the decrease in body fat by ET (307, 308). Various studies, however, propose a low duration (12 weeks) of ET or ET with diet restriction can decrease blood leptin level which is separated from the effect of body fat decrease (309). Numerous investigations have also focused on the effects of resistance exercise, like the bench press workout, on blood leptin concentrations. One analysis on postmenopausal obese females discovered that after accomplishment of three days per week of resistance exercise using machines and restricting diet for 16 weeks, blood leptin levels were decreased compared with pre training levels, but that resistance exercise per se had no effect on leptin (310). Conversely, when elderly persons were divided into low intensity (45–50% 1 repetition maximum [RM]), moderate intensity (60–65% 1 RM), and high intensity (80–85% 1 RM) groups and accomplished 60-minute training bouts three times per week for six months, blood leptin concentration was lower in all the groups in compared to pre training levels. The importance of this

reduction was significantly higher in the high intensity participants than in the participant having joined in low and moderate intensity (311).

2.4.6. Effects of Exercise on Adiponectin

Yokoyama has been reported no significant change in plasma level of adiponectin after 3 weeks 5 days per week and 40 min per session of aerobic exercise training at intensity of 50.6±8.9 VO2max. However, he found strong relationship among adiponectin and BMI, body weight, and fat percent following exercise protocol on diabetic's patients (312). Adiponectin level was significant increased following one week of aerobic exercise training (50-75% VO_2peak) on inactive abdominally obese men, and it remained up after 30 min of recovery time (313).

Hulver discovered that the blood level of adiponectin did not alter in obese adults after completed aerobic exercises running at 65–85% $V\dot{O}_2$ max four times per week over a phase of six months (314). An additional survey on diabetic men also discovered no alter in the blood level of adiponectin after eight weeks of aerobic exercise three times per week, even the total of visceral fat reduced (315). When obese males and females accomplished ET for 60 minutes on a treadmill or bicycle ergometer at 80–85% of their maximum heart rate five times per week for 12 weeks, there was no alter in the blood level of adiponectin in spite of the reduction in BMI and body fat (316). In a another study, the blood level of adiponectin has been enhanced along with decreased BMI and body fat mass after seven months of ET done four to five times per week in obese young females (307).

Some of studies support a significant and direct association between physical activity and adiponectin amounts in blood flow (317). For instance, exercise two or more sessions per week was related to increase adiponectin levels more than 4 μg/ml (318). Pierre et al also has been stated a positive relationship between adiponectin and physical activity (313). A 15-week ET along with diet therapy or 12 weeks of aerobic exercise has revealed an increase in the appearance of mRNA for adiponectin in the subcutaneous WAT of obese patients (319). In findings on rats shown that nine weeks of treadmill running has enhanced the mRNA extraction in visceral and subcutaneous adipocytes (320). Like with leptin, there have been numerous

investigations on the effects of ET on the blood concentration of adiponectin (311). Jurimae reported an increase in adiponectin level after exercise (321, 322). A graded treadmill walk or run practice in which subjects trained at 60, 75, 90, and 100% VO_2max provoked significant increase in adiponectin from 7.45 ± 1.10 μg/ml to 8.18 ± 1.20 μg/ml at post-exercise in skilled runners (322).

2.4.7. Effects of Exercise on CRP

Study of seven men and twenty women runners has been shown an increase in CRP instantly and 24 hours after marathon race. However, there were significant increases of IL-1following exercise (323). In another investigate; there was significant increase in the plasma level of CRP 24 hours after aerobic exercise in 18 athletes (324). The concentration of CRP was significantly increased following marathon race in 55 runners (325). Resistance, aerobic, and combination of both training did not significant reduced plasma concentration of CRP after aerobic training (0.16 mg/L), resistance exercise (-0.03 mg/L), and combination of both (-0.49 mg/L) versus to control group in 204 men with type 2 diabetes following 9 month exercise intervention (326). But no significant alteration was found in CRP levels in older women (282).

Twelve months of moderate-intensity exercise was decreased CRP level among females who were obese at the beginning of study. These results help the role of exercise in controlling inflammatory developments that are correlated to augmented risk of chronic disorder among obese females (327). Exercise training is accompanied with decreased of CRP activity in coronary artery disease (CAD) patients. C-reactive protein has decreased in those patients with higher level at baseline (328). Regular high-intensity interval training was associated with a significant decrease in levels of high-sensitivity C-reactive protein which decreased by -0.4±1.1 mg/L in the training group and increased by 0.1± 1.2 mg/L in the control group ($p = .03$) (329).

2.4.8. Effects of Exercise on Bone

Regular weight-bearing training is extensively stated to have helpful influences on bone mineral content and bone mineral density through growth and development. Effects of high-intensity sports have been revealed to improve bone construction on surfaces of long bones at overloaded skeletal positions (330).

2.4.9. Effects of Exercise on Osteocalcin

Plasma concentration of osteocalcin has been increased from following 8 week cycling at intensity of (60% VO_2max) in men and women (38-55 years) with subclinical hyperthyroidism (331). Kim et al were reported significant elevation of serum undercarboxylated osteocalcin after 8 weeks aerobic exercise intervention in 39 obese young man and the increase of osteocalcin has negative associations with alterations in body weight, BMI and body fat percentage as well as leptin level (332).

2.4.10. Effects of Exercise on BALP

Following a week of aerobic exercise training, the plasma level of BALP was increased in healthy postmenopausal women (333). Another study has revealed that the plasma level of BALP was significantly increased in 100 post-menopausal healthy women following of aerobic exercise training (334).

A study was conducted on the effects of high intensity resistance exercise training on bone metabolism in 17 young adult Oriental males (23–31 years) by measuring of bone formation and resorption markers. The training group followed a weight training program three times per week for 12 weeks. In the exercise group, plasma osteocalcin concentration and serum bone-specific alkaline phosphatase activity were significantly increased within the first month after the beginning of resistance exercise protocol, and the raised amounts were persisted during the training period. These results suggested that the resistance exercise training enhanced bone formation. Resistance exercise training increased markers of bone modeling, while it rapidly repressed a sign of bone resorption (335).

2.4.11. Effects of Exercise on CTX

Changes were statistically significant in the exercise group for CTX from baseline to post training following aerobic exercise protocol in healthy menopausal women. There was no significant change in the control group from baseline. When the groups were compared with each other, the exercise group was found to be superior to the control group for the change in CTX (334).

2.4.12. HIIT Training

High intensity interval training is a particular form of intermittent exercise that includes short intermittent of high intensity training with recovery time between every exercise bouts. The intense workout may vary from 5 seconds to 8 minutes duration, and are done at 80% to 95% of an individual's assessed maximum heart rate. The recovery cycles may take similarly as long as the workout and are usually complete at 40% to 50% of a person's estimated maximum heart rate (33, 59, 336).

In the recent years, it has been shown that compared to continues moderate exercise, high intensity interval training (HIIT) may result in superior or equal metabolic improvements (33, 59) due to its high intensity bouts and metabolic stimulation. On the other site, it has been well established that aerobic exercise training reduces body fat percentage and improves inflammatory state (337, 338).It also decreases obesity-induced dysregulated expression of the inflammation-related adipokines in plasma (6, 29) and white adipose tissue (339). Moreover, exercise training, particularly weight bearing exercise, improves bone health (340, 341).

Research has shown that high intensity interval training (HIIT) can burn adipose (fat) tissue more than up to 50% more effectively than low-intensity exercise and moderate intensity exercise (26, 342, 343). Boutcher et al found a significant loss in body fat in a group that exercised at a high intensity of 80-90% of maximum heart rate, whereas no significant modification in body fat mass was found in the lower intensity group which exercised at 60-70% of maximum heart rate; no significant difference in total work existed between groups (59, 336, 344). High intensity

interval training (HIIT) has also been shown to speed up metabolism which helps burn more calories throughout the day (345).

Studies have demonstrated that HIIT training has the same effect on body in compare with continues training in the long time (59, 346). In sport training, there are three different categorize of high intensity interval training. Long interval; about 3-15 minutes in an intensity corresponding to 80-90% of maximal oxygen uptake (VO_2max) by following of active recovery time of 30/60 second with (50% VO_2max), moderate interval intensity ; about 1-3 minutes in an intensity corresponding to 95-100% of maximal oxygen uptake (VO_2max) by following of active recovery time of 15/60 second (70% VO_2max), and short interval; 10 second to 1 minute in an intensity corresponding to 100-120% of maximal oxygen uptake (VO_2max) by following of active recovery time of 10/60 second (80% VO_2max) (346, 347). This type of exercise provide improved fat burning and is more effective to reducing subcutaneous abdominal body fat, and glucose metabolism and also decreases insulin resistance in obese individuals (39).

Vigorous intensity exercise is associated with low level of obesity markers in women (348). Likewise, a single bout of very high intensity exercise training may increase insulin sensitivity and fat oxidation in obese individuals (348, 349). In recent study revealed that the effects of HIIT on inflammatory cytokines after six months of HIIT the level of IL-1, TNF-α, CRP, and leptin has been reduced in patient with heart disease (350) and healthy students (336, 349). The levels of adiponectin were increased following six weeks of HIIT in healthy students (351). Thus, it may be concluded from these studies that exercise has possible potential effects on inflammatory cytokines directly and bone health promotion indirectly (351). Therefore, this type of exercise training is time efficient strategy to enhance skeletal muscle adaptations compared to traditional endurance training (352). However, In this study, subjects was performed of HIIT training on treadmill in long interval with intensity corresponding to 85-95% of maximal heart rate by following of active recovery time of three minutes with (50-60% MHR) due to their obesity status. Therefore, the aim of this study was to investigate the effect of HIIT on inflammatory cytokines and bone turnover markers in obese women.

3. METHODS

3.1 Subjects

All subjects were recruited through flyers, in the Urmia city, Iran. Initially 50 obese sedentary women responded. The responders were invited for an initial screening to determine their eligibility to the study. During the initial screening visit, all responders were received a detailed explanation of the potential benefits and possible risks associated with participation in the study. Then, a medical screening questioner (Appendix 1) and physical activity readiness questioner (Appendix 2) were administered to the participants and evaluated by a cardiologist. Additionally, body weight, height, and blood pressure of the responders were measured.

Thirty responders, who were eligible based on the initial screening, were invited to attend an orientation session, while 28 participated in the session. At the orientation session details of the study and procedures were explained further and the subjects were encouraged to ask their questions on the study. Only 24 of the responders accepted to participate in the study.

All participants were examined by a cardiologist to exclude participants with any cardiovascular risk contraindicated for exercise participation. Study procedures were carried out after obtaining subjects written informed consent (Appendix 3). Figure 3.1 summarizes the subject's recruitment and initial screening processes. Procedures of the study were approved by the Urmia University of Medical Sciences Ethic Committee (No.135 on 12/08/2014) (Appendix 4).

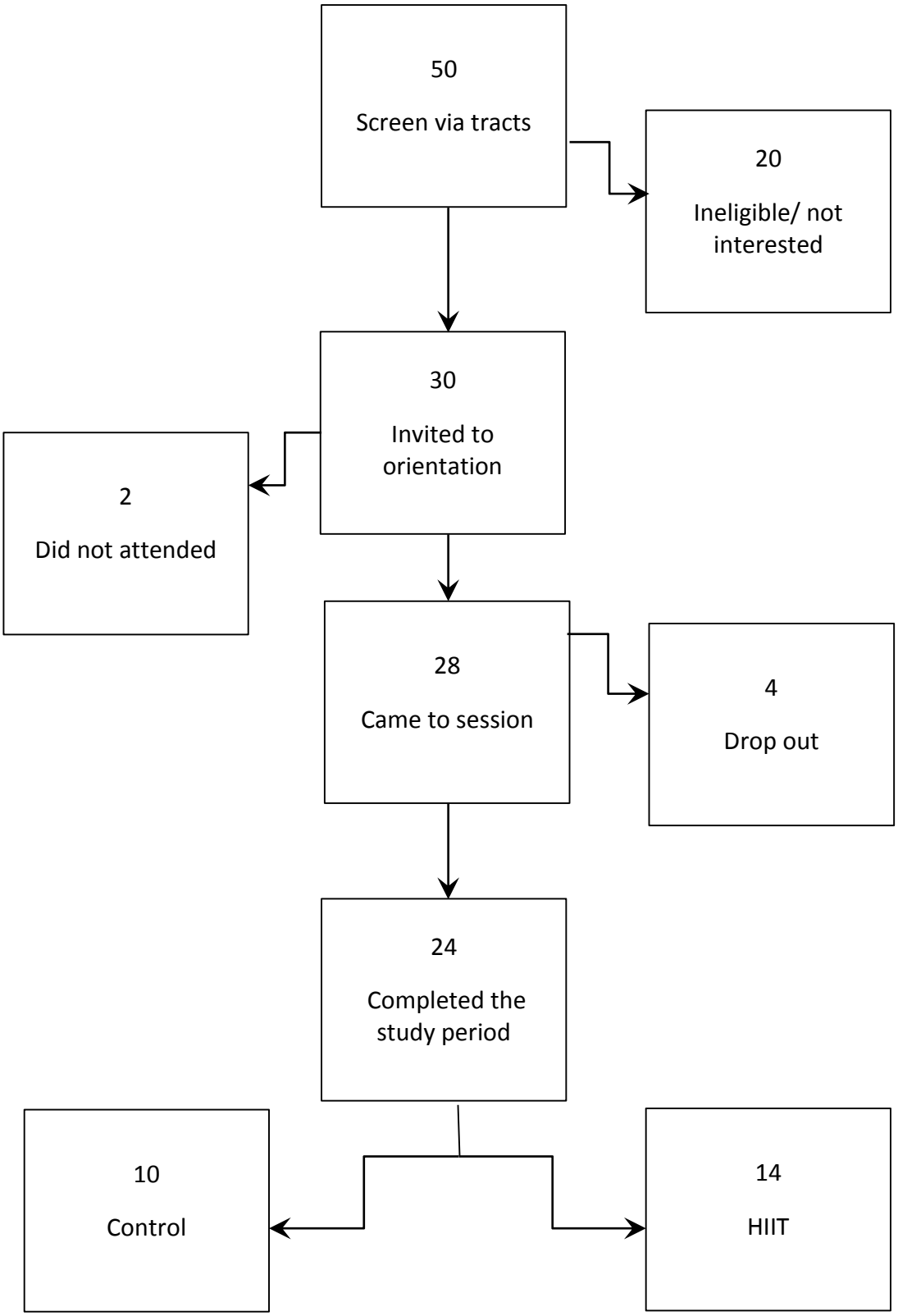

Figure 3.1. Subjects recruitment and initial screening process.

Eligibility of the subjects was determined according to the medical history (Appendix 1) and to the results of medical screening by a cardiologist. Inclusion and exclusion criteria were as follows:

3.1.1. Inclusion Criteria:

1. Healthy women between 30-40 years old
2. Obese, defined as having a BMI between 30 and 34.99 kg/m^2
3. Maintenance of stable body weight (less than 5 percent body weight change) for at least 12 months prior to enrollment
4. Being sedentary, consistent with the American College of Sports Medicine guidelines (353), was defined as not participating in regular exercise more than two sessions per week
5. Having normal menstrual cycles
6. Haven't had any fracture or orthopedic problem within the last year
7. Haven't used medication known to affect bone metabolism
8. Being a non-smoker

3.1.2. Exclusion Criteria

Subjects were left out from the study if they meet any of the below additional exclusionary criteria.

1. History of any chronic disease i.e. cardiovascular disease, diabetes mellitus, hypertension, cancer.
2. Taking any medication for known or unknown disease such as heart failure, respiratory problems, diabetes, obstructive pulmonary disease, multiple sclerosis etc.
3. Having any immobility condition, physical problem or any limitation that would prevent participation in exercise.
4. Pregnant at present time or pregnant in the previous 6 months, or plan on becoming pregnant in the next 3 months.
5. Having an abnormal menstrual cycle or being diagnosed with amenorrhea

3.2. Experimental Design

The current study was a randomized, controlled trial with pre and post-measures design. Subjects reported to the laboratory twice for both pre- and post-training measurements. A total of 24 obese women were randomly divided into two groups: Control group (n=10) and High intensity exercise training (HIIT) group (n=14). HIIT group participated in a 12-week, supervised high intensity training program 25 min/day, 3 days/week, at 85-95% of each subject's age-predicted maximal heart rate. Subjects in control group were instructed to maintain their current physical activity level and dietary habits during the study. All subjects underwent identical tests before and after the 12 weeks training program. Pre- and post-training evaluations including anthropometric measurements, heart rate, cardiorespiratory fitness test, and laboratory tests were performed within the one week before and after the training program. However, the post-training blood samples were collected 24 hours after the last exercise session.

On the first testing week, subjects arrived at Gavam laboratory to undergo a urine pregnancy test to ensure they were not pregnant prior to undergo further measurements. All tests were carried out in the morning between 8:00 and 11:00 am to avoid circadian rhythm variances. All subjects were asked to abstain from any physical activity for 72 hours. Laboratory tests (blood sampling for evaluating of TNF-α, IL-1α, leptin, adiponectin, estrogen receptor, BALP, CTX) were performed after 12 hour overnight fasting conditions. VO_2max was determined following consumption of a light breakfast at least 2 hour before testing. The study design and HIIT protocol were summarized in figure 3.2.

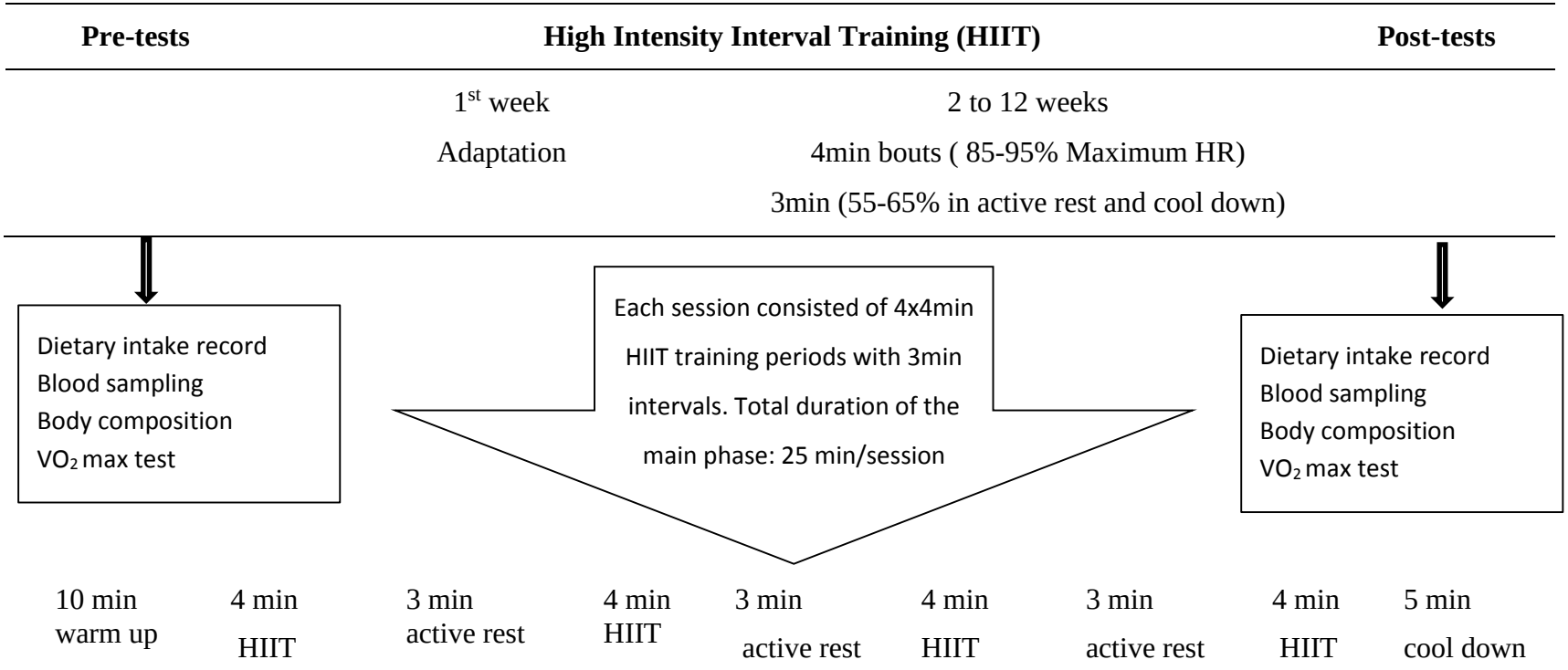

Figure 3.2. Study design and HIIT protocol.

3.3. Data Collection

3.3.1. Anthropometrics and Body Composition

Body composition of the subjects was measured to determine the eligible subjects as well as body composition changes throughout the study. In this respect, body weight, height, BMI, body fat percentage, fat mass, fat free mass, waist and hip circumferences and waist to hip ratio were measured before and after the HIIT program.

Height was measured to the nearest 0.1 cm using a wall mounted manual stadiometer (SECA 222 Corp, Hanover, Germany). Subjects were asked to remove their shoes and stand with their heels against the platform facing out towards the room. Body weight was measured to the nearest 0.01 kg by using a digital electronic scale (Ohaus DS44L, NJ). Subjects were asked to remove their shoes and any jewelry and step on the scale. Subjects were weighed after an overnight fasting in light clothing. Body mass index (BMI) was computed as the ratio of body weight divided by the square of the height in meters (kg/m^2).

Waist and hip circumferences were measured with a Gulick tape measure. Waist circumference was measured at the midpoint between the last rib margin and iliac crest with participants in a standing position, feet fairly close each other, weight equally distributed on each leg, and breathe out (354). Hip circumference was measured at the maximal protrusion of the greater trochanter (355). Waist to hip ratio (WHR) was calculated by dividing the waist circumference to hip circumference.

Body composition (including fat mass, fat free mass, and body fat percentage) was measured by bioelectrical impedance analysis (BOCA X1, South Korea) in the morning following a 12 hour overnight fasting. Subjects were asked to take off all jewelry and any metal before the measurement.

3.3.2. Resting Heart Rate and Blood Pressure

The subjects rested in a quiet room for at least 5 min prior to the measurements. Resting heart rate was measured using a heart rate monitor (Polar

810i, Finland). Brachial systolic and diastolic BPs, were measured on the right arm by a physician using a sphygmomanometer (EmsiG, SP98 Germany).

3.3.3. Determination of VO_2max

To determine subjects' baseline cardiorespiratory fitness level as well as the effect of 12-week HIIT training program on cardiorespiratory fitness, subjects were underwent a Bruce treadmill test (356) (Appendix 5) within the one week before and after the training program (357).

The Bruce Protocol test is a maximal graded exercise test where the participant works to complete up to exhaustion as the treadmill speed and incline are increased every three minutes (Table 3.1). The protocol consists of 10 stages each lasting 3 minutes. The speed and incline for the first stage are 2.74 km/h and 10%, respectively (Table 3.1). The total duration stayed on the treadmill is the test score and used to estimate the VO_2max value.

The test was performed on a treadmill (Nova fit 2400 Turbo, Taiwan) under the direct supervision of an attending cardiologist for each participant. During the test, heart rate, BP, and ratings of perceived exertion were monitored and recorded. All subjects were verbally encouraged to provide a true maximal effort.

The entire test consisted of a 3-min warm-up, main test and 5-min cool-down periods. The warm up and cool-down periods were performed at a treadmill speed of 2.74 km/h with no inclination.

Table 3.1. Bruce test protocol.

Stage	Time (min)	Slope (%)	Velocity (km/h)
1	0	10	2.74
2	3	12	4.02
3	6	14	5.47
4	9	16	6.76
5	12	18	8.05
6	15	20	8.85
7	18	22	9.65
8	21	24	10.46
9	24	26	11.26
10	27	28	12.07

Participants were asked to determine their perceived level of exertion every 3 minute and at the end of the test by indicating a number between 6 and 20 from a visual chart (358) (Appendix 6). The test was terminated when two of the following criteria were observed:

1) Possible effort of the subjects as judged by the attending cardiologist (357).

2) Rating of perceived exertion ≥ 18 on the Borg scale of 6–20 (359).

3) HR > 90% of age-predicted maximal HR (206 – (0.88 × age) (360).

Time to exhaustion was recorded and used to calculate the VO_2max value of the participants by using the following equation:

$$VO_2max = (4.38 \times T) - 3.9 \quad (3.1)$$

where, T= time (maximum time of effort up to exhaustion) (361).

Analysis of maximal heart rate attained during the VO_2max test confirmed that average HRmax attained during the test was 97.70±1.69% (range 95.70%-100.25%) of age predicted HRmax confirming that a true VO_2max test was performed.

3.3.4. High Intensity Interval Training (HIIT)

The participants completed a 12-week HIIT program with 3 weekly sessions. Throughout the training program a fixed exercise intensity and duration was followed. HIIT protocol was initiated the latest 1 week after the pre-training tests. The first week of the training program was considered as an adaptation week.

HIIT was performed on a treadmill (Nova fit 2400 Turbo, Taiwan) with a slope of 10 degree. Each HIIT session was consisted of warm-up, main exercise, and cool-down periods. A 10 min warm-up period at 55–65 % of age predicted maximum HR followed by 4×4 min training intervals at 85–95 % of maximum HR with 3 min active breaks between the intervals (Figure 3.2). Each active break was consisted of walking or jogging at 55–65 % of age predicted maximum HR. Main phase of the exercise session lasted 25 minutes (4x4 min = 16 min training plus 3x3 min = 9 min active breaks). The exercise session was terminated by a 5 min cool-down period (362). Each training session lasted 40 minutes.

Exercise intensity was based on each subject's target heart rate calculated as the 85% to 95% of age predicted maximal heart rate (360). Age predicted maximum heart rate and exercise target heart rate range of the participants were calculated according to the following equations:

$$\text{Age predicted maximum heart rate } (HR_{max}) = (206 - (0.88 \times \text{age})) \quad (360). \qquad (3.1)$$

$$\text{Exercise target heart rate range} = 85\% \times HR_{max} - 95\% \times HR_{max} \qquad (3.2)$$

Each participant was informed of her exercise target heart rate limits and instructed to regulate the speed of the treadmill according to the attained heart rate.

For example, if the attained HR is higher than the target heart rate the participant decreased the speed of the treadmill and if the attained heart rate is lower than the target heart rate the speed was increased.

Heart rate of each participant was monitored continuously by a heart monitor (Polar 810i, Finland) during the sessions and was recorded at the end of each 3 min and 4 min period to ensure that exercise target heart rate attained during the session.

All exercise sessions performed in the morning between 10:00 and 12:00 AM and were accompanied by a cardiologist and exercise physiologist. Since five treadmills were available in the sport center. Therefore, participants performed the exercise sessions in groups of 5.

3.3.5. Dietary Assessment

All subjects were instructed to maintain their usual diet during the study. However, to assess any change in nutrient intake throughout the study, participants completed two 1-day food records (on the days just before the pre and post training blood sample collections) and two 3-day food records in the 5^{th} and 9^{th} weeks of HIIT program. For the 3-day food record participants was recorded food and beverage intake on 2 weekdays and one weekend day. Food records were analyzed by a nutritionist using a dietary analysis software (the Food Processor dietary analysis version 07. 0.6, 2010, E-hakim Research, IR) to determine average daily intake of total energy (kcal), carbohydrate, protein, fat, and calcium intake (Appendix 7).

3.3.6. Blood Sampling and Assays

Blood samples were collected to determine plasma TNF-α, IL-1α, adiponectin, leptin, CRP, osteocalcin, BALP, and CTX levels. At the baseline measurements blood samples were collected during the luteal phase of the menstruation cycle. However, upon completion of the training program blood samples collected 24 hours after the last session.

Ten milliliters of peripheral venous blood were taken from the antecubital vein using standard venipuncture methods after 12 h of overnight fast between 8:00 and 11:00 am, by a registered nurse. Samples were collected into the Ethylene diamine tetra acetate (EDTA) vial. Plasma was separated by centrifugation at 3000 rpm for 10 minutes at 20°C of room temperature. For later analysis the serum was stored at -80°C.

Blood samples were analyzed at Gavam laboratory, Department of Medical Biochemistry, by using ELISA kits. Assays were performed in duplicate according to the manufacturer's instructions. Assay protocols for TNF-α, IL-1α, leptin, adiponectin, CRP, osteocalcin, BALP, and CTX were summarized below.

TNF-α Analysis

The 96 wells plate of TNF- α enzyme linked immunosorbent assay (Elisa) kit, (product no. E0082 Hu, Shanghai, China) was used to measure the serum level of TNF-α. First 50 µl standards, 50 µl Streptavidin-HRP were added into the wells. Then 40 µl of sample, and both TNF-α antibody 10 µl and Streptavidin-HRP 50 µl were added into the wells. Then the sealing membranes were sealed and gently shaked and incubated 60 minutes at room temperature. It was diluted 30 times with distilled water. The membranes were removed carefully. 50 µl chromogen solution A and 50 µl chromogen solution B were added to each well. Then solution was gently mixed and incubated for 10 min at room temperature away from light. In order to stop the reaction, 50 µl stop solution was added into each well. Within 15 min after adding the stop solution, optical density (OD) was measured by a plate reader (Awareness-Statfax 2100, USA) under 450 nm. CVs (%) for intra and inter assays were <10 and <12, respectively, with standard assay rang of 3-900 ng/L, and sensitivity of 1.5 ng/L.

IL-1α Analysis

The 96 wells plate of IL-1α enzyme linked immunosorbent assay (Elisa) kit, (product no. E0095 Hu, Shanghai, China) was used to measure the serum level of IL-1α. First of all 50 µl standard, 50 µl streptavidin-HRP were added into the wells.

Then 40 µl sample and both 10 µl IL-1α antibody and 50 µl streptavidin-HRP were added. Afterwards the sealing membranes were sealed, gently shaked and incubated 60 minutes at room temperature. It was diluted 30 times with distilled water as standby. The membranes were removed carefully. 50 µl chromogen solution A and 50 µl chromogen solution B were added to each well. Then the solution was gently mixed, and incubated for 10 min at room temperature away from the light. Finally, 50 µl stop solution was added to each well. The optical density (OD) was measured spectrophotometrically at a wavelength of less than 450 nm for 15 min (Awareness-Statfax 2100, USA). CV (%) for intra assay was <10 and for inter assay was <12 with standard assay range of (1-400 pg/ml), sensitivity of (0.54 pg/ml).

Leptin Analysis

The 96 wells plate of Leptin enzyme linked immunosorbent assay (Elisa) kit, (Product no. ME E-0300, Germany; LDN Labor Diagnostic Nord Gmbh & Co, KG Am Elchenhain 1, 48531 Northern Germany) was used to measure the serum level of leptin. 20 µL of each calibrator, control and serum samples were pipetted into the wells in duplicate. 80 µL of the monoclonal anti-leptin-biotin conjugate was pipetted into each well. The solution was incubated on a plate shaker (approximately 200 rpm) for 1 hour at room temperature. Then the wells were washed 3 times with prepared wash buffer. Afterwards 100 µL of the streptavidin-HRP-conjugate working solution was pipetted into each well. It was incubated on a plate shaker for 30 minutes at room temperature. Then the wells were washed 3 times with prepared 300 µL wash buffer and tap the plate to ensure that it was dry. Afterwards 100 µL of TMB substrate pipetted into each well at timed intervals and the plate was incubated on a plate shaker at room temperature for 10-15 minutes. Finally, 50 µl of stopping solution was pipetted into each well and the optical density (OD) was measured spectrophotometrically (Awareness-Statfax 2100, USA) at 450 nm within 20 minutes. CVs (%) for intra and inter assays were 3.7-5.5 and 5.8-6.8, respectively, with standard assay range of (0-100 ng/ml).

Adiponectin Analysis

The 96 wells plate of adiponectin enzyme linked immunosorbent assay (Elisa) kit (Product no. E09, Germany; LDN Labor Diagnostic Nord Gmbh& Co, KG Am Elchenhain 1, Northern Germany) was used to measure serum level of adiponectin. 100 µl dilution buffer VP was added in the first wells. Subsequently 100 µl standard was added to solution. Then the wells were covered with sealing tape and the plate was incubated for 1 h at room temperature. After incubation, the contents of the wells were aspirated and the wells were washed 3 times with 250 µl wash buffer. Following the last washing step, 100 µl of the antibody-POD-conjugate AK was pipetted in each well. Then the wells were covered with sealing tape and the plate was incubated for 30 minutes at room temperature. After incubation, the wells were washed 3 times with wash buffer. 100 µl of the TMB Substrate Solution was pipetted in each well and the plate was incubated for 15 minutes at room temperature (20 - 25°C) in dark. To stop the reaction, 100 µl of stop solution was added. Finally the color reaction was measured at 450nm wavelength within 30 minutes (Awareness-Statfax 2100, USA). CV (%) for intra assay was <4.7% and for inter assay was <6.7%.

CRP Analysis

The 96 wells plate of CRP enzyme linked immunosorbent assay (Elisa) kit, (Product no. DM E-4600; LDN Labor Diagnostic Nord Gmbh& Co, KG Am Elchenhain 1, 48531 Northern Germany), was used to measure the serum level of CRP. First each well was washed 5 times by dispensing 250 µl of diluted wash buffer into each well. Then 100 µl of standard sample and controls were added into each well. Afterwards the plate was covered and incubated for 1 hour at room temperature (15-30°C). Then each well washed for 5 times again by dispensing 250 µl of diluted wash buffer. 100 µl diluted conjugate was added into each well. Then the plate was covered and incubated for 1 hour at room temperature (15-30°C). Again each well washed 5 times by dispensing 250 µl of diluted wash buffer. 100 µl of substrate was added into each well, and incubated for 10 - 20 min at the dark room (temperature was 15-30°C). Finally 100 µl of stop solution was added into each well and mixed.

The absorbance was immediately read at 450 nm (Awareness-Statfax 2100, USA). CVs (%) for intra assay and inter assay were <6.1% and 13.9%, respectively.

Bone Alkaline Phosphatase Analysis

The 96 wells plate of BALP enzyme linked immunosorbent assay (Elisa) kit (Product no. E1493Hu, Shanghai, China), was used to measure the serum level of BALP. First of all 50 µl of 5mM *p*NPP solution was added into the wells. Then 10 µl of ALP enzyme solution was added to each *p*NPP standard well. Then the solution was mixed and incubated at 25°C room temperature for 60 min away from light. To stop the reaction, 20 µl stop solution was added to the wells. Finally optical density (OD) was measured under 405 nm (Awareness-Statfax 2100, USA). CVs (%) for intra assay and inter assay were 10% and 12%, respectively, with standard assay range of 0.52 IU/L.

Osteocalcin Analysis

The 96 wells plate of Osteocalcin enzyme linked immunosorbent assay (Elisa) kit (Product no. AC-11F1, Germany; Immunodiagnosticsystem, Northern Germany), was used to measure the serum level of osteocalcin. 10 mL of conjugate diluent solution was added both the peroxidase conjugated antibody solution and the biotinylated antibody solution, and the two conjugate solutions were mixed in equal volumes. 20µL of either standard samples or unknown samples were pipetted into wells followed by 150 µL of the antibody solution. The immunostrips were covered with sealing tape and incubated for 120 minutes at 18-22°C without mixing. The immunostrips were washed 5 times manually with 300µL diluted washing buffer. 100µL of the substrate solution TMB was pipetted into each well and incubated for 15 minutes at 18-22°C in the dark without mixing. To stop color reaction, 100 µL of the stopping solution H_2SO_4 was pipetted into each well. The absorbance was measured at 450 nm wavelength within two hours (Awareness-Statfax 2100, USA). CV (%) for intra assay was <2.2% and for inter assay was <5.1% with standard assay range of 0.5-100 ng/ml.

CTX Analysis

The 96 wells plate of Carboxy terminal crosslinking telopeptides type 1 Collagen enzyme linked immunosorbent assay (Elisa) kit (Product no. AC-02F1, Germany; Immune diagnostic system, Northern Germany) was used to measure serum level of CTX. 50 µL of standards was pipetted into wells followed by 150 µL of the antibody solution. Then the immunostrips were covered with sealing tape and incubated for 120 minutes at room temperature (18-22°C) on a microtitre plate mixing apparatus (300 rpm). The immunostrips were washed 5 times manually with 300 µL diluted washing buffer 50 and diluted 1+50 in distilled water. 100 µL of the substrate solution TMB was pipetted into each well and incubated for 15 minutes at room temperature in the dark room on the mixing apparatus (300 rpm). 100 µL of the stopping solution H_2SO_4 was pipetted into each well to stop reaction. The absorbance was measured at 450 nm wavelength within two hours (Awareness-Statfax 2100, USA). CVs (%) for intra assay and inter assay were <3% and <11%, respectively.

3.4. Data Analyses

Data was presented as means and standard deviations (Mean ± SD). The Smirnov Kolmogorov test was used to test the normal distribution of data. Baseline differences between the groups were determined using student's t-test. Due to the statistical differences between the groups in some of the dependent variables (TNF- α and IL-1α) at baseline, analysis of covariance (ANCOVA) was used to correct for initial group differences. In this regard, pre-test values were used as covariate and the post – test scores were used as the outcome variables. MANCOVA was used to determine the group differences in three inflammatory cytokines (IL-1α, TNF-α, and leptin) which were found to be associated with each other according to the Bartlet analysis test. Two Factorial (group x time) Repeated measure of ANOVA was used for the analysis of energy intake variables.

Partial Eta squared (η^2) was used to calculate effect sizes for significant main effects and interactions according to Hopkins (2002) guidelines (403). For this mean, the following standards used to determine the magnitude of mean effect size: 0.2–0.6

represented a small effect size; 0.6–1.2, a medium effect size; and >1.2, a large effect size.

Pearson correlation coefficient product was used to determine the associations among the percent changes in bone turnover and inflammatory variables. All data were analyzed using SPSS statistical software (version 19.0, Chicago, Illinois, USA). All statistical analysis, significant level was accepted at $p < 0.05$.

4. RESULTS

The purpose of this study was to evaluate the effect of HIIT on inflammatory cytokines and bone turnover markers in obese women. In this chapter findings of the study on subjects' demographic variables; effectiveness of HIIT program in improving cardiorespiratory fitness and body composition; dietary intake changes throughout the study; and effects of HIIT program on pro- inflammatory cytokines (TNF-α, IL-1α, leptin,), anti-inflammatory cytokine adiponectin, bone turnover markers of (osteocalcin, BALP, and CTX), and CRP were presented.

4.1. Baseline Comparisons of the Experimental Groups

A total of 24 (BMI: 30.0- 34.99; age: 30-40 years) obese women completed baseline assessments and post-training measurements. Participants involved in this study were assigned to either HIIT group (n=14) or Control group (n=10).

Participant's characteristics at baseline with respect to experimental groups were compared by independent samples t-test. Findings on baseline comparisons of the groups in body composition, cardiorespiratory fitness, dietary intake, pro and anti-inflammatory cytokines, and bone turnover markers were presented with subheadings.

4.1.1. Baseline Demographic and Body Composition Variables

Analysis of independent samples t-test revealed that HITT and Control group were similar with respect to demographic and body composition variables at baseline ($p>0.05$) (Table 4.1).

Table 4.1. Baseline demographic and body composition variables.

	Control (n=10)	**HIIT (n=14)**		
	Mean ± SD	**Mean ± SD**	**t**	**p**
Demographic variables				
Age (years)	36.6 ± 3.34	35.57 ± 3.41	-0.734	0.471
Body composition				
Height (cm)	157.90 ± 6.98	159.86 ± 4.9	0.809	0.427
Weight (kg)	80.30 ± 7.27	82.68 ± 5.81	0.890	0.383
BMI (kg/m^2)	32.17 ± 1.46	32.36 ± 1.89	0.262	0.796
Hip circumference (cm)	109.90 ± 7.11	113.29 ± 6.24	1.237	0.229
Waist circumference (cm)	95.40 ± 6.40	99.07 ± 5.15	1.557	0.134
Waist to hip ratio	0.86 ± 0.01	0.87 ± 0.01	1.194	0.245
Fat mass (kg)	33.93 ± 3.50	35.69 ± 5.27	0.920	0.368
Body fat (%)	42.14 ± 2.57	42.20 ± 3.50	0.046	0.964
Soft lean mass (kg)	42.98 ± 6.37	44.54 ± 5.51	0.642	0.527
Soft lean percent (%)	54.27 ± 2.91	54.20 ± 3.39	-0.049	0.962

BMI, body mass index; SD, standard deviation.

BMI was greater than 30 kg/m^2, body fat percent was greater than 40% and waist to hip ratio was greater than 0.85 (Table 4.1) classifying the participants of both groups as obese and as well as abdominally obese (75).

4.1.2. Baseline Cardiorespiratory Fitness and Physiological Variables

Comparisons of baseline group differences in cardiorespiratory fitness and physiological variables were presented in (Table 4.2). According to the independent samples t-test analysis no significant differences were noted between the groups at baseline ($p>0.05$).

Table 4.2. Baseline cardiorespiratory fitness and physiological variables.

	Control (n=10)	**HIIT (n=14)**		
	Mean ± SD	**Mean ± SD**	**t**	**p**
SBP (mmHg)	116 ± 5.16	117.86 ± 4.26	0.965	0.345
DBP (mmHg)	78 ± 3.50	77.50 ± 3.80	-0.328	0.746
Resting heart rate (bpm)	83.20 ± 11.61	89.21 ± 11.62	1.250	0.224
Maximum heart rate (bpm)*	170 ± 3.77	170.78 ± 3.49	0.526	0.604
Time to exhaustion (min)	6.01 ± 2.07	6.23 ± 2.54	0.225	0.824
VO_2max (ml/kg/min)	23.04 ± 9.03	24.27 ± 11.06	0.289	0.776
RPE	16.21 ± 0.59	16.34 ± 1.63	0.236	0.816

SBP, systolic blood pressure; DBP, diastolic blood pressure; bpm, beat per minute; min, minute; VO_2max, maximal oxygen uptake; RPE, rate of perceived exertion; *Maximum heart rate attained during VO_2max test; SD, standard deviation.

SBP and DBP values of both groups were within the normal range according to the blood pressure classification. Maximum heart rate attained during the VO_2 max test was greater than 95% of the age predicted maximum heart rate indicating that participants showed their maximal effort during the test. Predicted VO_2 max values of HIIT and Control group at baseline were 24.27±11.06 and 23.04±9.03 ml/kg/min, respectively (Table 4.2), clearly indicating that the participant's cardiorespiratory fitness levels in both groups were poor for age and gender (404).

4.1.3. Baseline Inflammatory Cytokines and Bone Turnover Markers

Comparisons of baseline group differences in inflammation and bone turnover markers were presented in (Table 4.3).

Table 4.3. Baseline inflammatory cytokines and bone turnover markers.

Variables	**Control (n=10)**	**HIIT (n=14)**		
	Mean ± SD	**Mean ± SD**	**t**	**p**
IL-1α (ng/L)	3.29 ± 1.10	4.55 ± 0.43	3.900	0.001
TNF-α (ng/L)	18.45 ± 3.74	19.62 ± 11.20	0.315	0.755
CRP (ng/mL)	8.01 ± 3.32	9.37 ± 3.52	0.956	0.350
Adiponectin (μg/ml)	26.78 ± 4.78	24.05 ± 11.67	-0.696	0.494
Leptin (ng/mL)	53.61 ± 20.54	60.60 ± 18.43	0.874	0.392
BALP (IU/L)	12.06 ± 2.70	8.03 ± 2.18	-4.041	0.001
CTX (ng/mL)	0.45 ± 0.20	0.50 ± 0.23	0.610	0.548
Osteocalcin (ng/mL)	9.63 ± 4.59	7.17 ± 3.77	-1.441	0.164

IL-1α, interleukin 1 alpha; TNF-α, tumor necrosis factor alpha; CRP, C-reactive protein; BALP, bone alkaline phosphatase; CTX, C-terminal crosslinking telopeptide type 1 collagen.

With respect to inflammatory cytokines and bone turnover markers no significant differences were noted between the groups at baseline ($p>0.05$), except IL-1α and BALP ($p<0.05$). These findings showed that experimental groups were similar with respect to most of the variables measured before the training ($p>0.05$) while IL-1α was higher and BALP was lower in HIIT than control group ($p<0.05$) (Table 4.3).

4.2. Dietary Intake

Comparisons of baseline differences and changes from pre to 12 weeks post-HIIT in dietary intake between groups were presented in Table 4.4 and 4.5 respectively. According to the independent samples of t-test analysis no significant difference was noted between the groups at baseline ($p>0.05$). These findings showed that experimental groups were similar with respect to CHO, protein, fat, calcium and total energy intake before the training (Table 4.4).

Table 4.4. Baseline daily dietary intake.

	Control (n=10)	**HIIT (n=14)**		
Variables	**Mean ± SD**	**Mean ± SD**	**t**	**p**
CHO (g)	132.50 ± 15.07	141.87 ± 11.56	1.725	0.099
Protein (g)	105.70 ± 16.33	94.12 ± 39.52	-0.871	0.393
Fat (g)	87.93 ± 9.18	89.93 ± 9.91	0.500	0.622
Calcium (mg)	1042.66 ± 95.77	1005.83 ± 99.13	-0.910	0.373
TEI (kcal)	1744.27 ± 150.20	1753.34 ± 167.71	0.136	0.893

TEI, total energy intake; CHO, carbohydrate.

There was no significant difference in food intake variables within and between groups at the end of 12 weeks HIIT protocol ($p>0.05$) (Table 4.5). These results indicated that groups were similar in CHO, protein, fat, calcium, and total calorie intake at the end of the study, and no alteration was observed over the 12 weeks of HIIT intervention.

Table 4.5. Dietary intake at baseline and after HIIT intervention. Comparison of the differences in total energy intake.

Variables	Pre-training	5th week	9th week	Post-training	Group Effect		
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	F	p	η^2
CHO (g)							
Control	132.50 ± 15.07	141.10 ± 10.41	140.62 ± 23.60	146.60 ± 21.66	0.104	0.750	0.005
HIIT	141.87 ± 11.56	141.87 ± 11.14	144.28 ± 10.70	138.30 ± 10.58			
Protein (g)							
Control	105.70 ± 16.33	98.21 ± 7.10	98.41 ± 13.04	108.45 ± 17.73	0.001	0.937	0.000
HIIT	94.12 ± 39.52	106.10 ± 21.99	105.31 ± 20.81	104.55 ± 19.49			
Fat (g)							
Control	87.93 ± 9.18	87.23 ± 8.55	84.73 ± 15.65	81.62 ± 12.52	1.564	0.224	0.066
HIIT	89.93 ± 9.91	84.21 ± 13.71	78.03 ± 10.89	73.53 ± 12.66			
Calcium (mg)							
Control	1042.66 ± 95.77	1037.36 ± 157.00	1030.52 ± 47.05	1038. 53 ± 101.55	0.336	0.568	0.015
HIIT	1005.83 ± 99.13	1034.74 ± 91.60	1038.41 ± 115.10	1003.20 ± 82.36			
TEI (kcal/day)							
Control	1744.27 ± 150.20	1742. 39 ± 102.42	1718.72 ± 193.17	1754.83 ± 122.39	0.725	0.404	0.032
HIIT	1753.34 ± 167.71	1749.86 ± 141.96	1700.58 ± 108.32	1633.22 ± 132.90			

TEI, total energy intake; $\eta^{2,}$ partial eta squared.

4.3. High Intensity Interval Training

In this study a-12 week HIIT program consisting of four 4 min high intensity intervals interspersed with 3 min low intensity walking/jogging was performed 3 days per week. Each active break was consisted of walking or jogging at 55–65 % of age predicted maximum heart rate. Intensity of the exercise was set as 85–95 % of age predicted maximum heart rate for training intervals. Each exercise session began with a warm-up and cool-down sessions at subjects 55-65% of age predicted maximum heart rate.

Age predicted maximum heart rate of the participants was 174.82 ± 2.92 bpm via VO_2max test. Accordingly, target heart rate range for 85% and 95% of age predicted maximal heart rate were calculated as 148.60 ± 2.48 bpm and 166.08 ± 2.78 bpm, respectively. Analysis of HR measurements recorded during each session revealed that average training heart rate for the 4 min and 3 min periods for 12 weeks were 164.56 ± 1.39 bpm and 111.82 ± 1.64 bpm, respectively. Thus, the average training intensity for 4 min periods was 94.13 ± 0.80% and for 3 min periods was 63.96 ± 0.94% of age predicted maximum heart rate (Figure 4.1). These data clearly indicated that a true HIIT training program was implemented.

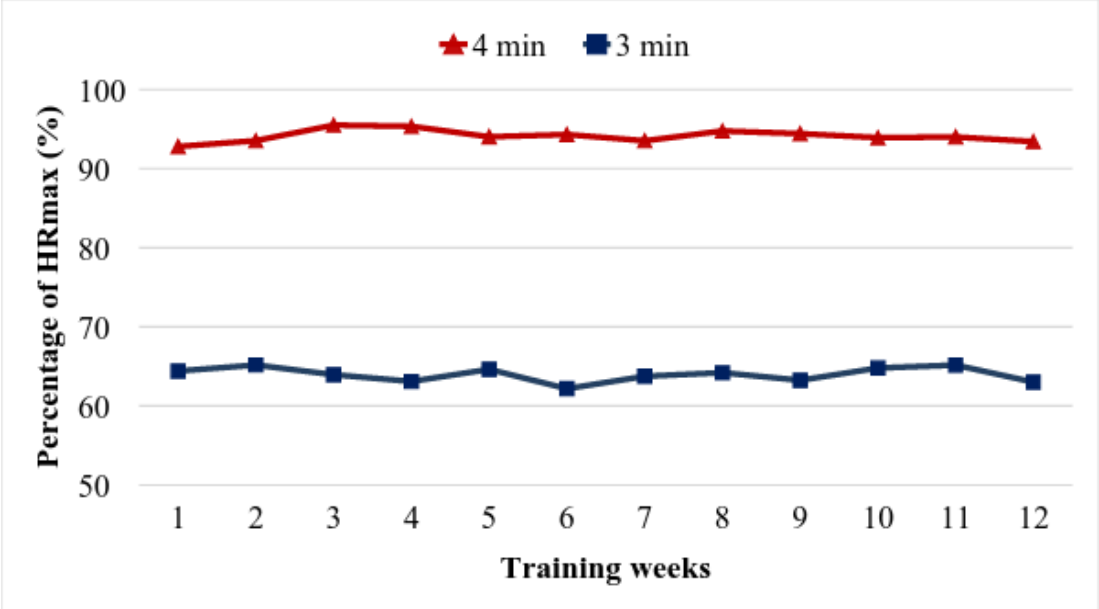

Figure 4.1. Percentage of age predicted maximum heart rate attained during HIIT protocol over 12 weeks.

Average treadmill speed was 5.6 ± 1.6 km/h (range 4-7.2 km/h). Exercise adherence to the program was quite high, for 12 participants was 100% (36 sessions), and for the remaining 2 participants was 94.44% (34 sessions).

4.4. Effects of HIIT Program

To determine the effects of high intensity interval training on body composition, cardiorespiratory fitness, inflammatory and bone turnover markers post-training values were compared by ANCOVA and MANCOVA by using pre-values as covariate. The findings were presented below under related subheadings.

4.4.1. Effect of HIIT on Body Composition

Comparisons of the differences in body composition variables from pre- to 12 weeks post HIIT protocol between groups were presented in Table 4.6.

Table 4.6. Body composition at baseline and after HIIT intervention.

Variables	**Pre-training**	**Post-training**	**Group Effect**		
	Mean ± SD	**Mean ± SD**	**F**	**P**	**η^2**
Weight (kg)					
Control	80.30 ± 7.27	81.10 ± 6.854	4.918	0.038	0.190
HIIT	82.68 ± 5.81	78.61 ± 5.241			
BMI (kg/m^2)					
Control	32.17 ± 1.46	32.63 ± 1.32	15.727	0.001	0.428
HIIT	32.36 ± 1.89	30.75 ± 1.50			
Body fat (%)					
Control	42.14 ± 2.57	42.83 ± 2.43	7.390	0.013	0.260
HIIT	42.2 ± 3.50	39.84 ± 3.70			
WC (cm)					
Control	95.40 ± 6.40	94.90 ± 2.00	7.247	0.014	0.257
HIIT	99.07 ± 5.15	95.79 ± 1.54			
HC (cm)					
Control	109.90 ± 7.11	110.20 ± 2.32	3.418	0.079	0.140
HIIT	113.29 ± 6.24	111.79 ± 1.66			
WHR					
Control	0.86 ± 0.01	0.85 ± 0.01	0.064	0.803	0.003
HIIT	0.87 ± 0.01	0.86 ± 0.01			

BMI, body mass index; WC, waist circumference; HC, Hip circumference; WHR, waist to hip ratio.

There were significant decreases in body weight [$F_{(1, 22)} = 4.918$; $p= 0.038$; $\eta^2 = 0.190$], BMI [$F_{(1, 22)} = 15.727$; $p= 0.001$; $\eta^2 = 0.428$], fat percentage [$F_{(1, 22)} = 7.390$; $p= 0.013$; $\eta^2 = 0.260$] and waist circumference [$F_{(1, 22)} = 7.247$; $p= 0.014$; $\eta^2 = 0.257$]. No significant difference was found between the groups in waist to hip ratio [$F_{(1, 22)} = 0.064$; $p= 0.803$; $\eta^2 = 0.003$] and hip circumference [$F_{(1, 22)} = 3.418$; $p= 0.079$; $\eta^2 = 0.140$] after HIIT intervention (Table 4.6). BMI and body fat percent decreased significantly from 32.36 to 30.75 kg/m^2 and 42.2% to 39.84% in HIIT group, respectively ($p < 0.05$) (Figures 4.2 and 4.3).

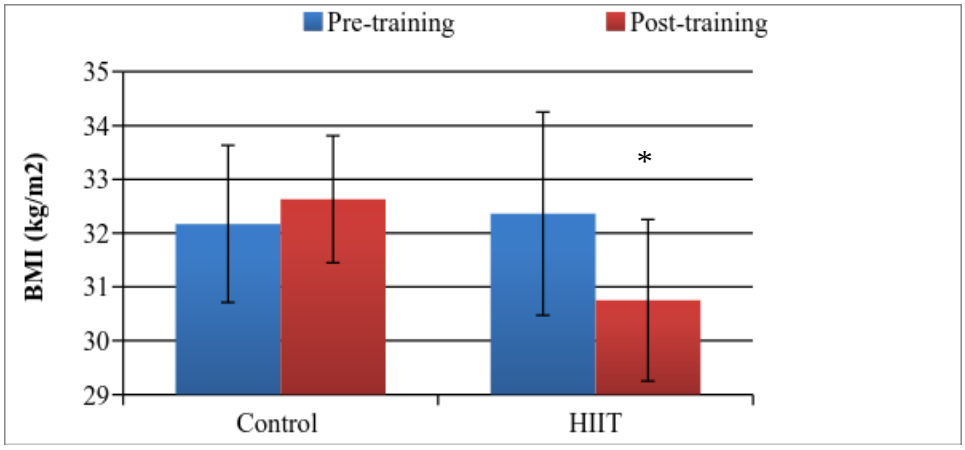

Figure 4.2. BMI at baseline and after training ($^*p<0.01$).

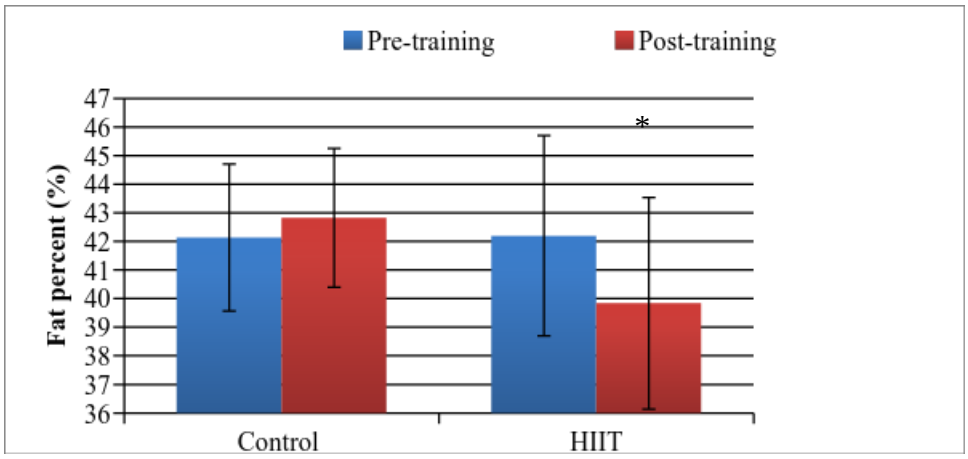

Figure 4.3. Fat percent at baseline and after training ($^*p<0.05$).

4.4.2. Effect of HIIT on Cardiorespiratory Fitness

Table 4.7. Cardiorespiratory fitness at baseline and after HIIT intervention.

Variables	Pre-training	Post-training	Group Effect		
	Mean ± SD	Mean ± SD	F	P	η^2
Resting HR (bpm)					
Control	83.2 ± 11.6	85.4 ± 0.61	25.130	0.000	0.545
HIIT	89.21 ± 11.6	87.4 ± 10.34			
Maximal HR (bpm)					
Control	170 ± 3.77	173.1 ± 2.99	98.180	0.000	0.824
HIIT	170.8 ± 3.49	164.3 ± 3.36			
Time to exhaustion (min)					
Control	6.01 ± 2.07	5.94 ± 1.63	37.610	0.000	0.642
HIIT	6.23 ± 2.55	9.23 ± 2.55			
VO_2max (ml/kg/min)					
Control	23.04 ± 9.03	22.20 ± 6.90	44.057	0.000	0.677
HIIT	24.27 ± 11.06	37.41 ± 11.07			

HR, heart rate, VO_2max, maximal oxygen consumption.

Twelve weeks of HIIT training significantly decreased maximum heart rate [$F_{(1, 22)} = 98.180$; $p = 0.000$; $\eta^2 = 0.824$] compared to control group. Although resting HR decreased in HIIT group compared baseline values it was significantly higher than that of control group [$F_{(1, 22)} = 25.130$; $p = 0.000$; $\eta^2 = 0.545$]. HIIT significantly increased both time to exhaustion [$F_{(1, 22)} = 37.610$; $p = 0.000$; $\eta^2 = 0.642$] and VO_2max level [$F_{(1, 22)} = 44.057$; $p = 0.000$; $\eta^2 = 0.677$] compared to control group (Table 4.7).

VO_2max increased significantly (from a baseline value of 24.27 ± 11.06 to 37.41 ± 11.07) in HIIT group compared to control group (22.04 ± 9.03 to 22.20 ± 6.90) ($p < 0.05$) (Figure 4.4).

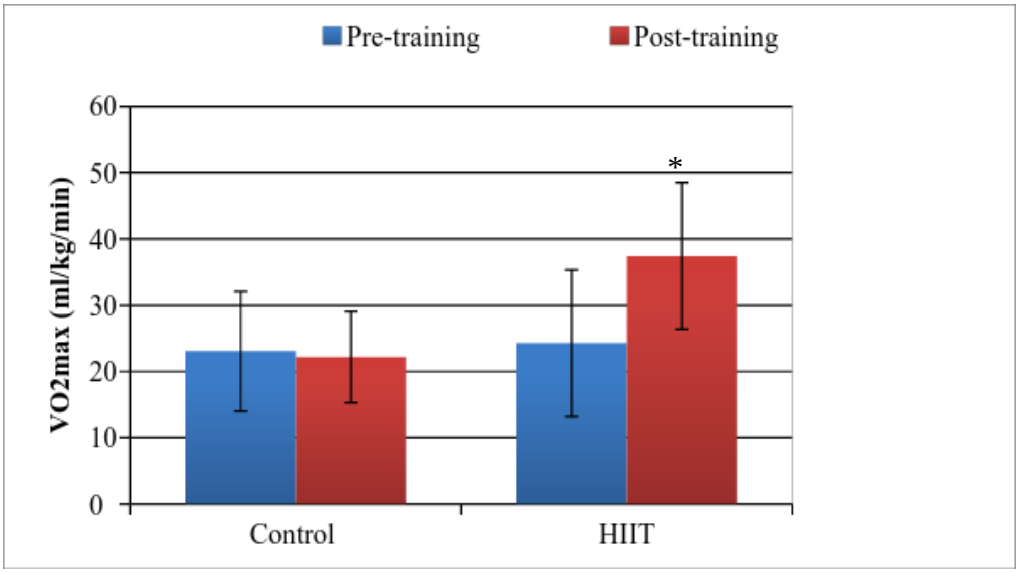

Figure 4.4. VO_2max at baseline and after training ($^{*}p<0.001$).

4.4.3. Effect of HIIT on Inflammatory Cytokines

Comparison of the differences in IL-1α, TNF-α, leptin, CRP and adiponectin from pre- to 12 weeks post-exercise between control and HIIT groups are illustrated in table 4.8.

MANCOVA analysis revealed significant decreases in IL-1α ($F_{(1, 21)}$ = 27.312, p = 0.000, η^2 =0.590), TNF-α ($F_{(1, 21)}$ = 14.125, p = 0.001, η^2 =0.426), and leptin ($F_{(1, 21)}$ = 14.818, p = 0.001, η^2 =0.438) and CRP level ($F_{(1, 21)}$ = 5.365, p = 0.031, η^2 =0.203) in HIIT group compared to control group. Moreover, ANCOVA analysis showed significant increase in adiponectin level ($F_{(1, 21)}$ = 12.275, p = 0.002, η^2 =0.369) in HIIT group compared to control group after exercise training. Magnitude of the decreases in inflammatory cytokines in HIIT group compared to baseline values for IL-1α, TNF-α, leptin and CRP were 15%, 39%, 16% and 14%, respectively. HIIT increased adiponectin level by 12%. Changes in IL-1α, TNF-α, leptin, CRP, and adiponectin from pre- to 12 weeks post-exercise in control and HIIT groups were illustrated in Figures 4.5-4.9.

Table 4.8. Inflammatory cytokines at baseline and after HIIT intervention.

Variables	Pre-training	Post-training			
	Mean ± SD	**Mean ± SD**	**F**	**P**	**η^2**
IL-1α (ng/L)					
Control	3.29 ± 1.10	4.09 ± 1.22	27.317	0.000	0.590
HIIT	4.55 ± 0.43	3.88 ± 0.52			
TNF-α (ng/L)					
Control	18.45 ± 3.74	18.75 ± 3.64	14.125	0.001	0.426
HIIT	19.62 ± 11.20	11.96 ± 3.18			
Leptin (ng/ml)					
Control	53.61 ± 20.54	61.63 ± 20.94	14.818	0.001	0.438
HIIT	60.60 ± 18.43	50.86 ± 16.42			
CRP (ng/L)					
Control	8.01 ± 3.33	8.15 ± 3.23	5.365	0.031	0.203
HIIT	9.37 ± 3.52	8.03 ± 3.60			
Adiponectin (µg/ml)					
Control	26.78 ± 4.78	26.71 ± 5.20	12.275	0.002	0.369
HIIT	24.05 ± 11.67	27.04 ± 15.30			

IL-1α: Interleukin 1 alpha, TNF-α: Tumor necrosis factor alpha, CRP: C reactive protein, η^2: partial eta squared.

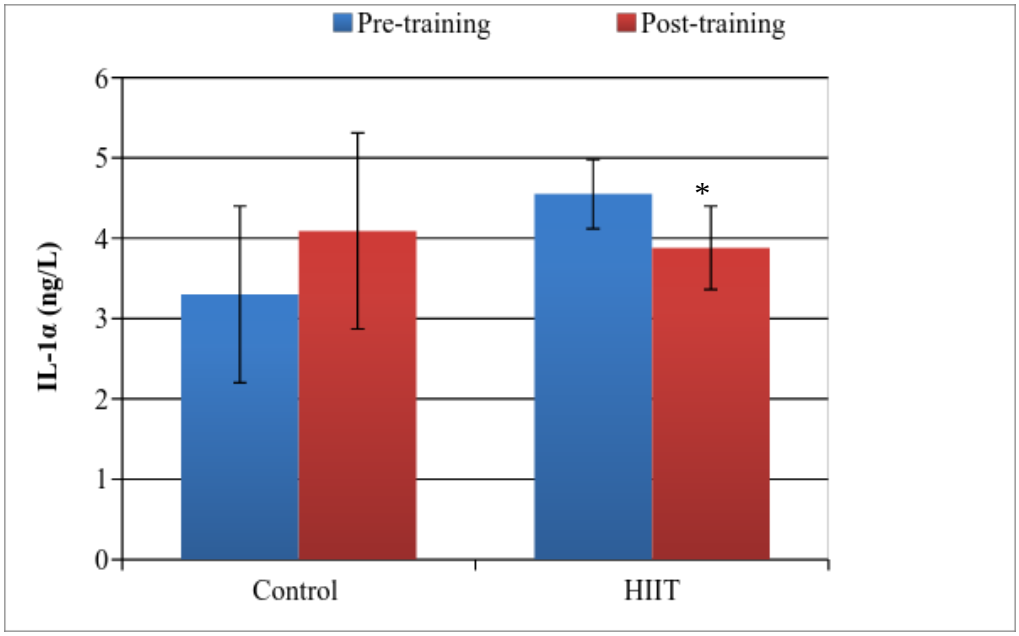

Figure 4.5. Serum levels of IL-1α at baseline and after training ($^{*}p<0.001$).

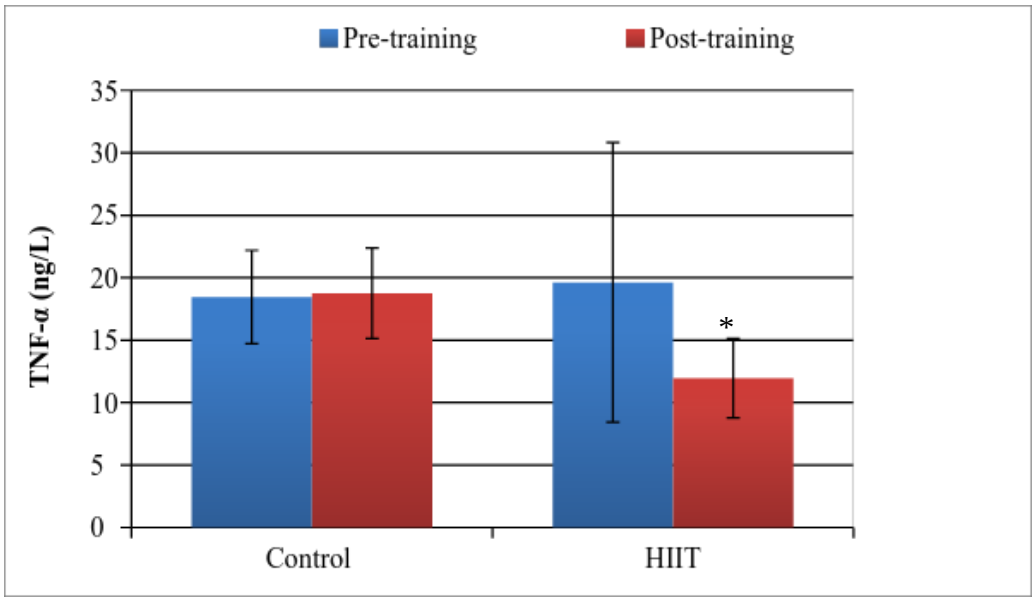

Figure 4.6. Serum levels of TNF-α at baseline and after training ($^{*}p<0.01$).

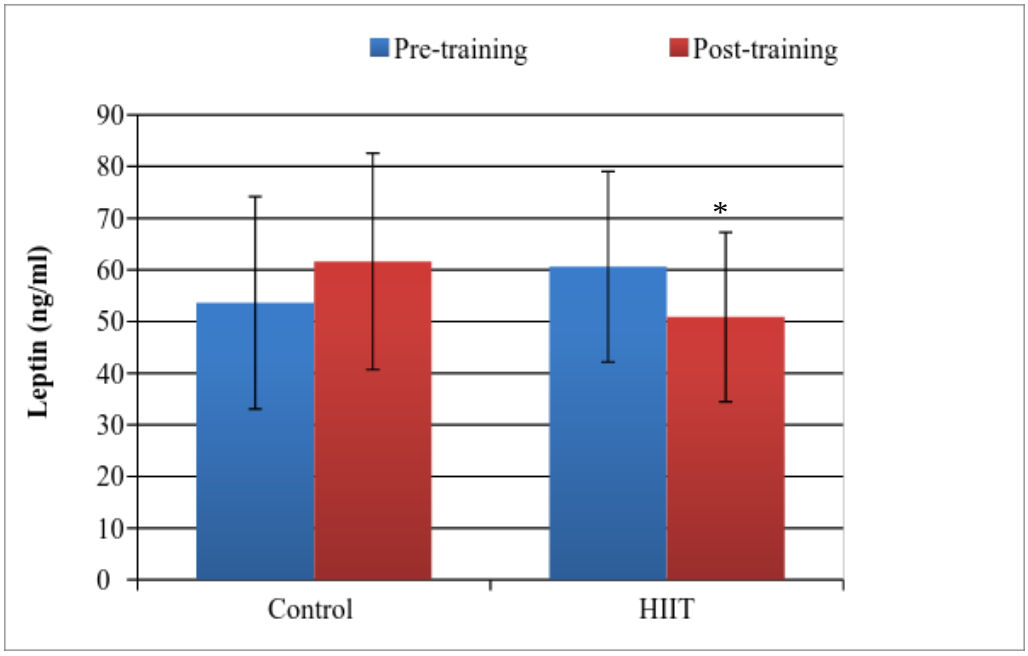

Figure 4.7. Serum levels of leptin at baseline and after training (*p<0.01).

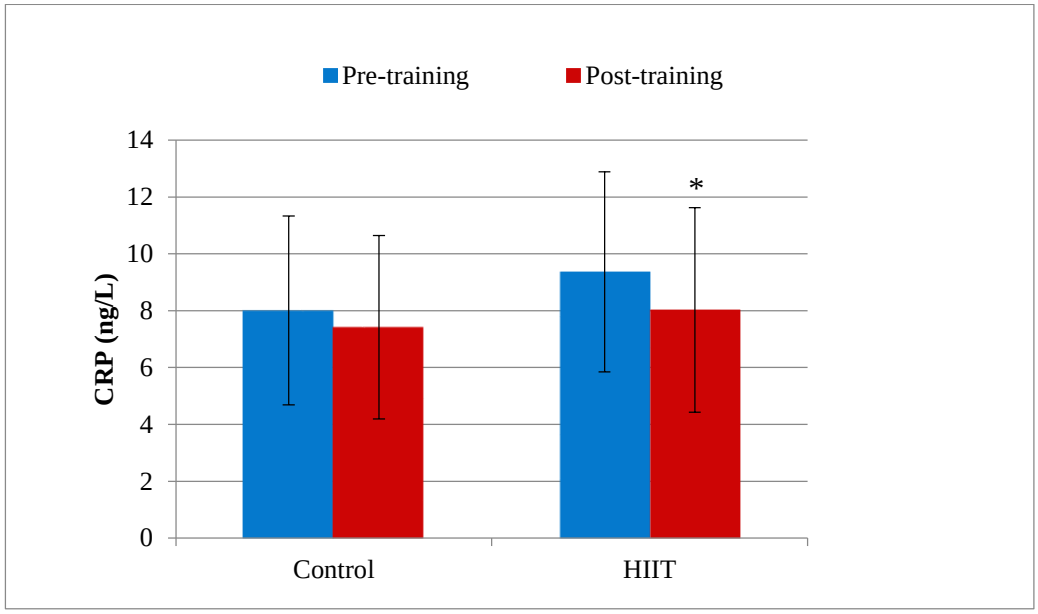

Figure 4.8. Serum levels of CRP at baseline and after training (*p<0.05).

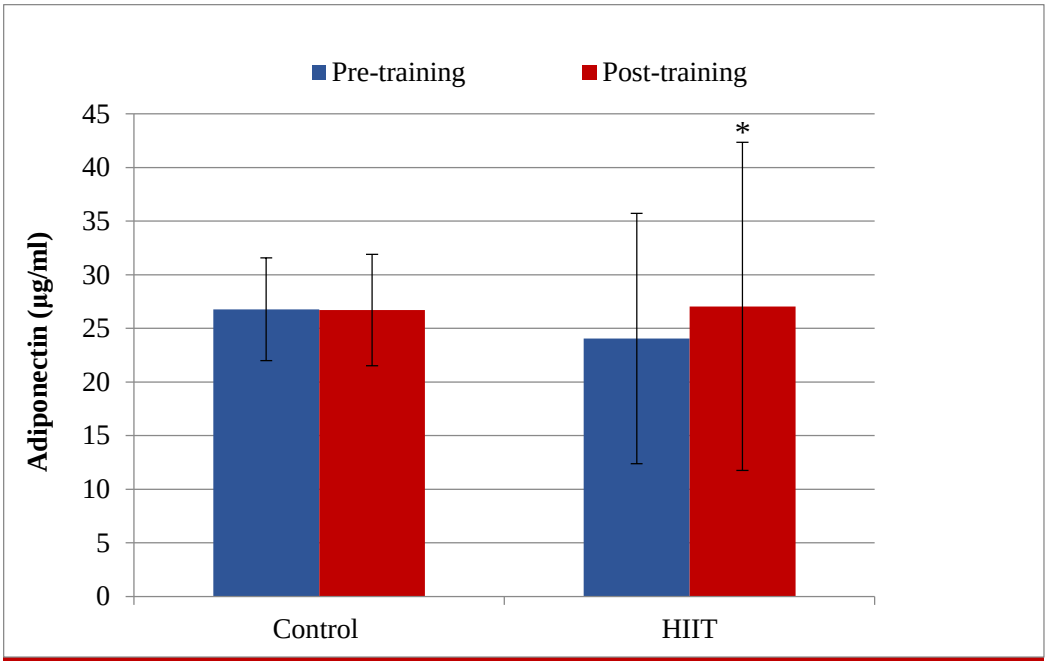

Figure 4.9. Serum levels of adiponectin at baseline and after training ($^{*}p<0.01$).

4.4.4. Effect of HIIT on Bone Turnover Markers

Concentrations of bone turnover markers in serum were different between two groups. HIIT group had higher concentration of bone formation marker for osteocalcin and lower concentration of bone resorption marker for CTX. Analysis of ANCOVA has shown statistically significant increase in osteocalcin [$F_{(1, 22)} = 8.390$, $p=0.009$, $\eta^2 =0.285$] in HIIT group than control group after exercise intervention, and statistically significant decrease in CTX [$F_{(1, 22)} = 45.908$, $p= 0.000$, $\eta^2 = 0.686$] in HIIT group compared to control group. Despite of increased in the level of BALP as a bone formation marker, the difference was not statistically significant [$F_{(1, 22)} = 3.983$, $p= 0.059$, $\eta^2 = 0.159$] between the groups. The data was presented in Table 4.9 and Figures of 4.10-4.12.

Table 4.9. Bone turnover markers at baseline and after HIIT intervention.

Variables	Pre-training	Post-training	Group Effect		
	Mean ± SD	Mean ± SD	F	P	η^2
OC (ng/ml)					
Control	9.63 ± 4.59	7.42 ± 2.72	8.039	0.009	0.285
HIIT	7.17 ± 3.77	8.81 ± 5.12			
BALP (IU/L)					
Control	12.06 ± 2.7	10.84 ± 2.00	3.983	0.059	0.159
HIIT	8.03 ± 2.18	9.57 ± 1.35			
CTX (ng/ml)					
Control	0.45 ± 0.202	0.64 ± 0.28	45.908	0.000	0.686
HIIT	0.50 ± 0.23	0.43 ± 0.19			

HIIT, high intensity interval training; OC, osteocalcin; BALP, bone alkaline phosphatase; CTX, carboxy-terminal telopeptide cross-linking type one collagen; η^2, partial eta squared. The percentages for bone markers are (Osteocalcin (22.87% increase), BALP (19.17% increase but not significant), CTX (14% decrease).

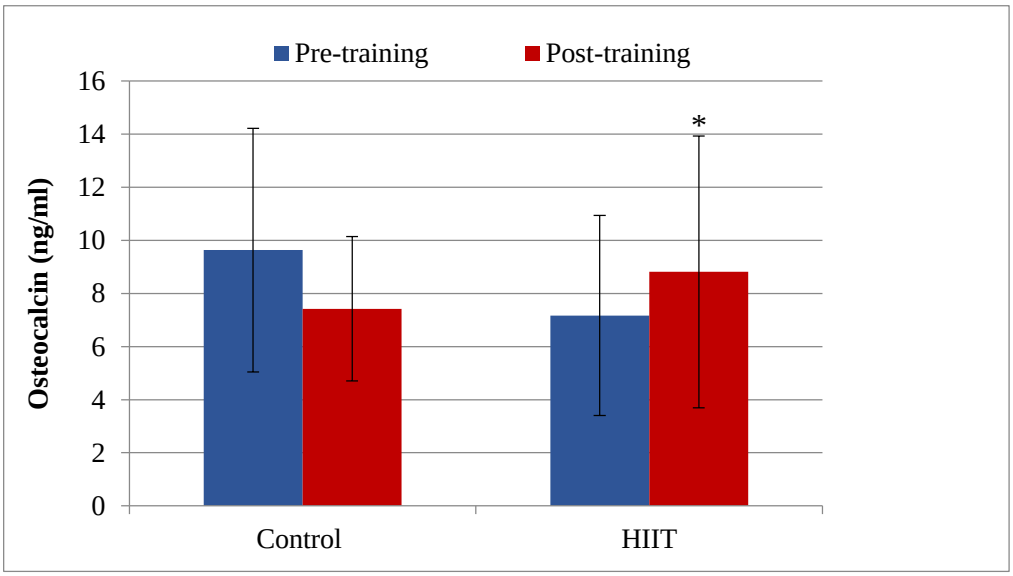

Figure 4.10. Serum levels of osteocalsin at baseline and after training ($^*p<0.01$).

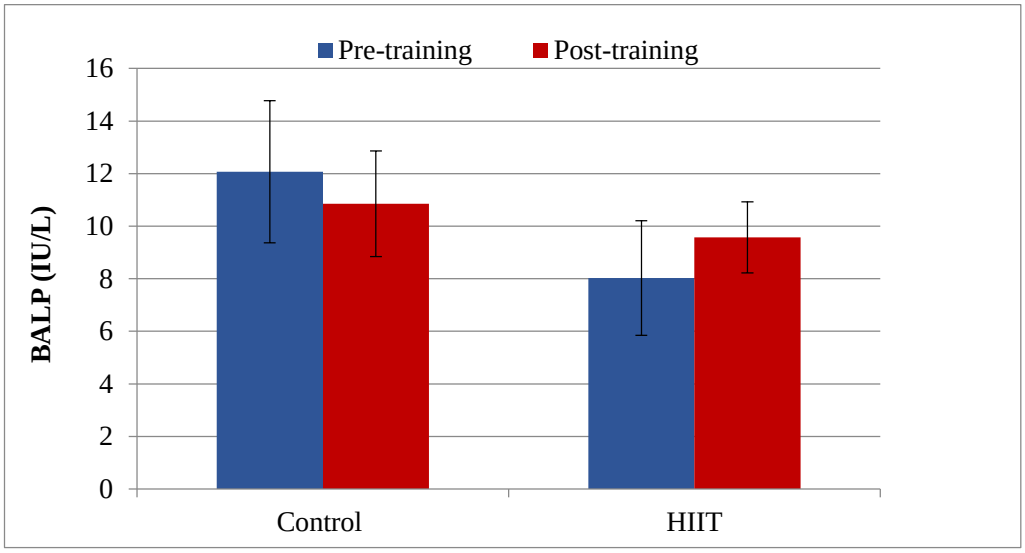

Figure 4.11. Serum levels of BALP at baseline and after training.

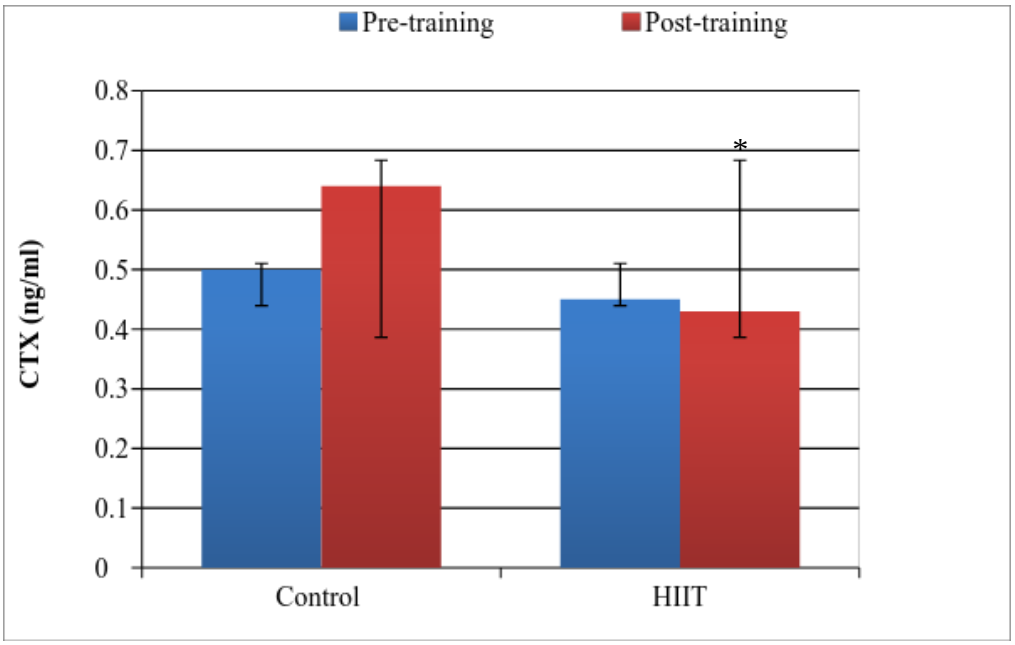

Figure 4.12. Serum levels of CTX at baseline and after training ($^{*}p<0.001$).

4.5. Relationships between Inflammatory Cytokines and Bone Turnover Markers

To determine the associations among inflammatory cytokines and bone turnover markers after HIIT intervention, Pearson correlation analysis was used for percent changes of the inflammatory cytokines and bone turnover markers. Results were presented in Table 4.10. IL-1α, TNF-α, leptin and CRP were positively associated with CTX ($p<0.001$) and negatively associated with OC (Table 4.10, $p<0.05$). Correlation between CRP and OC didn't reach statistical significance ($p>0.05$). BALP inversely associated with IL-1α, TNF-α, leptin and CRP, while only the associations with IL-1α and CRP were significant (Table 4.10, $p<0.05$). However, changes in adiponectin levels didn't correlate significantly with any bone turnover marker ($p>0.05$).

Table 4.10. Associations among the changes in inflammatory cytokines and bone turnover markers.

Variables	OC	BALP	CTX
IL-1α	-0.594**	-0.467*	0.818**
TNF-α	-0.535**	-0.385	0.594**
Leptin	-0.513*	-0.379	0.839**
CRP	-0.293	-0.642**	0.526**
Adiponectin	0.302	0.062	-0.280

*p<0.05 **p<0.001 TNF-α: tumor necrosis factor alpha, IL-1α: interleukin 1 alpha, CRP: C- reactive protein, OC: osteocalcin, BALP: bone alkaline phosphatase, CTX: carboxy-terminal crosslinking telopeptid type 1 collagen.

5. DISCUSSION

The main findings of the present study are that the 12 weeks of HIIT program in obese women increased VO_2max (54.14%); decreased body weight (4.92%), BMI (4.98%), and body fat percentages (5.59%); decreased plasma levels of inflammatory cytokines (TNF-α, IL-1α, leptin and CRP) and increased anti-inflammatory adiponectin level; decreased bone resorption marker of CTX and increased bone formation markers of osteocalcin and BALP. Furthermore, changes in inflammatory cytokines (TNF-α, IL-1α, leptin and CRP) over 12 weeks positively correlated with the changes in bone resorption marker of CTX and negatively correlated with the changes in bone formation markers (osteocalcin and BALP). All these associations were statistically significant except the correlations between CRP and osteocalcin, and between TNF-α and BALP and CTX. These findings show that 12 weeks of HIIT program was beneficial in improving cardiorespiratory fitness and body composition in obese women. Regarding with the aims of the study findings clearly indicate that HIIT program was effective in reducing inflammatory state, improving anti-inflammatory adiponectin, and improving bone formation and reducing bone resorption markers.

Although there are numerous studies reporting the beneficial effects of exercise training on either inflammatory and anti- inflammatory cytokines or bone health the interest in the interconnections among obesity, inflammation, bone health and exercise is relatively new. Therefore, studies on these interactions are very limited. Even up to now only three studies (30, 40, 42) have reported the effects of exercise training (either aerobic interval, aerobic continues, resistance or combination of aerobic and resistance exercise training) on both bone metabolism and inflammatory state in obese individuals. Two of these studies conducted on obese adolescents (40, 42), and one on overweight men and women (30). There is no study in the literature investigating the effects of HIIT on inflammatory and bone turnover markers at the same time either in lean or obese individuals. Thus, this study is unique in investigating the effects of HIIT on both the inflammatory and bone turnover markers in obese women.

Regarding the effects of HIIT program on inflammatory and anti-inflammatory cytokines there is limited studies in the literature (27, 43, 44) and only two (27, 44) were conducted on overweight or obese individuals.

Our main hypothesis was that "exercise training will improve bone turnover by reducing inflammatory state in obese women". In this regard findings of the study support our hypothesis. However, several mechanisms might have been involved in improving both bone turnover and inflammatory state. These are: independent effects of exercise training and decreased body fat percent in improving inflammatory state; independent effects of exercise in improving bone metabolism; and effect of improved inflammatory state on bone metabolism. The following section will discuss the details of the effect of 12 weeks of HIIT program on pro-inflammatory, anti-inflammatory, bone formation and resorption markers and the associations among the changes in these markers.

5.1. Experimental Design

In the present study the effects of a 12-week HIIT program on inflammatory cytokines and bone turnover markers in obese women were investigated. Twenty four participants were randomly assigned into either Control (n=10) or HIIT (n=14) groups. In order to control the effects of dietary intake on the studied variables dietary intake of the subjects were recorded 4 times throughout the study. Analysis of dietary intake data revealed that Control and HIIT groups were similar in CHO, protein, fat, calcium and total calorie intakes at the beginning and at the end of the study, and no change has occurred in dietary intake over time (Table 4.5, $p>0.05$). Moreover, these findings indicate that improvement in body composition in HIIT group was due to exercise training not due to the potential caloric restriction.

A 12-week HIIT program with long intervals was effective in improving cardiorespiratory fitness and body composition in obese women. As discussed in detail below these findings clearly indicate the effectiveness of the HIIT intervention in inducing aerobic adaptations.

Strengths of the present study are; this is the first study comparing the effects of HIIT on both inflammatory and bone turnover markers in healthy, obese women. In most of the studies examining the effects of exercise training on inflammatory cytokines one or a few inflammatory cytokines were investigated while in this study TNF-α, IL-1α, leptin, and CRP, as well as an anti-inflammatory marker adiponectin were investigated together. Furthermore, this study is the first study in reporting the chronic effect of HIIT on IL-1α and leptin.

In the present study we didn't include a dietary treatment group which would have contributed the study in discussing the effects of exercise training and fat loss on inflammatory cytokines independently. Furthermore, including a lean control and HIIT group would have allowed us to compare the responsiveness of lean and obese individuals to HIIT program with regard to inflammatory and bone turnover markers as well as compare the baseline values of cytokines and bone turnover markers.

Although initially we have planned to measure bone mineral density, bone mineral content, and body composition by dual X-ray absorptiometry (DXA) we were unable to accomplish this due to the technical error with the DXA device during the study. Therefore, we were unable to identify the baseline BMD and BMC of the participants. Since observable changes in BMD and BMC with exercise interventions require 6 to 12 months we weren't expecting any change in these parameters after HIIT program. However, baseline information on these parameters would have contributed in discussing the individual differences in bone turnover markers in response to HIIT program.

Measurement of body composition by DXA would have allowed us to determine fat distribution, visceral and subcutaneal body fat and their relation to inflammatory and bone turnover markers. It has been shown that visceral fat mass is closely associated with inflammatory cytokines not with subcutaneal fat mass. Moreover, increased visceral fat adversely affect bone metabolism but not subcutaneal fat.

In spite of these weaknesses findings of this study clearly revealed that a 12-week HIIT program was effective in decreasing inflammatory and bone resorption

markers, and in increasing anti-inflammatory adiponectin and bone formation markers of osteocalcin and BALP.

5.2. Effectiveness of HIIT Program

Twelve weeks of HIIT program (4x4 min intervals performed at 85-95% of age predicted HRmax with 3 min active rest, jogging at 55-65% of age predicted HRmax) in obese women significantly improved VO_2max by 54.4% (pre-training: 24.27±11.06 ml/kg/min vs post-training: 37.41±11.07 ml/kg/min) and time to exhaustion by 48.15%. This finding clearly indicates that HIIT program was effective in inducing aerobic adaptations of either peripheral or central. Significant decreases in both resting (2.03%) and maximal heart rate (3.81%) in HIIT group also support this finding. High increase in VO_2max and time to exhaustion can be explained by the initial lower fitness level of the participants. These findings are in agreement with Sijie and colleagues (363), reporting that HIIT protocol is more effective in improving VO_2max compared to moderate intensity continues training.

HIIT program also improved body composition by reducing body weight (4.92%), BMI (4.98%), and body fat percentages (5.59%) (Table 4.6, $p < 0.05$). These findings are in agreement with the findings in the literature reporting significant decreases in BMI and fat percentage by HIIT program in overweight or obese individuals (270, 364). Similarly, Buchan et al. reported a decrease in BMI in healthy adolescents following a 7-week HIIT program (365).

Several previous investigations indicated that HIIT increases fat oxidation by increasing metabolic enzymes such as hydroxyacyl-COA dehydrogenase (366), free fatty acid level in plasma (367), content of triacylglycerol in muscle (368) in normal and healthy individuals. Moreover, HIIT exercise has been shown to be more effective in enhancing metabolic enzyme functions and activities (366). All these peripheral adaptations as well as central adaptations by HIIT might account for the improvement in cardiorespiratory fitness and body composition in the present study. As a result, we can conclude that HIIT protocol performed in this study was effective in inducing aerobic adaptations.

5.3. The Effects of HIIT on Inflammatory and Anti- Inflammatory Cytokines

One of the aims of this study was to investigate the effects of 12 week HIIT on several inflammatory (TNF-α, IL-1α, leptin, CRP) and anti- inflammatory cytokines (adiponectin) in obese women. The results of the study showed that 12 weeks of HIIT program significantly decreased TNF-α, IL-1α, leptin and CRP levels, and increased adiponectin levels. Discussions of the findings based on the related literature were given with respect to effects of exercise and weight loss for each inflammatory and anti-inflammatory marker. Discussion has been mainly based on the human studies and circulating levels of the markers, particularly, in overweight and obese individuals. Where there is no available literature in humans, findings of the animal studies and the changes in the levels of studied markers in other tissues were also included.

5.3.1. The Effects of HIIT on TNF-α

This study showed that 12 weeks of HIIT significantly decreased TNF-α level (39.04%) in obese women compared to control group ($p<0.05$). This finding supports our hypothesis that "HIIT training will decrease TNF-α level in obese women".

There are conflicting results on the effects of exercise training on circulatory TNF-α levels in humans. Study design, intensity, duration and type of the exercise training, and subject characteristics (healthy or with chronic disease, age, obese or lean, male or female, pre or post-menopausal etc.) might be accounted for the inconsistencies.

There are limited data on the effects of HIIT program on TNF- α level (27, 43, 44) in the literature and only two (27, 44) were conducted on overweight or obese individuals. It has been shown that relatively short duration (2 weeks) of HIIT program was not successful in inducing anti-inflammatory adaptations in young recreationally active men (43) or reducing plasma TNF-α level in overweight and obese males (27). In contrast to our findings, an exercise training protocol quite similar to performed in the present study (12-week of aerobic interval training consisted of running on a treadmill (4x4 minutes at 80–90% maximal heart rate, with

3-minute recovery intervals at 55–65% maximal heart rate) didn't alter TNF- α levels in obese middle aged men (44).

In agreement with our findings others have shown that moderate intensity aerobic exercise training decreased TNF- α levels in overweight and obese women (289, 369, 370), and adolescents (285). Study by Ho et al (369) showed that aerobic exercise training, resistance training and the combination of both were effective in reducing TNF-α levels after 12 weeks compared to baseline by 20.8%, 26.9%, and 32.6%, respectively, in overweight and obese individuals. TNF-α levels in the combination group were significantly lower (−22.6%) compared to the control when adjusting for baseline levels (369). Similarly, a 10-week of treadmill exercise training program (30 to 40 min at an exercise intensity of 55-65% of peak heart rate) decreased TNF- α levels in obese young women with Down syndrome (370). Schultz et al (285) showed that 16 week of resistance training in obese adolescents decreased TNF-α concentration.

Fisher et al (284) reported that decreased TNF- α levels induced by a life style modification consisting of diet with and without exercise training, was associated with weight loss, particularly with intra-abdominal fat loss, and that exercise didn't result in further decrease in TNF- α level.

In contrast to our findings several studies reported no change in TNF- α level in obese individuals in response to either a combination of diet therapy or exercise training for 15 weeks (29), a 3-month aerobic exercise training (289) or a 14-week exercise training at either 50% or 70% maximal oxygen consumption although body fat percent decreased (371).

On the contrary of inflammation, monocyte activity may decrease (372) or unchanged (373) during exercise, low making of monocytes an unlikely contribute to the decrease plasma concentrations of TNF-α. Studies have been reported circulating of lipopolysaccharides (LPS) are decreased during exercise too (374, 375), which are known as stimulator of TNF-α. In this study we didn't measure monocytes and lipopolysaccharides concentration. Studies have revealed that muscle may damage during exercise, therefore it is normally to see accumulation of pro-inflammatory

substrates such as TNF-α in skeletal muscle due to infiltration of neutrophils (19), but doing exercise with high intensity for a long time (12 weeks) may cause to decrease infiltration due to decrease of neutrophil activity and may adaptation of muscle to exercise, therefore muscle damage may decreased at the end of exercise and provide less concentration of TNF-α. This finding is differing from other studies that report no change in TNF-α (30) with 60 min of cycling at 70% of VO_2peak. Sixty minutes of treadmill running at 75% VO_2max˙results no changes in TNF-α compared with 55% and 65% VO_2max (276).

In epidemiological study of cytokines with obesity (282) suggested that serum TNF-α, was significantly associated with the type and phase of exercise in subjects initial training level, age, sex, place and time of day for blood sampling, heredity, drug uses, and the sensitivity of assessment tools are the factors for determining production (282) that could be the reason for inconsistencies in the findings on the effect exercise training on TNF-α level in the literature.

5.3.2. The Effects of HIIT on IL-1α

IL-1α is expressed constitutively in non-hematopoietic cells under physiological conditions and its expression increases in response to hypoxia (376, 377). It is released from the damaged cell and triggers an inflammatory response (378). IL-1α increases bone metabolism by regulating osteoclast formation under both physiological and pathological conditions (16).

In the present study IL-1α levels decreased significantly (14.73%) after 12 weeks of HIIT program which supports our hypothesis. This is the first study reporting the effect of exercise training on plasma IL-1α levels in humans. Very recently (379) reported the acute effects of high intensity low impact (bicycle) exercise on plasma IL-1α levels in recreationally active young males. They (379) reported that IL-1α levels significantly increased 5 min after exercise and returned to the baseline levels after 24h. There is no study investigating the effect of exercise training on IL-1α levels. However, currently (368) have shown that a 16-week low intensity aerobic exercise training on treadmill (25-30 min/day, up to 55% of

HRmax, 3-4 days/week) significantly decreased IL-1β levels in postmenopausal women.

IL-1β effects bone metabolism almost very similarly with IL-1α (16). Released IL-1α from the damaged cells facilitates neutrophil recruitment to the site of injury and induces IL-1β and other cytokines and chemokines from neighboring cells (376, 377). Therefore, decreased IL-1α levels after HIIT program can be considered as an anti-inflammatory adaptive response.

5.3.3. The Effects of HIIT on Leptin

The level of leptin in the HIIT group decreased significantly (16.07%) in the present study supporting our hypothesis that "HIIT will decrease plasma leptin levels in obese women". With regard to time course responses of leptin to an acute exercise session Essig et al, (380) showed that leptin concentrations did not change soon after or 24 h after a single exercise session performed at an intensity of 70% VO_2max but it decreased 30% after 48 hours. Thus a decreased leptin level in the present study is due to the adaptation not because of the last exercise session.

The findings from the present study are consistent with those reported in the literature. Gutin (381) demonstrated that plasma leptin concentrations in obese children decreased after a 4-month exercise program (40 min/day for 5 days a week) and then increased after the next 4 months of detraining. He concluded that leptin levels reflect changes in energy balance. Similarly, two months aerobic training program with moderate intensity decreased leptin levels in obese adolescent boys (382). Another study (383) found that 12 weeks of regular aerobic training decreased the potential risk of coronary heart disease by improving plasma levels of adiponectin, leptin and CRP.

The effects of exercise training on leptin levels are shown to be mediated by the sympatho-adrenergic system (384, 385). Changes in serum levels of leptin depends on exercise intensity and volume, and the amount of energy expended (218) as well as baseline levels. In this respect, Fatourose and coworkers (311) observed a significantly greater reduction in leptin in the HIIT group compared with the low and

moderate intensity exercise group, even following adjusting for body mass index and sum of skinfold (311). Exercise intensity in the present study was high which could have resulted in high energy expenditure and in the long term adaptation with decreased leptin levels.

Weight loss and/or body fat reduction is another mechanism responsible for the changes in leptin and adiponectin following exercise training (209). An inverse associate has been demonstrated between body composition indices (weight, BMI, waist circumference, waist-to-hip ratio and fat mass) and adiponectin, and a positive association was observed between these factors and leptin (386, 387).

As leptin is mainly synthesized and released by adipose tissue, and the plasma leptin levels are associated positively with body fat mass (388) the decrease in serum levels of leptin in the exercise group at the present study was likely to be related to the decrease in fat percent, body weight and BMI. Furthermore, since leptin levels are positively correlated with TNF-α level, decrease in TNF-α level in the present study might be another reason for decreased leptin levels. On the other hand, long-term changes in lifestyle consisting of decreased intake of dietary fat and increased physical activity reduced plasma leptin concentrations in humans beyond the reduction expected as a result of changes in fat mass (389) indicating independent effects of life style changes.

5.3.4. The Effects of HIIT on Adiponectin

Plasma adiponectin level significantly increased in HIIT group (12.43%) supporting our hypothesis. This finding is in agreement with the current literature reporting that exercise training increases adiponectin levels in humans (382, 383, 386, 390). Furthermore, short-term exercise training increased circulating adiponectin levels which is accompanied with improved insulin sensitivity in overweight males (391). Similarly, significant increases in circulating adiponectin and a trend for reduced leptin levels (35% decrease) were reported following 12 weeks of combined endurance and resistance exercise training with intensity of 60%–70% of maximum heart rate in elderly (392). Increased blood adiponectin levels were also found after flexibility and aerobic exercise intervention (393, 394).

Although studies on the effect of HIIT on adiponectin levels are rare Racil et al (351) demonstrated a decrease in adiponectin levels in obese adolescent females following HIIT protocol. Moreover, this study showed that better modifications in plasma adiponectin levels were attained by high compared to moderate exercise training intensity. Adiponectin enhances fat oxidation and skeletal muscle glucose uptake (395). Therefore, higher circulating adiponectin during high intensity exercise need more adiponectin to regulate metabolic fluxes (396).

It was suggested that weight loss induced by exercise training might be the main mechanism for increases in adiponectin levels (390). Indeed, decreases in body weight and fat result in a significant increase in adiponectin gene expression (315). In a review (397) it was noted that training longer than 2 months that employs enough exercise volume (frequency, intensity, and duration) which decreases body weight increases adiponectin levels. On the contrary, plasma adiponectin level didn't change despite reduced abdominal fat and increased insulin sensitivity without a significant alteration in body weight after 8 weeks exercise intervention in 16 middle-aged men with T2DM (398).

However, there are numerous studies in the literature reporting no change after a single exercise session or exercise training in healthy lean or overweight/obese individuals as summarized in a review by Golbidi et al (388). They suggested that in interpreting the findings in the literature it is important to consider the intensity, duration and the volume of the exercise program, characteristics of the participants (obese, lean, age, healthy or with chronic disease), and the form of measured adiponectin (total or multimers) (388). HIIT program in the present study performed at high intensity (94.13% of HRmax) for 4 min long intervals intercepted with active rest periods at 64% of HRmax, 3 times week for 12 weeks. Therefore, the intensity and the volume of exercise were high. Additionally, participants of the study were obese and body fat percent, body weight, and BMI decreased after HIIT program. All these factors may explain increased adiponectin levels in our participants.

5.3.5. The Effects of HIIT on CRP

In the present study, performing HIIT exercise in obese women significantly decreased CRP levels (14.30%) compared to baseline values supporting our hypothesis.

There are inconsistent results regarding the effects of exercise training on CRP levels in healthy or diseased conditions, in lean or obese individuals, reporting either no change or significant decreases. Randomized trials of combined exercise and dietary interventions reduced CRP levels (399-401). However, independent effects of exercise on inflammatory cytokines are not clear (402-405). A meta-analysis study indicated a non-significant 3% decrease in CRP from five studies with aerobic exercise interventions of eight weeks or longer (405). In this regard, a 12-week lifestyle and dietary intervention program decreased CRP levels by 30% in obese adolescents (393). In addition, both resistance and aerobic exercise training for 10 weeks reduced CRP levels by 32.8% ($p=0.05$) and 16.1% ($p=0.06$), respectively (406) (452).

In contrast several other studies have reported no significant changes in CRP level in response to training (407). In obese girls 12 week of aerobic exercise training didn't change adiponectin or CRP levels although a significant decrease in both BMI and body weight (408). Similarly, another meta-analysis study examining the effects of aerobic exercise on CRP levels in adults 18 years and older showed that although significant improvements in body composition and VO_2max were noted, CRP levels didn't change across a variety of patient and training program characteristics (405).

Differences between studies may be due to lack of congruity between training modalities. The effects of HIIT on CRP and other inflammatory cytokines are similarly conflicting in obese intervention studies (409). Several reasons may impede fundamental deductions made in human training studies. CRP level may be changed by a variation of potential confounding variables including tobacco and environmental smoke exposure, recent dietary intake variables, sex hormones, sleep duration, and exposure to viruses, bacteria, and environmental toxins (410). Moreover it seems that differences in exercise intensity, duration, weight loss, health

status, diet, heredity, and dietary supplementations are the critical issues on inflammatory concentrations, especially in CRP.

5.3.6. Mechanisms Responsible for Improved Inflammatory State after HIIT

Regular exercise training causes many changes in skeletal muscle, adipose tissues, endothelial cells, and immune cells which can be accounted for the reduction in pro-inflammatory cytokines and increase in anti-inflammatory cytokines. The mechanisms of changes are summarized below:

Regular exercise training increases the level of IL-6 cascade from skeletal muscle as an anti-inflammatory myokines, which increases the secretion of anti-inflammatory cytokine like IL-10 and IL-1ra in circulation. So the systemic inflammation decreases after acute exercise training due to an increase of IL-6, anti-inflammatory cytokines, and decrease of pro-inflammatory cytokine such as TNF-α in circulation (93). Furthermore increases in the production of IL-6 as an anti-inflammatory cytokine in skeletal muscle may cause to decrease the secretion of IL-6 from adipose tissue as a pro-inflammatory cytokine which is potent factor to production of CRP in liver (411). Due to the reduced release of IL-6 from adipose tissue after regular exercise intervention results in decreased CRP production. Consequently, all these mechanisms are involved in decreased systemic inflammation (412).

The other mechanisms that is involved in reducing inflammation after regular exercise training, is the considerable changes in adipose tissue functions. Regular exercise training increases angiogenesis and blood flow, decreases hypoxia and vasoconstriction in adipose tissue, as well as reduces the production of pro-inflammatory cytokines by macrophages (289, 412). Furthermore, angiogenesis in adipose tissue as well as decreased adipocyte size improves blood flow and thus decrease hypoxia (413). All these mechanisms are responsible for decreased systemic inflammation after regular exercise training in obese individuals. With respect to HIIT protocol in the present study, decrease in fat percent and weight loss might result in improved macrophage functions. As a result, pro-inflammatory cytokine production by macrophages, such as TNF-α, was reduced (414, 415).

Damage occurred in endothelial cells causes to infiltrate variety of tissues. There is mechanism that involved in decreasing of systemic inflammation in obesity with consider of endothelial cells. Regular exercise training causes to changes in endothelial cells by two mechanisms. First; increasing of progenitor endothelial cells derived from bone marrow which they can differentiate into endothelial cells (416). Exercise promotes vascular ability to regenerate endothelial cells after damage. Consequently decreases vascular wall inflammation and systemic inflammation in obese individuals. And second; increased blood flow in tissues leads to a decrease in the capacity of endothelial cells to produce adhesion molecules (417). These effects of exercise training decreases the migration of leukocytes into vessel wall and consequently it reduces inflammation in obese individuals (418).

Exercise training changes pro and anti-inflammatory cytokines in individuals by effect on toll-like cell surface. Reduce of the Toll-like 4 receptor activation caused to decreases secretion of pro-inflammatory monocytes (419). Chronic exercise also reduces the number of pro-inflammatory monocytes in order to decrease the production of pro- inflammatory cytokines in individuals. Ten percent of monocytes are pro-inflammatory monocytes, but they are the main producer of pro-inflammatory cytokines in body. Furthermore this effect of exercise decreases the secretion of CRP and TNF-α in lean and obese persons (93). Exercise increases the number of T cells producing anti-inflammatory cytokines such as IL-10 in lean and obese persons.

In summary, in the present study HIIT program might have affected skeletal muscle, adipose tissue, endothelium cells, and immune cells in the same mechanisms explained above and decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines.

5.4. Effects of HIIT on bone turnover markers OC, BALP, and CTX

High intensity interval training increased OC by 22.87% and decreased CTX by 14% ($p<0.05$). In addition, BALP level increased (19.18%) non-significantly ($p=0.06$) in HIIT group. In this study we failed to assess BMD and BMC. Therefore we can’t associate the changes in bone turnover markers with the changes in BMD or

BMC. However, since detectable changes in BMD requires longer durations (6 to 12 months) and bone markers react to exercise training more rapidly (420) changes in bone turnover markers in the present study adequate to determine the effectiveness of HIIT program on bone metabolism.

In the literature there is only one study (379) investigating the effects of HIIT on bone turnover markers and cytokines. However, they used a low impact high intensity exercise and determined the post exercise effects of a single session in male university students (379). They have shown that bone formation markers of BALP and osteoprotegerin (OPG) and bone resorption marker of RANKL significantly increased from baseline to 5 min after exercise by 10.9%, 13.5%, and 34.2%, respectively. Only BALP remained significantly higher 1h and 24h after exercise. Bone resorption marker of NTX was significantly lower 24h after exercise. They also reported significant increases in IL-1α, IL-1β, IL-6, and TNF-α 5 min after exercise, which returned to baseline, levels 1h after exercise. The post-exercise changes in bone formation markers correlated positively with the anti-inflammatory cytokine (IL-10) and negatively with the pro-inflammatory cytokines while NTX correlated positively with a pro-inflammatory cytokine, TNF-α. In the present study we found significant increase in bone formation marker of osteocalcin and a non-significant increase in BALP. Additionally, bone resorption marker of CXT decreased significantly. We also found significant correlations among bone turnover markers and inflammatory cytokines. In the present study IL-1α, TNF-α, leptin and CRP were positively associated with CTX ($p<0.001$) and negatively associated with OC ($p<0.05$). Correlation between CRP and OC didn't reach statistical significance. Inverse associations were found between BALP and IL-1α, CRP, TNF-α and leptin while only the associations to IL-1α and CRP were significant. Findings of both Mezil's (379) study and ours suggest that HIIT elicits favorable changes in both bone turnover markers and inflammatory cytokines with regard to bone health.

Marques and colleagues (30) investigated the effects of 32 weeks of exercise intervention including both resistance and weight-bearing impact exercise on BMD and serum levels of bone metabolism and inflammatory cytokines in older adults. They (379) reported a decrease in hs-CRP and IFN-g levels while no change in TNF-

α levels both in men and women. IL-6 levels decreased only in men. No change has been reported in bone metabolism markers of OC, CTX, OPG, and RANKL either in women or men. In contrast, Lester et al. (421) found significant increases BALP and OC following a 8-week resistance training alone and combined resistance and aerobic training. Additionally, they Lester et al., (421) reported significant increases in volumetric and areal BMD in the aerobic and combined groups, respectively.

Evans et al. (422) investigated the effects of a 4-month military program including running, marching, jumping, battle drills, walking and standing for prolonged periods of time on 153 premenopausal women and P1NP, sCTx, PTH, Ca2+, and 25-OH Vitamin D levels were measured. CTX increased from baseline to 2 months and decreased from 2 to 4 months. These findings provide some evidence for the ability of CTX to increase with exercise training, and these changes occurred in the first 2 months of training. We also found increased CTX after 3 months of HIIT. In line with our findings Fujimura et al. (423) also reported increases in serum OC and BALP, but in contrast to our finding they found no change in CTX after 4 months resistance training.

Shah and colleagues (10) determined the independent and combined effects of weight loss and exercise training on bone metabolism in relation to BMD in obese older adults. CTX and OC concentrations increased in the diet group (31% and 24%, respectively) while they decreased in the exercise group (−13% and −15%). No change was observed in serum CTX and OC levels in the diet-exercise group. They (10) concluded that inclusion of exercise training to weight-loss program in obese older adults prevents weight-loss-induced increase in bone turnover and attenuates weight loss- induced reduction in hip BMD.

Villareal and colleagues (424) reported the effects of weight loss therapy composed of diet and exercise on bone metabolism and mass in obese older adults. Total hip BMD (-2.4%), CTX (101%), OC (66%), leptin (-30%) and estradiol (-14%) changed significantly in treatment group compared to control group. Changes in weight, bone markers, and leptin correlated with changes in hip BMD. Kim et al (332) reported significant elevation in serum OC level after 8 weeks aerobic exercise intervention in obese young men.

Current literature proposes several mechanisms for improvements in bone turnover markers in response in the literature. In terms of increased bone formation markers exercise intensity, increased muscle cross-sectional area and increased extracellular fluid within bone are the proposed mechanisms. Indeed, exercise intensity has been found to be positively associated with serum BALP and OC (423, 425). Increased muscle cross-sectional area due to resistance training stimulates skeletal re-modeling. In addition dynamic loading of bone increases extracellular fluid within the bone which triggers new bone formation both in trabecular and cortical bones (423). On the other site, bone resorption markers such as CTX in decrease following resistance exercise training. It has been proposed that adaptation of bone resorption markers to exercise may be mediated by age, sex, or exercise intensity (426).

Whether HIIT protocol could stimulate these processes is unknown. It is hypothesized that increased levels of several hormones, such as 1.25 dihydroxy vitamin D and PTH, are associated with increased osteocalcin and BALP after exercise training (423, 427). Literature reports that concentration of 1.25 dihydroxy vitamin D increases during exercise intervention thus it may increase the expression of bone formation markers while immobilization suppresses its activity (428).

5.4.1. Mechanisms Responsible for Improved Bone Turnover Markers

Several mechanisms are involved in bone turnover markers alteration after regular exercise training. First; acute response of inflammatory cytokines (429) such as TNF-α and IL-1 and bone turnover markers to exercise is raising the concentration of these markers in circulation immediately after exercise (376, 430). The mechanism is increases the production of IL-6 from muscle in response to muscle contraction and liver and adipose tissue cause to elevate of the level of IL-1 and TNF-α which both of them are act on muscles. IL-6 cause to up-regulation the receptors of IL-1 antagonist IL-1ra and soluble TNF-α which they prevent the pro- inflammatory effects of TNF -a and IL-1 (376).

The next mechanism is related to activity of RANKL and RANK bindings with each other and also OPG functions. While RANKL binds to its receptor on

osteoclasts surface it leads to increase the osteoclast activation for bones resorption, however OPG acts as a decoy receptor for RANKL (431). Therefor when the concentration of inflammatory cytokines such as TNF-α and IL-1α decrease after exercise training the secretion of RANKL and OPG increased in osteoblast cells. This process caused to decrease the RANKL binding to RANK on osteoclast. And then bone resorption become reduces. Regular exercise was increased the production of OPG on osteoblast cells, and OPG plays an inhibitor role on RANKL to bind RANK on osteoclast cells, consequently they lead to blunted bone resorption and increased bone formation (376).

5.5. Relationship among Inflammatory Cytokines and Bone Turnover Markers

Findings of this study showed that IL-1α, TNF-α, leptin and CRP were positively associated with CTX ($p<0.001$) and negatively associated with OC ($p<0.05$). While the association between CRP and OC didn't reach statistical significance. Moreover, BALP inversely and significantly associated with IL-1α and CRP. Non-significant correlations were also observed between BALP and TNF-α and leptin.

In the present study bone resorption marker of CTX was positively associated with IL-1α, TNF-α, leptin and CRP ($p<0.05$). All these cytokines known to be pro-inflammatory and are associated with mainly bone resorption. HIIT significantly reduced all these inflammatory cytokines and CTX indicating an association between exercises induced improvement in inflammatory state and reduced bone resorption.

It is proposed that decrease in leptin level directly alters bone remodeling affecting both osteoblastic and osteoclastic activity (432). It has dual functions in that leptin inhibits bone formation through a hypothalamic relay and promotes bone formation through actions in the periphery (433). This suggests that decrease in leptin concentration may decrease bone resorption. Decrease in leptin concentration in plasma prevents osteoclastogenesis and increase osteoblastic differentiation and proliferation of stromal cells in human and lead to mineralization of matrix (434).

Contradictory findings have been reported in the literature. In obese children higher leptin levels led to more advanced bone maturation than non-obese children of similar pubertal stage (435). An inverse association between bone resorption (CTX levels) and serum leptin levels at baseline was reported in response to weight loss and weight regain. In menopausal women circulating leptin exerts its bone protective effect by both limiting the excessive bone resorption and promoting bone formation (434).

These contradictory findings are clearly related to dual functions of leptin on bone metabolism mediated by its peripheral and central actions. Leptin is supposed to exert positive effects on bone formation by its peripheral actions, while it is believed to decrease bone formation via a central control mechanism by binding to its specific receptors located on the hypothalamic nuclei (436). Although circulating leptin may contribute to maintenance of bone mass these effects may occur independent of bone biochemical markers (437). Supporting this no correlation was found between leptin and bone turnover markers in Chinese adolescent dancers or control group. In one more study, Blain (438) has reported that serum leptin level was positively associated with body weight, fat mass, BMI, E2, and BALP level and inversely correlated with urine CTX.

There is limited data regarding the relationship between adiponectin and bone turnover markers in humans. Increased adiponectin levels are associated with increased bone formation markers. Supporting these findings *in vitro* studies have shown that adiponectin stimulates differentiation and proliferation of human osteoblasts (439). Furthermore, recent in vivo studies showed that osteocalcin leads to increased adiponectin expression in adipocytes suggesting an association between bone and energy metabolism (121). Supporting this finding, bone formation rate was slower in obese women, as measured by the biochemical marker propeptide of type I collagen, indicating that an increase in body fat may suppress new collagen formation (440).

On the basis of these studies, it has been hypothesized that an increase in adiponectin levels should have beneficial effects on bone health (144). In the present study we found a non-significant inverse association between the changes in

adiponectin and CTX levels. Additionally, adiponectin levels increased significantly in the present study. These findings indicate a potential association between increased adiponectin levels and decreased bone resorption. Indeed adiponectin was found to be associated with a decrease in osteoclast numbers and NTX serum levels (441). However, several studies (442, 443) reported a positive association between adiponectin and CTX in women suggesting that circulating adiponectin can stimulate bone resorption and thus affect bone mass negatively.

Actually, adiponectin has multifunctional properties and a dual effect on bone metabolism. It both stimulates osteoblast formation and osteoclast RANKL pathway by inhibiting its decoy receptor, OPG (444). RANKL is a potent stimulus for bone resorption and osteoprotegerin has been shown to prevent RANKL-induced bone loss (444) (491). Thus, the negative effect of adiponectin on bone metabolism might be related to promotion of bone-resorbing RANKL pathway. Misra (445) showed that increased adiponectin levels were associated with a decrease in BMD and were positively associated with serum OC (442).

Contradictory to these findings indicating an inverse association between adiponectin and bone metabolism, others have reported that adiponectin promotes human osteoblast proliferation and differentiation (439, 444, 446). In this regard, it has been reported that adiponectin increased bone mass by suppressing osteoclastogenesis and stimulating osteoblastogenesis (442). A more complex effect of adiponectin was found in adiponectin-null mice and mice overexpressing adiponectin in the liver (447) who had normal bones. Based on the findings of this study authors suggested three distinct actions of adiponectin on bone formation which two actions were positive through the autocrine/paracrine pathway by locally produced adiponectin in indirect pathway by circulating adiponectin enhancing insulin signaling. The third action was negative and was exerted through the direct pathway by circulating adiponectin. Although the mechanism mediating adiponectin action on bone turnover remains controversial in the present study we found a positive correlation between adiponectin and CTX.

6. CONCLUSION and RECOMMENDATIONS

Conclusion

This study showed that the 12 weeks of HIIT program in obese women increased VO_2max (54.14%); decreased body weight (4.92%), BMI (4.98%), and body fat percentages (5.59%); decreased plasma levels of pro-inflammatory cytokines (TNF-α, IL-1α, leptin and CRP) and increased anti-inflammatory cytokine adiponectin; decreased bone resorption marker of CTX and increased bone formation markers of osteocalcin and BALP. In addition, changes in pro-inflammatory cytokines (TNF-α, IL-1α, leptin and CRP) over 12 weeks positively associated with the changes in bone resorption marker of CTX and negatively associated with the changes in bone formation markers (osteocalcin and BALP). All these associations were statistically significant except the correlations between CRP and osteocalcin, and between TNF-α, and BALP and CTX. These findings indicate that 12 weeks of HIIT program was beneficial in improving cardiorespiratory fitness and body composition in obese women. Moreover, HIIT program was effective in reducing pro-inflammatory cytokines, increasing anti-inflammatory adiponectin, and improving bone formation and reducing bone resorption markers. Consequently, the results of this study suggest that HIIT is an effective way to improve both inflammatory state and bone health in obese women.

Recommendations

1. It is worth to determine the effects of HIIT on inflammatory cytokines and bone turnover markers in different populations with high risk including elderly, post-menopausal women, individuals with sarcopenic obesity and osteosarcopenic obesity.
2. It is recommended to determine the effects of low impact exercise (i.e. bicycle, swimming) on inflammatory cytokines and bone turnover markers in whom high impact exercise is contraindicated. For example, individuals with type 2 diabetes, elderly, osteoporosis and other inflammatory diseases.
3. Inclusion of lean control and lean exercise groups would allow the comparison of the responsiveness of obese and lean groups to HIIT with regard to bone turnover, and inflammatory and anti-inflammatory cytokines.
4. Determination of bone mineral density is recommended since bone turnover responses to exercise training may vary according to baseline BMD.
5. Determination of visceral and abdominal fat percent is recommended to associate the changes in both inflammatory cytokines and bone turnover markers with the changes in subcutaneal, abdominal and visceral fat percent separately.
6. It would be interesting to determining the effects of HIIT on the other inflammatory and bone turnover markers in overweight, obese, and healthy population in the future investigations.

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APPENDIX 1

Medical Screening Questioner

Name: ……. Date of birth: …… Phone No: …

E- Mail: ……. Single □ Married □

Please indicate if any of these statements apply to you by marking **YES** or **NO** in the space provided (*past or current):

Statements	**YES**	**NO**
1. History of heart problem (chest pain, heart murmur or stroke)	□	□
2. Diabetes mellitus	□	□
3. Asthma, breathing, or lung problems	□	□
4. Allergies	□	□
5. Cancer (other than skin)	□	□
6. Seizures, seizure medication, neurological problems, dizziness	□	□
7. High blood pressure	□	□
8. Do you have any musculoskeletal problem	□	□
9. Have you had any bone fracture or orthopedic problem within the last year	□	□
10. Recent surgery (last 12 months)	□	□
11. Hernia or any condition that may be aggravated by exercise	□	□
12. Physician's advice not to exercise	□	□
13. History of high cholesterol	□	□
14. Do you have normal menstrual cycle	□	□
15. Do you have any hormonal imbalance	□	□
16. Are you on a regular medication for any chronic disease	□	□
17. Have you ever used any medication known to affect bone metabolism	□	□
18. Do you smoke or have ever smoked	□	□
19. Did you experienced any change more than 5% of your normal body weight during the last year?	□	□
20. Do you participate in regular physical activity more than 2 times a week?	□	□

APPENDIX 2

Physical Activity Readiness Questionnaire (PAR-Q)

Name: …….. Date:……....

Weight (kg):…….. Height (cm):…… Age: ……

Physicians name: …… Phone: …….

Questions	Yes	No
1. Has your doctor ever said that you have a heart condition and that you should only perform physical activity recommended by a doctor?	□	□
2. Do you feel pain in your chest when you perform physical activity?	□	□
3. In the past month, have you had chest pain when you were not performing any physical activity?	□	□
4. Do you lose your balance because of dizziness or do you ever lose consciousness?	□	□
5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?	□	□
6. Is your doctor currently prescribing any medication for your blood pressure or for a heart condition?	□	□
7. Do you know of any other reason why you should not engage in physical activity?	□	□

If you have answered "Yes" to one or more of the above questions, consult your physician before engaging in physical activity. Tell your physician which questions you answered "Yes" to. After a medical evaluation, seek advice from your physician on what type of activity is suitable for your current condition.

APPENDIX 3

Informed Consent Form for Exercise Group

Dear participant

You are being invited to participate in a research study conducted by Assistant Professor Dr. Şükran Nazan Koşar in Faculty of Sport Sciences, Hacettepe University, Associate Professor Dr. Bakhtyar Tartibiyan in Urmia University and Masoumeh Alizadeh.

Obesity is a prevalent epidemic problem over the world, and it is accompanied with chronic low grade inflammation which is associated with several diseases such as diabetes type 2, cardiovascular disease, and bone disorders. Although obesity has long been considered as a protective factor for bone health due to its mechanical loading effect, current findings suggests that increased body fat may have deleterious effects on bone metabolism by decreasing bone formation and increasing bone resorption by several mechanisms including increased inflammatory state. Therefore, countermeasures targeted to reduce body fat percent and improve inflammatory status may improve bone health in obese individuals.

In this regard, exercise training as a non-pharmacological approach known reduce body fat percentage and improve inflammatory state. Therefore, the purpose of this study is to evaluate the effects of high intensity interval training on inflammatory state and bone turnover markers in obese women. The results of this study will be published in an academic journal. We hope that the findings of this study is to be useful to verify the effect of exercise training on inflammatory cytokines and bone turnover markers in obese women.

If you agree to participate in this study, you will participate in 12 weeks of high intensity interval training (HIIT). First, your medical history will be taken and you will undergo a medical examination. You are required to visit laboratory twice for pre- and post-training measurements. Pre- and post-training evaluations include anthropometric measurements (body weight, height, waist and hip circumferences, and body fat percentage), heart rate, cardiorespiratory fitness test, and collection of blood samples which will be performed within the one week before and after the training program. However, the post-training blood samples will be collected 24 hours after the last exercise session. Pre and post-tests will last about 1.5 hours in the laboratory center for each of tests. At least one week prior to these tests, you will be asked to avoid participation in exercise and consumption of alcohol and caffeine. Blood samples analysis and measurement of body composition will be carried out at Gavam laboratory.

Each time 10ml blood will be drawn from your antecubital vein by using a small gauge needle to assess biochemical changes in inflammatory cytokines and bone turnover markers. This procedure will be done by certified physician's assistant.

You will be asked to take a quick urine pregnant test and to confirm that you are not pregnant. Body composition will be assessed using bioelectrical impedance (BIA) two times for baseline and at the end of study period 12 weeks.

Upon completion of the pre-tests you will participate in 12 weeks of HIIT, 3 times/week, under direct supervision of exercise physiologist. HIIT protocol lasts approixmately 40 minutes and consists of 10 minutes warm up with 55-65% HRmax, 4 repetition of 4 minutes HIIT program on treadmill (85-95% HR max) and 3 minutes of active rest at the end of each 4 min, and lastly 5 minutes cooling down with 55-65% HRmax on treadmill. During the 12 weeks of exercise training, you will be asked to refrain from making any significant change in your diet and physical activity level.

You will be asked to record your daily dietary intake four times (totally for 8 days). Please consume the same diet in the first and last day of dietary record which will be performed just prior to blood sampling.

All information obtained during the study is strictly confidential and your anonymity will be protected at all times. All data will be available for you and researchers. This information will be used only for educational and research purposes. During this study, the privacy of your information will be approached with great care and respect.

The results will not be reported immediately since getting the results does not provide any benefit to you. However if you wish, you will be informed about the results of your tests at the end of study. There will be no costs for participating in the research. You will not be paid for your participation either.

Potential Risks Discomfort

The methods used for the determination of your waist and hip circumference as well as body fat percentage have not any risk for you. Measurement of maximal oxygen uptake tests will be performed on a treadmill. The degree of difficulty on the treadmill test will be increased every 3 minutes interval and test will be continued until exhaustion. Your maximal effort during determination of maximal oxygen uptake is extremely important for the reliability of the findings obtained from the study.

During the test, you may feel tiredness, increase in your heart rate, breathless and sweating. However, these are normal body responses given to exercise. In addition, during the test, heart rate, blood pressure, ratings of perceived exertion will

be monitored and recorded. Test will be performed under direct supervision of a cardiologist. You may feel tired or even exhausted after the test.

During blood sampling, you may feel temporary pain due to needling. The needle prick can be painful and temporary burning can be felt. Somewhat nausea and fainting are also possible when drawing blood. Blood sampling will be performed in a hygienic manner. The material used for others will not be used in any way for you.

During the exercise training, exercise intensity will be monitored through heart rate monitor placed around your chest. During each exercise session, your heart rate and breathing frequency will increase and you will sweat. You may feel tired at the end of the training sessions. However, these are normal physiological responses given to exercise. Sore muscle and joint stiffness may be seen at the initial stages of the HIIT protocol intervention. The optimal form to do HIIT protocol to reduce muscle skeletal injuries during training will be explained to you by the investigator.

With regard to the above-mentioned notification, these are potential risks that may be experienced during participation in this study. However, all precautions will be taken to minimize possible problems by the preliminary examination and constant surveillance during tests and training sessions. Participation in this study is completely voluntary. You can refuse to participate in this study. If you even decide to participate, you can withdraw from the research at any time. If you choose not to participate or to withdrawal from the study at any time, there will be no penalty for you.

Participant / Patient's Declaration

I have been given sufficient information about the study conducted by principal researcher Assistant Professor Dr. Ş. Nazan Koşar and Associate Professor Dr. Bakhtyar Tartibian and Masoumeh Alizadeh (Num:+09143469281). After obtaining this information, I will be invited to participate in this study.

If I participate in this study, I am sure that all my information obtained during the course of this study will be kept confidential. I have got enough confidence that my personal information will be protected during the use of research results for educational and scientific purposes.

I can withdraw from this study at any time without making excuses. However, in order not to cause any problem for research process, I will be expected to inform to researchers that I will be withdrawing from the study.

In case of injuring from participation in the study, first aid will be provided. If additional medical care is necessary, payment will be provided by researchers.

Principal Researcher	**Assistant Researcher**	**Assistant Researcher**
Assist. Prof. Dr.	Phd Student	Assoc. Prof.
Ş. Nazan Koşar	Masoumeh Alizadeh	Dr. Bakhtyar Tartibian
Work phone: 2976890/117	Work phone:	Work phone:
Mobile: 05387612924	+09143469281	4432753174
	Mobile: +04433449535	Mobile: 9126090551

By signing this form, I hereby agree to the following:

1. The aim of the study was explained to me.
2. My participation in this study is completely voluntary.
3. My questions were answered adequately.
4. My participation in this study is completely voluntary. I am free whether to participate in this study or not. I have not encountered any coercive behavior to participate in this research.

I have completely understood the purpose of the research and all explanations were made to me in detail. By signing this consent form, I am indicating that I agree to participate in this study. I accept the invitation for participation in this study in great satisfaction and volunteering. A signed copy of this form will be given to me.

Participant	**Interview witnesses**
Name & Surname:	Name & Surname:
Adress:	Adress:
Tel:	Tel:
Sign:	Sign:

Informed Consent Form for Control Group

Dear participant

You are being invited to participate in a research study conducted by Assistant Professor Dr. Şükran Nazan Koşar in Faculty of Sport Sciences, Hacettepe University, Associate Professor Dr. Bakhtyar Tartibiyan in Urmia University and Masoumeh Alizadeh.

Obesity is a prevalent epidemic problem over the world, and it is accompanied with chronic low grade inflammation which is associated with several diseases such as diabetes type 2, cardiovascular disease, and bone disorders. Although obesity has long been considered as a protective factor for bone health due to its mechanical loading effect, current findings suggests that increased body fat may have deleterious effects on bone metabolism by decreasing bone formation and increasing bone resorption by several mechanisms including increased inflammatory state. Therefore, countermeasures targeted to reduce body fat percent and improve inflammatory status may improve bone health in obese individuals.

In this regard, exercise training as a non-pharmacological approach known reduce body fat percentage and improve inflammatory state. Therefore, the purpose of this study is to evaluate the effects of high intensity interval training on inflammatory state and bone turnover markers in obese women. The results of this study will be published in an academic journal. We hope that the findings of this study is to be useful to verify the effect of exercise training on inflammatory cytokines and bone turnover markers in obese women.

If you agree to participate in this study, you will be assigned to control group of the experiment. Medical history will be taken and you will undergo a medical examination. You are required to visit laboratory twice for pre- and post-study measurements. Pre- and post-study evaluations include anthropometric measurements (body weight, height, waist and hip circumferences, and body fat percentage), heart rate, cardiorespiratory fitness test, and collection of blood samples which will be performed within the one week before and after the training program. However, the post-study blood samples will be collected 24 hours after the last exercise session. Pre and post-tests will last about 1.5 hours in the laboratory center for each of tests. At least one week prior to these tests, you will be asked to avoid participation in exercise and consumption of alcohol and caffeine. Blood samples analysis and measurement of body composition will be carried out at Gavam laboratory.

Each time 10ml blood will be drawn from your antecubital vein by using a small gauge needle to assess biochemical changes in inflammatory cytokines and bone turnover markers. This procedure will be done by certified physician’s assistant.

You will be asked to take a quick urine pregnant test and to confirm that you are not pregnant. Body composition will be assessed using bioelectrical impedance (BIA) two times for baseline and at the end of study period 12 weeks.

Upon completion of the pre-tests you are free for 12 weeks. However, during this period you will be asked to refrain from making any significant change in your diet and physical activity level. You will be asked to record your daily dietary intake four times (totally for 8 days). Please consume the same diet in the first and last day of dietary record which will be performed just prior to blood sampling.

All information obtained during the study is strictly confidential and your anonymity will be protected at all times. All data will be available for you and researchers. This information will be used only for educational and research purposes. During this study, the privacy of your information will be approached with great care and respect.

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Assist. Prof. Dr.	Phd Student	Assoc. Prof.
Ş. Nazan Koşar	Masoumeh Alizadeh	Dr. Bakhtyar Tartibian
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1. The aim of the study was explained to me.
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I have completely understood the purpose of the research and all explanations were made to me in detail. By signing this consent form, I am indicating that I agree to participate in this study. I accept the invitation for participation in this study in great satisfaction and volunteering. A signed copy of this form will be given to me.

Participant	**Interview witnesses**
Name & Surname:	Name & Surname:
Adress:	Adress:
Tel:	Tel:
Sign:	Sign:

APPENDIX 4

Ethical Approval (English)

Urmia University of Medical Sciences
and Health Services
1986

6 December, 2014

Dear Dr. S.Nazan Kosar

Re: Ethical Committee Approval

This is to certify that the Ethic Committee in Biomedical Researches of Urmia University of Medical Sciences has considered the thesis of Ms. Masumeh Alizadeh Osalou Ph.D. student in faculty of Sports Sciences & Technology of Hacettepe University entitled: **Effects of high intensity interval training (HIIT) on inflammatory and bone turnover markers in obese adult**" in session No.135 on 12/8/2014. Finally the thesis has been approved by this committee with Ethical Code No.umsu.rec.1393.122

Sincerely,

Iraj Mohebbi, M.D
Vice Chancellor for Research Affairs

INTERNATIONAL AFFAIRS
ESTABLISHED 1986

Djahad Ave, P.O.Box 1138 Urmia, Iran 57147-83734 Tel: (441) 32222010-32232296
E-mail: research@umsu.ac.ir Fax: 32221841

APPENDIX 4

Ethical Approval (Persian)

بسمه تعالی

شماره: ۶/۹۳/۴/۱۰۳۰۷۵پ

تاریخ: ۱۳۹۳/۸/۱۹

پیوست:

دانشگاه علوم پزشکی و خدمات بهداشتی درمانی استان آذربایجان غربی

سرکار خانم معصومه علیزاده

با سلام.

با کمال احترام به استحضار می‌رساند موضوع پایان نامه دوره PHD تکنولوژی ورزشی سرکار عالی در دانشگاه حاجت تپه ترکیه تحت عنوان **"اثر تمرینات خیلی شدید اینتروال بر نشانگرهای التهابی و ترمیم وتجزیه استخوان بزرگسالان چاق"** در جلسه شماره ۱۳۵ مورخ ۹۳/۵/۲۱ کمیته منطقه‌ای اخلاق در پژوهش‌های علوم پزشکی دانشگاه علوم پزشکی استان آذربایجان غربی (ارومیه) بررسی وضمن انجام کلیه اصلاحات درخواست شده از مجری محترم با کد umsu.rec.1393.122 مورد تصویب قرار گرفته است.

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این نامه بدون مهر دبیرخانه فاقد اعتبار می باشد.

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کد پستی: ۵۷۱۴۷-۸۳۷۳۴ صندوق پستی: ۱۱۳۸ نمابر: ۳۲۲۲۹۰۵۹ تلفن: ۳۲۲۳۴۸۹۷
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APPENDIX 5

Bruce Protocol Data Collection Form

Name: …… Date: …….

Age (years): …… Height (cm): …… Weight (kg): ……..

Age-predicted HR_{max}: ……. beat/min; Equation: (206 – (0.88 × age))

90% of age-predicted HR_{max}: …….. beat/min

Resting HR: …….. beat/min Maximal HR: ……..beat/min

Resting BP: …… mmHg

Time to exhaustion (min): ……. $VO_{2\ max}$ (ml/mg/min): ……

Time (min)	**Speed** (mph)	**Grade** (%)	**HR** (bpm)	**RPE**
1	1.7	10		
2				
3				*
4	2.5	12		
5				
6				*
7	3.4	14		
8				
9				*
10	4.2	16		
11				
12				*
13	5.0	18		
14				
15				*

*Where RPE recorded

VO_2max equation for women: VO_2max (ml/kg/min) = 4.38 x T-3.9, where T is time to exhaustion. APMHR: Age predicted maximum heart rate

APPENDIX 6

Borg's Rate of Perceived Exertion Scale

Borg's Scale	Perceived Exertion
6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

APPENDIX 7

24-Hour Diet Recall Form

Name: …… **Date**: …… **Weight (kg):** ……

Instructions:

Please maintain your usual diet during the study. In order to assess any change in your nutrient intake throughout the study we kindly ask you to complete two 1-day food records (on the days just before the pre and post training blood sample collections) and two 3-day food records in the 5th and 9th weeks of the study. For the 3-day food record please fill out the dietary record form on 2 weekdays and one weekend day. Please specify the day of your record by checking the corresponding day listed below.

□ Saturday □ Sunday □ Monday □ Tuesday □ Wednesday □ Thursday □ Friday

Time	Place	Meal	Food/Beverage Item	Ingredients/ Preparation	Amount