IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 13, Issue 8 Ver. II (Aug. 2014), PP 15-22
www.iosrjournals.org

Leptin and Adiponectin Increase MMP-9 and MMP-13 Secretion and PPAR-γ Expression in IL-1β-induced Chondrocyte

Handono Kalim[1], Rena Normasari[2], Sri Fauziyah[3], Ardani Galih[4], Rasjad Indra[4], Putra Suryana[1]

[1](*Department of Internal Medicine, Saiful Anwar Hospital, Faculty of Medicine, Brawijaya University, Malang, Indonesia)*
[2](*Department of Pathological Anatomy, Faculty of Medicine, Jember University, Jember, Indonesia)*
[3](*Department of Physiology, Faculty of Medicine, Islamic University of Malang, Malang, Indonesia)*
[4] *(Department of Physiology, Faculty of Medicine, Brawijaya University, Malang, Indonesia)*

Abstract: *Obesity is a major risk factor for OA. Adipose tissue, which is abundant in individuals with obesity, is an active endocrine organ that secretes cytokines and other molecules, including adipokines, such as leptin and adiponectin. Osteoarthritis is the most common chronic musculoskeletal disorders. The disease is characterized by the breakdown of cartilage matrix. MMP-9 and MMP-13 are biomarkers in matrix degradation in osteoarthritis. Until now there is still no therapy to stop the progression of osteoarthritis. Recent studies suggest that activation of PPAR-γ is an interesting target for the disease. PPAR-γ is a transcription factor that is also expressed in chondrocytes. The aim of this study is to determine the effects of leptin and adiponectin on MMP-9 and MMP-13 secretion and PPAR-γ expression by chondrocytes. This study used chondrocyte cell line induced with IL-1β then exposed to leptin and adiponectin. MMP-9 and MMP-13 were measured using ELISA meanwhile PPAR-γ expression was measured by real time PCR. Leptin and adiponectin exposure increased MMP-9 and MMP-13 significantly, and in contrast they are also increased PPAR-γ expression in all groups. This results show that leptin and adiponectin have a role in the pathogenesis of osteoarthritis by increasing catabolic response of chondrocyte, but the mechanism of increased PPAR-γ expression still needs further research. The regulation of PPAR-γ by adipokines seems complex in that it varied in relation to dose in vivo, and may be impacted by in vitro vs. in vivo circumstances.*

Keywords: *adiponectin, leptin, MMP-9, MMP-13, PPAR-γ*

I. Introduction

Osteoarthritis is the most common chronic musculoskeletal disorders and the most common form of arthritis. The disease is characterized by the breakdown of cartilage matrix, where chondrocytes is the only cell in cartilage responsible for the synthesis and degradation of cartilage matrix. In early osteoarthritis, chondrocytes induced by cytokines such as IL-1 and TNFα derived from synovial cells or macrophages. IL-1 is a potent pro-inflammatory cytokine which is able to stimulate chondrocytes to synthesize more IL-1 and other proinflammatory cytokines such as IL-6 and synthesize degradative enzymes MMP[1].

IL-1 suppress the synthesis of type II collagen and proteoglycans, and inhibits proliferation of chondrocytes stimulated by transforming growth factor-β (TGF-β). Induction of IL-1 activates several transcription factors, such as NF-κB, AP-1, c-jun N-terminal kinase (JNK) and p38 MAPK. NFκB activation induces the transcription of several target genes involved in inflammation and the immune system, cell proliferation, cell cycle, and apoptosis. NFκB activation also induces several MMP genes, such as MMP-9 and MMP-13 [2,3,4].

Obesity is one of several risk factors of osteoarthritis. Adipose tissue, which is abundant in individuals with obesity, is an active endocrine organ that secretes cytokines and other molecules, including adipokines, such as leptin and adiponectin. Leptin, adiponectin, and their receptors have been identified in human chondrocytes. This hormone is associated with increased catabolic factors involved in the progression of osteoarthritis joints and cartilage destruction. Adiponectin seems to have a role as proinflammatory agents involved in joint cartilage matrix degradation. Therefore, leptin and adiponectin, considered to affect the pathogenesis of osteoarthritis [5,6,7].

Until now, there is still no therapy to stop the progression of osteoarthritis. Recent studies suggest that activation of PPAR-γ (peroxisome proliferator-activated receptor gamma) is an interesting target for the disease.

PPAR-γ is a transcription factor that is also expressed in chondrocytes. Isoforms of PPAR-γ1 expression is decreased in osteoarthritis compared to normal cartilage. These findings suggest that the decreased expression of PPAR-γ in osteoarthritis cartilage reflects an increase in the expression of inflammatory and the catabolic factors[1].

II. Material and Methods

2.1 Chondrocyte cell culture

Chondrocytes cell line were obtained from NHAC-Kn, Lonza. Cell line were thawed in Chondrocyte basal media supplemented with R3-IGF1, bFGF, transferrin, insulin, FBS, and gentamicin/amphotericin-B. Cells were then seeded in 25cm^2 flask at a density of 10.000 cells/cm^2. The cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2, and the medium was changed once a week. The confluent cells were dispersed by trypsinization and were collected by centrifugation at 200g for 5 min. The cells were resuspended in alginate solution, aspirated into syringe, and released dropwise into 30ml polymerization solution. The cells will entrapped into alginate bead. Cells were fed every 2 to 3 days with differentiation media supplemented for 2 -3 weeks. At the end of incubation cells induced with IL-1β for 24 hours continued with exposure of leptin or adiponectin for 18 – 24 hours.

2.2 RNA isolation and cDNA synthesis

Total RNA was extracted from confluent chondrocytes using TriReagent according to the manufacturer's procedures (Promega). RNA was recovered in DEPC-water and quantified by nano-spectrophotometry at 260 and 280 nm. Then, RNA samples were reverse-transcribed to cDNA using GoScript™ Reverse transcription System (Promega, Cat. A5000). Experimental RNA, primer and nuclease-free water were mixed and centrifuged into RNA tube. The tube preheated into 70ºC then immediately chill in ice water for 5 minutes. The reverse transcription mix was prepared by combining the following component: reaction buffer, $MgCl_2$, PCR nucleotide mix, reverse transcriptase, and nuclease-free water. The PCR conditions were as follows: annealing 25ºC for 5 minutes, 42ºC for 60 minutes, and 70ºC for 15 minutes. Then the tube immediately chilled on ice, and stored in -70ºC

2.4 Real Time PCR analysis

PPAR-γ expression levels were also determined by quantitative real-time RT-PCR using fluorescence-labeled (Light Cycler-Fast Start DNA Master SYBR Green I, Roche Applied Science) primers and LightCycler software (Roche Applied Science). The primers used were: PPAR-γ forward 5 – TGA CCA GGG AGT TCC TCA AAA – 3 and PPAR-γ reverse 5 – AGC AAA CTC AAA CTT AGG CTC CAT – 3; GAPDH forward 5 – CAG AAC ATC ATC CCT GCC TCT – 3 and GAPDH reverse 5 – GCT TGA CAA AGT GGT CGT TGA – 3. PCR conditions were: pre-incubation 95ºC 10 minutes 1 cycle; amplification 45 cycles of denaturation at 95ºC for 10 s, annealing at 56ºC for 20 s, and extension at 72ºC for 25 s; melting curve analysis 1 cycle of denaturation 95ºC 0 s, annealing 65ºC 15 s, melting 95ºC 0 s with slope 0,1ºC/s. Normalized gene expression was calculated as the ratio between PPAR-γ and glyceraldehyde-3-phosphate dehydrogenase copy number.

2.5 MMP measurement

The chondrocytes culture media was preserved for MMP-9 and MMP-13 measurement. MMP-9 were assayed using Elisa kit from R&D System, Inc. This assay employs the quantitative sandwich enzyme immunoasssay technique. A monoclonal antibody spesific for MMP-9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and MMP-9 is bound by the immobilized antibody. After washing away unbound substances, an enzyme-linked polyclonal antibody spesific for MMP-9 is added to the wells. Following a wash to remove unbound antibody-enzyme reagent, a substrate solution is added to the wells and color development in proportion to the amount of MMP-9 bound in the initial step. The color development is stopped snd the intensity of the color is measured. MMP-13 were assayed in the conditioned culture media using ELISA kits from Abnova, cat no. KA0182 according to the manufacturer's protocol.

2.6 Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences, v 16 (SPSS) using the appropriate parametric tests. The MMP-9, MMP-13, PPAR-γ level were measured and presented as mean ± SEM. The mean value was analysed by Kolmogorov Smirnov to determine the data distribution. The data distribution was normal ($p > 0,05$), then analysed by one way anova to determine the differences between groups. The differences in each groups analyzed using post hoc tukey test. The analysis of statistical correlation was performed by perason correlation test. A P value less than 0,05 was considered significant for the differences and correlation.

III. Result

The study was performed in Biomedic Laboratory, Physiology Laboratory, Parasitology Laboratory of Medical Faculty of Brawijaya University, Life Science Central Laboratory of Brawijaya University, and Science and Technology Laboratory of Islamic State University Maulana Malik Ibrahim Malang. This study used a chondrocyte cell line - Normal Human Articular Chondrocyte (Lonza), which is divided into 2 major groups: leptin group and adiponectin group, each group divided into 6 groups. Leptin group were: group 1, normal control; group 2, induced by IL-1β 10ng/ml; group 3, induced by IL-1β 10ng/ml and exposed to 100ng/ml leptin; group 4, induced by IL-1β 10ng/ml and exposed to leptin 1 µg / ml; group 5, induced by IL-1β 10ng/ml and exposed to leptin 10µg/ml; and group, induced by IL-1β 10ng/ml, leptin 10µg/ml and pioglitazone 10µM. Adiponectin group were : group 1, normal control; group 2, induced by IL-1β 10ng/ml; group 3, induced by IL-1β 10ng/ml and exposed to 100ng/ml adiponectin; group 4, induced by IL-1β 10ng/ml and exposed to adiponectin 1µg/ml; group 5, induced by IL-1β 10ng/ml and exposed to adiponectin 5µg/ml; and group 6, induced by IL-1β 10ng/ml, adiponectin 5µg/ml and pioglitazone 10µM. Cells used when reached confluency 70-80% then treated with alginate solution. The study was conducted from March to July 2014.

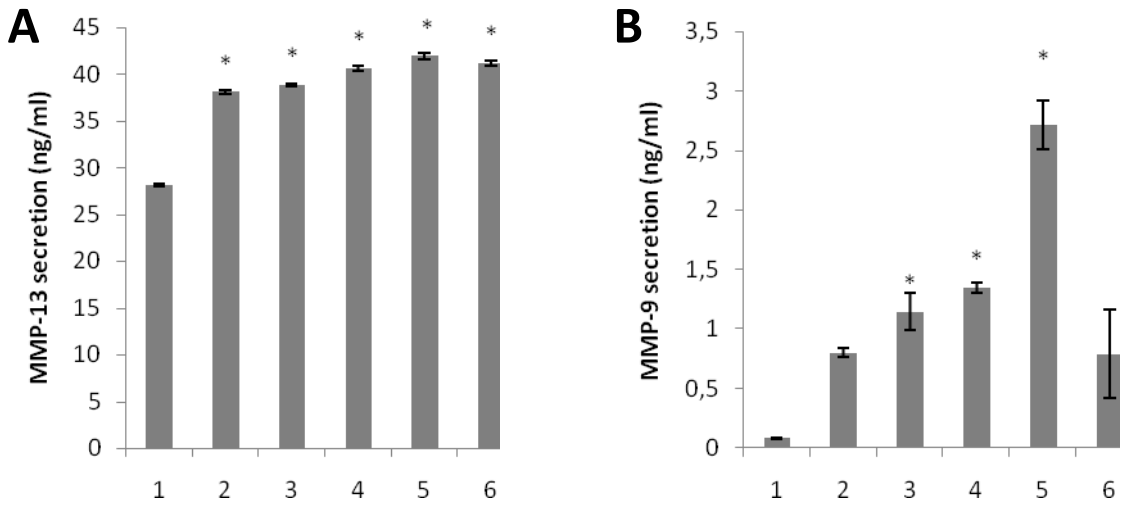

Figure 1. Effect of leptin in MMPs secretion by chondrocyte in various tratment groups. (A) Leptin increased IL-1β-induced MMP-13 secretion on chondrocytes in dose dependent manner, significant effect seen in higher dose (leptin 1µg/ml and 10µg/ml). Addition of pioglitazone slightly decrease MMP-13 secretion. **(B)** Leptin increased IL-1β- induced-MMP-9 secretion also in dose dependent manner. Significant effect at leptin dose 10µg/ml, the pioglitazone addition decreased IL-1β-induced MMP-9 significantly. (1 : no treatment; 2 : chondrocyte induced by IL-1β 10ng/ml; 3 : chondrocyte induced by IL-1β 10ng/ml then treated with leptin 0,1µg/ml; 4 = chondrocyte induced by IL-1β 10ng/ml then treated with leptin 1µg/ml; 5 : chondrocyte induced by IL-1β 10ng/ml then treated with leptin 10µg/ml; 6 : chondrocyte induced by IL-1β 10ng/ml then treated with leptin 10µg/ml and pioglitazone 10µM). The histogram with error bars represents the mean and the standard error of the mean. *Asterisks* (*) denote a significant effect of increased MMP compared to normal ($p < 0.05$).

Figure 1A showed that IL-1β increased MMP-13 secretion significantly. Addition of leptin also increased, significant effect found after L2 administration ($p < 0,05$). In the last group L3, pioglitazone was added to determine PPAR-γ pathway in increasing IL-1β-induced MMP-13 secretion. Our investigation showed no significant changes in IL-1β-induced MMP-13 secretion after pioglitazone addition ($p = 0,447$). Pioglitazone was one of the PPAR-γ ligands. A positive correlation was seen between leptin and MMP-13 secretion (R =

0,927; p < 0,05). Figure 1B also revealed that leptin increased IL-1β-induced MMP-9 secretion, though the significant effect found in the highest dose of leptin. Addition of pioglitazone decreased IL-1β-induced MMP-9 secretion significantly (p = 0,018). Leptin and MMP-9 also have positive correlation (R = 0,870; p = 0,024).

Figure 2A showed that adiponectin also increased MMP-13 secretion, but there was no significant changes after IL-1 induction (p = 0,651) and adiponectin 0,1µg/ml (p = 0,387) addition. Significant changes was seen after addition of adiponectin 1µg/ml. Pioglitazone addition decreased MMP-13 secretion significantly (p < 0,05). A positive correlation was seen between adiponectin and MMP-13 secretion (R = 0,943; p < 0,05). In Figure 2B IL-1 induction increased MMP-9 significantly (p < 0,05). Addition of adiponectin increased the secretion significantly at dose adiponectin 1µg/ml (p = 0,004) and dose adiponectin 5µg/ml (p = 0,006). A positive correlation was also seen between adiponectin and MMP-9 secretion (R = 0,788; p < 0,05).

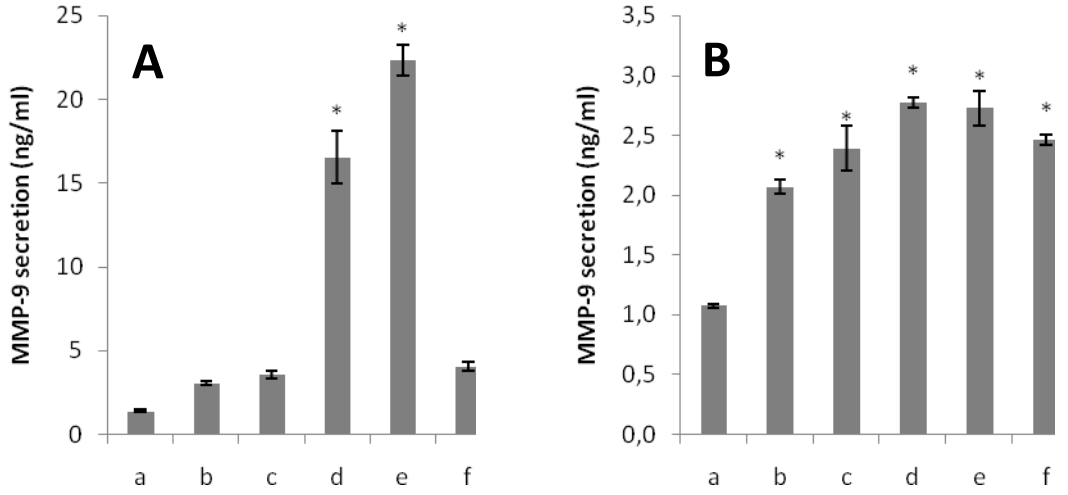

Figure 2. Effect of adiponectin in MMPs secretion by chondrocyte in various tratment groups. (A) Adiponectin increased IL-1β-induced MMP-13 secretion on chondrocytes in dose dependent manner, significant effect seen in higher dose (adiponectin 1µg/ml and 5µg/ml). Addition of pioglitazone decrease MMP-13 secretion significantly. **(B)** Adiponectin increased IL-1β-induced MMP-9 secretion also in dose dependent manner. Significant effect at leptin dose 10µg/ml, the pioglitazone addition decreased IL-1β-induced MMP-9 significantly. (a : no treatment; b : chondrocyte induced by IL-1β 10ng/ml; c : chondrocyte induced by IL-1β 10ng/ml then treated with adiponectin 0,1µg/ml; d = chondrocyte induced by IL-1β 10ng/ml then treated with adiponectin 1µg/ml; e : chondrocyte induced by IL-1β 10ng/ml then treated with adiponectin 5µg/ml; f : chondrocyte induced by IL-1β 10ng/ml then treated with adiponectin 5µg/ml and pioglitazone 10µM). The histogram with error bars represents the mean and the standard error of the mean. *Asterisks* (*) denote a significant effect of increased MMP compared to normal ($p < 0.05$).

PPAR-γ expression was measured by Real time PCR. Figure 3A showed that induction of IL-1β decreased PPAR-γ expression significantly (p < 0,05). Exposure of intermediate dose of leptin increased PPAR-γ expression significantly compared to IL-1β group (p < 0,05). Exposure of high dose of leptin PPAR-γ expression start to decrease. Addition of pioglitazone decreased the expression significantly (p < 0,05). And the correlation between leptin and PPAR-γ expression was positive (R = 0,867, p = 0,00). Figure 3B also showed that induction of IL-1β decreased PPAR-γ expression significantly (p < 0,05). Exposure of adiponectin increased PPAR-γ expression significantly at dose A3 compared to IL-1β group (p = 0,01). Addition of pioglitazone slightly decreased the expression of PPAR-γ (p = 0,146). And the correlation between adiponectin and PPAR-γ expression was positive (R = 0,834, p = 0,01).

IV. Discussion

Osteoarthritis is the most common joint disease and often accompanied by pain and joint dysfunction. The presence of cartilage destruction is an important factor onset of osteoarthritis. The articular cartilage damage is characterized by degeneration of the extracellular matrix (ECM). Enzymes that degrade the matrix, such as matrix metalloproteinase (MMP) plays an important role in this process because of its ability to damage type II collagen or aggrecans, which are two important components of ECM [8]. Changes in chondrocyte

metabolism caused by the presence of proinflammatory mediators. The cytokines, mechanical trauma and genetic changes involved in the pathogenesis of osteoarthritis, and these factors may initiate the degenerative cascade that generates a lot of changes in the characteristics of articular cartilage in osteoarthritis. One of the pro-inflammatory cytokine that is a potent and play an important role on the pathogenesis of osteoarthritis is IL-1. IL-1 is able to induce chondrocytes to synthesize MMPs [9].

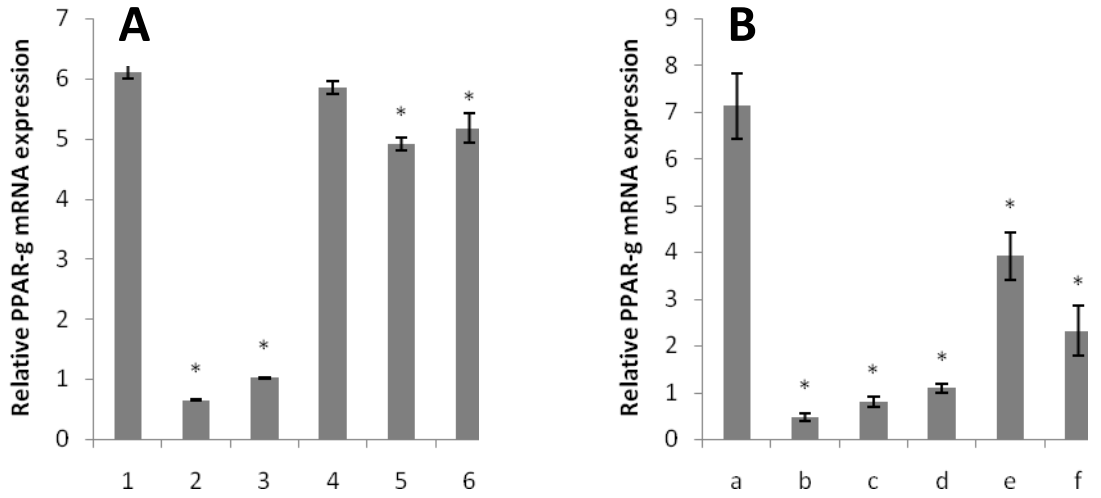

Figure 3. Effect of leptin (A) and adiponectin (B) in PPAR-γ expression. (A) Leptin increased IL-1β-induced PPAR-γ expression. At leptin dose 1μg/ml, PPAR-γ increased significantly. And at higher dose of leptin, PPAR-γ expression start to decrease. Addition of pioglitazone slightly increase PPAR-γ expression in chondrocyte. **(B)** Adiponectin increase PPAR-γ expression in dose dependent manner. Addition of pioglitazone slightly decrease PPAR-γ expression. The histogram with error bars represents the mean and the standard error of the mean. (1 : no treatment; 2: chondrocyte induced by IL-1β 10ng/ml; 3 : chondrocyte induced by IL-1β 10ng/ml then treated with leptin 0,1μg/ml; 4 = chondrocyte induced by IL-1β 10ng/ml then treated with leptin 1μg/ml; 5 : chondrocyte induced by IL-1β 10ng/ml then treated with leptin 10μg/ml; 6 : chondrocyte induced by IL-1β 10ng/ml then treated with leptin 10μg/ml and pioglitazone 10μM; a : normal; b : IL-1β 10ng/ml; c : chondrocyte induced by IL-1β 10ng/ml then treated with adiponectin 0,1μg/ml; d = chondrocyte induced by IL-1β 10ng/ml then treated with adiponectin 1μg/ml; e : chondrocyte induced by IL-1β 10ng/ml then treated with adiponectin 5μg/ml; f : chondrocyte induced by IL-1β 10ng/ml then treated with adiponectin 5μg/ml and pioglitazone 10μM). *Asterisks* (*) denote a significant effect of PPAR-γ expression compared to normal group ($p < 0.05$). The histogram with error bars represents the mean and the standard error of the mean.

This study revealed that induction of IL-1β increase the secretion of MMP-13 significantly. Induction of IL-1 activates several transcription factors, such as NF-κB, AP-1, c-jun N-terminal kinase (JNK) and p38 MAPK. The activation of NFκB induces several target genes transcription involved in the inflammation and immune system, cell proliferation, cell cycle, and apoptosis. Activation of NFκB also induces several MMP genes, such as MMP-3 and MMP-13 [3,4].

Obesity is one of several risk factors of osteoarthritis. Obesity can increase the stiffness of collagen, mechanical changes of extracellular matrix, also can decrease the proteoglycans synthesis, where it may be become the beginning of cartilage degradation. Adipose tissue is able to secrete a hormone adipokines such as leptin, adiponectin, resistin and visfatin. Leptin, adiponectin and their receptors have been identified on the human chondrocytes. Leptin can increase catabolic factors, such as matrix metalloproteinase [10,11]. This study showed that addition of leptin on IL-1β-induced chondrocytes increased the secretion of MMP-13 in a dose-dependent manner. Leptin plays an important role in the pathophysiology of osteoarthritis. Leptin has powerful proinflamation effects [12]. Otero et al. showed that, in cultured human and murine chondrocytes, type 2 nitric oxide synthase (NOS2) and *interferon-γ,* and NOS2 activation by IL-1 β was increased by leptin via a mechanism that involved JAK2, PI3K, and mitogen-activated kinases (MEK1 and p38)[13]. Leptin also induced MMPs expression involved in osteoarthritis cartilage damage, such as MMP-9 and MMP-13. Other evidence also suggests that leptin alone and in combination with IL-1β is able to regulate the production of MMP-1 and MMP-3 in osteoarthritis cartilage through transcription factors NF-κB, protein kinase C, and MAP kinase pathways. In addition, administration of leptin can increase gene expression of ADAMTS-4 and -5 significantly and lowered proteoglican in cartilage[14].

Another adipokine is adiponectin. It has been found to improve insulin sensitivity and to have antiarthrogenic properties. Interestingly, adiponectin has also been identified as a regulatory factor in inflammation and arthritis. Tissues in the joint of OA patient including synovium, meniscus, osteophytes, cartilage, bone and fat, have been reported to produce adiponectin. In the present study adiponectin increased IL-1β-induced MMP-9 and MMP-13 significantly. In this study, we also found positive correlations between adiponectin and MMP-13 secretion and between adiponectin and MMP-9 secretion. This finding supports the role of adiponectin in cartilage degradation of OA cartilage.[15] The biological effects of adiponectin are mediated through two adiponectin receptor subtypes, adiponectin receptor type 1 (AdipoR1) and adiponectin receptor type 2 (AdipoR2) which have been shown to be expressed in articular cartilage. The AdipoR1-AMPK pathway could be associated with cartilage catabolism. Lago *et al* reported that the AMPK/Akt signaling pathway is involved in iNOS and MMP-3 induction by adiponectin in the murine chondrocyte ATDC5 cell line. While Kang *et al* reported that AMPK/JNK pathways are the major signaling pathway involved in adiponectin-mediated induction of iNOS and MMPs in human OA chondrocytes, whereas the AMPK/Akt or AMPK/p38 pathway is partially involved in MMP-13 or MMP-3 induction, respectively [15, 16, 17].

In this study, we also observed the expression of PPAR-γ. PPAR-γ activated by its agonist decreased the synthesis of catabolic response, inflammatory factors and reduce cartilage degradation in vivo and in vitro in osteoarthritis animal model. PPAR-γ is expressed in chondrocytes and the expression in osteoarthritis was decreased compared to normal cartilage. These findings suggest that the decreased expression of PPAR-γ in osteoarthritis cartilage reflect an increase of the inflammatory and catabolic factor expression [1,18]. In this study, it was found that the addition of pioglitazone, as an activator of PPAR-γ, on leptin-treated chondrocyte slightly decreased the secretion of MMP-13. This suggests that leptin increased secretion of MMP-13 is influenced by the activation through many pathways. PPAR-γ interacts with other transcription factors and are not directly involved in binding to DNA to regulate gene transcription. For example, PPAR-γ have interaction with AP-1 (activator protein-1), STAT (signal transducers and activators of transcription), and NF-κB, which all transcription factors also play a role in regulation of gene expression. Proinflammatory transcription factor NF-κB has a central role in immune responses and inflammation, which is NF-κB is the main target of PPAR-γ to suppress inflammation [19]. In some studies, PPAR-γ was shown to interact with several co-activators such as cyclic adenosine monophosphate (cAMP)-responsive element binding factor (CREB)-binding protein (CBP), p300, steroid receptor co-activator-1 (SRC-1), and thyroid hormone receptor-associated protein (TRAP220). Some of the co-factors required for transcriptional activity of AP-1, NF-κB, Egr-1 (early growth response-1), STAT (signal transducers and activators of transcription), and NF-AT [20].

Transactivation function of PPAR-γ is suppressed by IL-1 through NF-κB activated by the TAK1/TAB1/NF-κB-inducing kinase (NIK) cascade, a downstream cascade associated with IL-1 signalling [21]. Results in this study also showed that induction of IL-1β decreased the expression of PPAR-γ significantly and the addition of pioglitazone on IL-1β-induced chondrocytes was not able to increase the expression of PPAR-γ. Study by Mracek proved that administration of IL-1β inhibit brown adipocyte differentiation followed by a decrease in PPAR-γ mRNA levels[22]. In addition, research by Shan also explained that the PPAR-γ expression is regulated by IL-1β. In that study the induction of IL-1β may decrease the expression of PPAR-γ after 6 hours of induction[23]. In patients with osteoarthritis found that the expression of PPAR-γ decreased in the cartilage. The other studies have reported a that PPAR-γ expression decreased in mono-iodoacetate induced rat cartilage. These findings suggest that decreased expression of PPAR-γ in osteoarthritis cartilage will increase the gene expression of inflammatory and catabolic response, causing inflammation and degradation of articular cartilage. Effect of human osteoarthritis chondrocytes with IL-1β administration resulted in decreased expression of PPAR-γ [24].

Surprisingly, the other results in this study showed that leptin addition in low dose and intermediate dose was increased the expression of PPAR-γ, but addition in high dose of leptin start to decreased PPAR-γ expression. The finding that the addition of leptin only slightly decreased the expression of PPAR-γ at the mRNA level suggests that leptin may suppress the activity of PPAR-γ is also due to posttranslational modifications [25]. But on the other hand, study by Qian showed that leptin administration on adipose tissue increased PPAR-γ expression. Leptin administered via intracerebroventricular (ICV) induced adipose tissue apoptosis to determine its role in lipid metabolism. Expression of PPAR gamma in epididymal fat tissue was examined by Western immunoblot and in situ immunocytochemical analysis after 5 days of i.c.v. leptin treatment. Young and old rats (3 and 8 months old) were treated with or without 5 micrograms/d leptin. Leptin treatment increased PPAR gamma expression by 70-80% ($P < 0.01$) in both age groups. The study suggests that expression of PPAR gamma may be directly involved in the leptin-induced adipocyte apoptosis signal pathway[26]. Studies by Ajuwon showed that leptin in high dose decreased PPAR-γ expression in adipocyte. Two in vivo studies were conducted with pigs to determine the effects of exogenous leptin on the expression of peroxisome proliferator activated receptors (PPAR), and on serum concentrations of selected metabolites and

hormones. Initially, leptin was administered i.m. to young pigs for 15 days at 0 (control), 0.003 (low), 0.01 (medium) and 0.03 (high) mg · kg^{-1} · day^{-1}. Leptin at the intermediate and high doses depressed adipose expression of both PPARγ1 ($P < 0.06$) and PPARγ2 ($P < 0.01$). The data presented here in indicate that leptin modulates lipid metabolism in the pig. And it varied in relation to dose in vivo[27].

Other studies also demonstrate that leptin activates PPARs in C2C12 muscle cells. C2C12 pre-treated with $AACOCF_3$, a specific inhibitor of cytosolic phospsolipase A_2 ($CPLA_2$), an enzyme that supplies ligands of PPARs. The result is leptin treatment significantly increased $cPLA_2$ activity. Furthermore, MEK1 inhibitor PD98059 showed that leptin activates $cPLA_2$ through ERK induction. These results support a direct effect of leptin on skeletal muscle cells, and suggest that the hormone may modulate muscle transcription also by precocious activation of PPARs through ERK–cPLA2 pathway [28]. High levels of leptin is not always associated with the destructive effect of the chondrocytes. The intra-articular injection of leptin can strongly stimulate the synthesis of insulin-like growth factor-1 (IGF-1) and transforming growth factor-β (TGF-β) at both the messenger RNA (mRNA) and protein levels which can exert anabolic activities in cartilage metabolism. Leptin concentrations in synovial fluid were also significantly correlated with BMI in people with severe osteoarthritis. These findings have suggested that high circulating leptin levels in obese individuals may protect cartilage from osteoarthritic degeneration [29].

Our study also found that exposure of higher dose of adiponectin significantly increased PPAR-γ expression. Adiponectin is a fat-specific adipokine whose expression is tightly regulated by PPAR-γ, itself induces PPAR-γ 2 expression in a variety of cells. Transduction of the adiponectin signal by AdipoR1 and AdipoR2 involves the activation of AMP-activated protein kinase peroxisome proliferator activated receptors and seemingly other signal molecules [29,30]. Increased PPAR-γ activity associated with increased adiponectin level [31]. But there are limited studies that correlate the level of adiponectin with increased PPAR-γ 1 expression. Present study demonstrate the first time that adiponectin stimulate PPAR-γ 1 expression in IL-1 induced chondrocytes. This result is linear to a study by Sagheb that showed that adiponectin significantly increased Pdx1 and PPAR-γ transcription and insulin secretion in rat islets [32].

In addition to abundant expression in fat, PPAR-γ is detectable in wide range of cells. In light PPAR-γ's pleiotropic physiological roles, its relative abundance is predicted to have an important modulating influence on a variety of homeostatic responses. A large number of soluble mediators are known to effect PPAR-γ expression, stability and activity in a tissue and cell type-specific manner. Transcription factors shown to regulate PPAR-γ expression include C/EBPs, EBF proteins, inhibitor of DNA binding (ID) 2 and NF-E2 related factor 2 (Nrf2). Several cytokines and chemokines regulate PPAR-γ expression in mesenchymal cells. TGF-β is a potent inhibitor of PPAR-γ expression in fibroblasts and hepatic stellate cells. In contrast to fibroblasts, in monocytes and macrophages TGF-β stimulated PPAR-γ expression. The inflammatory cytokines TNF-α and IL-13 inhibit adipocyte differentiation by suppressing PPAR-γ expression [33].

From the results it can be concluded that leptin and adiponectin increased MMP-9 and MMP-13 secretion, but this increase is not always followed by decreased of PPAR-γ expression. The regulation of PPAR-γ by adipokines seems complex in that it varied in relation to dose in vivo, and may be impacted by in vitro vs. in vivo circumstances. The increasing knowledge about the role of PPARs and adipokin in normal and osteoarthritis cartilage may help to decrease cartilage degradation.

V. Conclusion

Leptin and adiponectin could increase MMP-9 and MMP-13 secretion in IL-1β-induced chondrocyte. This results show that leptin and adiponectin have a role in pathogenesis of osteoarthritis by increasing catabolic response of chondrocyte. This study still needs further research using an animal model of osteoarthritis.

References

[1]. H. Fahmi, J. Martel-Pelletier, JP. Pelletier, and M. Kapoor, Peroxisome proliferator-activated receptor gamma in osteoarthritis, Mod Rheumatol, 21, 2011, 1–9
[2]. T. Aigner, S. Soeder, and J. Haag, IL-1beta and BMPs--interactive players of cartilage matrix degradation and regeneration, Eur Cell Mater, 12, 2006, 49-56
[3]. A. Weber, P. Wasiliew, and M. Kracht, Interleukin-1 (IL-1) pathway, Sci Signal, 3 (105), 2010 Jan 19
[4]. JA. Roman-Blas and SA. Jimenez, NF-kB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis, Osteoarthritis and Cartilage, 14, 2006, 839-848
[5]. T. Simopoulou, KN. Malizos, and D. Iliopoulos, Differential expression of leptin and leptin's receptor isoform (Ob-Rb) mRNA between advanced and minimally affected osteoarthritic cartilage; effect on cartilage metabolism,, 15, 2007, 872–883.
[6]. EH. Kang, YJ. Lee, and TK. Kim, Adiponectin is a Potential Catabolic Mediator in Osteoarthritis Cartilage, Arthritis Research & Therapy, 12, 2010, R231

[7]. K. Robinson, J. Prins, and B. Venkatesh, Clinical Review : Adiponectin Biology and its Role in Inflammation and Critical Illness, Critical Care, 15, 2011, 221

[8]. C. Yu, WP. Chen, and XH. Wang, MicroRNA in Osteoarthritis, Journal of International Medical Research, 39, 2011, 1 – 9

[9]. MA. Karsdal, AC. Bay-Jensen, K. Henriksen, and C. Christiansen, The Pathogenesis of Osteoarthritis involves bone, cartilage and synovial inflammation: may estrogen be a magic bullet?, Menopause Int, Vol 18 No 4, 2012, 139 – 146

[10]. J. DeGroot, The AGE of the matrix: chemistry, consequences and cure, Curr Opin Pharmacol, 4, **2004**, 301–305.

[11]. M. Kimata, T. Michigami, and K. Tachikawa, Signaling of extracellular inorganic phosphate up-regulates cyclin D1 expression in proliferating chondrocytes via the Na/Pi cotransporter Pit-1 and Raf/MEK/ERK pathway, Bone, vol. 47, no. 5. 2010, pp. 938–947

[12]. MR. Gandhi, M. Takahashi, R. Rizek, O. Dessouki, and N. Nizar, Obesity-related Adipokines and Shoulder Osteoarthritis, The Journal of Rheumatology, Vol. 39 No.10, 2012, 2046-2048

[13]. M. Otero, R. Lago, F. Lago, JJ. Reino, and O. Gualillo, Signalling pathway involved in nitric oxide synthase type II activation in chondrocytes: synergistic effect of leptin with interleukin-1, Arthritis Research & Therapy, vol. 7, no. 3, 2005, pp. R581–R591.

[14]. E. Toussirot, G. Streit, and D. Wendling, The contribution of adipose tissue and adipokines to inflammation in joint diseases. Current Medicinal Chemistry, vol. 14, no. 10, 2007, pp. 1095–1100

[15]. A. Koskinen, S. Juslin, R. Nieminen, T. Moilanen, K. Voulteenaho, and E. Moilanen. Adiponectin associates with markers of cartilage degradation in osteoarthritis and induces production of proinflammatory and catabolic factrors through mitogen-activated protein kinase pathways. Journal of Arhtritis Research and Therapy, 2011, 13:R184, 6-11

[16]. R. Lago, R. Gomez, M. Otero, F. Lago, R. Gallego, C. Dieguez, JJ. Gomez-Reino, O. Gualillo: A new player in cartilage homeostasis: adiponectin induces nitric oxide synthase type II and pro-inflammatory cytokines in chondrocytes. Osteoarthritis Cartilage 2008, 16:1101-1109

[17]. EH. Kang, et al. 2010. Adiponectin is a Potential Catabolic Mediator in Osteoarthritis Cartilage. Arthritis Research & Therapy 2010, 12:R231

[18]. H. Afif, M. Benderdour, L. Mfuna-Endam, J. Martel-Pelletier, JP. Pelletier, and N. Duval, Peroxisome proliferator-activated receptor gamma1 expression is diminished in human osteoarthritic cartilage and is downregulated by interleukin-1beta in articular chondrocytes. Arthritis Res Ther. 9, 2007, R31.

[19]. SZ. Duan, MG. Usher, and RM. Mortensen, Peroxisome Proliferator-Activated Receptor-γ–Mediated Effects in the Vasculature, Circulation Research, 102, 2008 283-294

[20]. DE. Green, RL. Sutliff, and CM. Hart, Is peroxisome proliferator-activated receptor gamma (PPAR-γ) a therapeutic target for the treatment of pulmonary hypertension?. Pulmonary circulation, vol 1 No 1, 2011

[21]. M. Suzawa, I. Takada, J. Yanagisawa, F. Ohtake, S. Ogawa, T. Yamauchi, T. Kadowaki, Y. Takeuchi, H. Shibuya, Y. Gotoh, K. Matsumoto, and S. Kato, Cytokines suppress adipogenesis and PPAR-γfunction through the TAK1/TAB1/NIK cascade. Nature Cell Biology, 5, 2003: 224 - 230

[22]. T. Mrácek , B. Cannon , and J. Houstek , IL-1 and LPS but not IL-6 inhibit differentiation and downregulate PPAR gamma in brown adipocytes, Cytokine, 26(1), 2004 Apr 7, 9-15

[23]. Z. Shan, K. Masuko-Hongo, S. Dai, H. Nakamura, T. Kato, and K. Nishioka, A Potential Role of 15-Deoxy-$\Delta^{12,14}$-prostaglandin J2 for Induction of Human Articular Chondrocyte Apoptosis in Arthritis, The Journal of Biological Chemistry, 279 (36), 2004 Sep 3, 37939-50.

[24]. H. Fahmi, JP. Pelletier, JA. diBattista, HS. Cheung, JC. Fernandes, and J. Martel-Pelletier, Peroxisome proliferator-activated receptor γ activators inhibit MMP-1 production in human synovial fibroblasts likely by reducing the binding of the activator protein 1, Osteoarthritis Cartilage, 10, **2002**, 100–8.

[25]. L. Wang, YY. Shao, and RT. Ballock, Leptin Antagonizes Peroxisome Proliferator-Activated Receptor-γ Signaling in Growth Plate Chondrocytes, PPAR Res, 2012, 2012, 756198

[26]. H. Qian , GJ. Hausman , MM. Compton , MJ. Azain , DL. Hartzell , and CA. Baile, Leptin regulation of peroxisome proliferator-activated receptor-gamma, tumor necrosis factor, and uncoupling protein-2 expression in adipose tissues, Biochem Biophys Res Commun, 246(3), 1998 May 29, 660-7

[27]. KM. Ajuwon, JL. Kuske, DB. Anderson, DL. Hancock, KL. Houseknecht, O. Adeola, and ME. Spurlock, Chronic leptin administration increases serum NEFA in the pig and differentially regulates PPAR expression in adipose tissue, Journal of Nutritional Biochemistry, 14, 2003, 576–583

[28]. P. Bendinelli, R. Piccoletti, and P. Maroni, Leptin rapidly activates PPARs in C2C12 muscle cells, Biochemical and Biophysical Research Communications, 332, 2005, 719–725

[29]. J. Conde, M. Scotece, R. Gomez, V. Lopez, JJ. Gomez-Reino, and O. Gualillo, Adipokines and Osteoarthritis: Novel Molecules Involved in the Pathogenesis and Progression of Disease, Arthritis, vol 2011, 2011, Article ID 203901, 8 pages doi:10.1155/2011/203901

[30]. J. Wei, S. Bhattacharyya, M. Jain, J. Varga. 2012. Regulation of Matrix Remodelling by Peroxisome Proliferator-Activated Receptor-γ: A Novel Link Between Metabolism and Fibrogenesis. The Open Rheumatology Journal. 2012,6, (Suppl1:M6) 103-115.

[31]. R. Gomez, F. Lago, JG. Reino, C. Diugez, O. Gualilo. 2009. Adipokines in the skeleton: influence on cartilage function and joint degenerative diseases. Research Laboratory 9 (NEIRID LAB, Laboratory of Neuro Endocrine Interactions in Rheumatology and Inflammatory Diseases), Santiago University Clinical Hospital, Calle Choupanas/n, Edificio C, Planta -2, 15706 Santiago de Compostela

[32]. AVW Nunn, J. Bell, adn P. Barter. The integration of lipid-sensing and anti-inflammatory effects: how the PPARs play a role in metabolic balance, 2007, Journal of Nuclear Receptor 2007, **5**:1

[33]. MM Sagheb, N. Azarpira, M. Mokhtary, SE. Hosseini, R. Yaghobi. The effects of Leptin and Adiponectin on Pdx1, Foxm1, and PPARγ Transcription in Rat Islets of Langerhans, Hepatmon. 2013; 13(6): e9055. DOI: 10.5812/hepatmon.9055