UNIVERSITY OF CALIFORNIA SAN DIEGO

The effect of deficiency of AMP-activated protein kinase (AMPK) in osteoarthritis development associated with obesity

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in

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by

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The thesis of Roseanna Wallace is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

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Chair

University of California San Diego

2020

DEDICATION

I would like to dedicate this thesis to my parents & spouse. My late father Robert Loya Ardilla Fitzpatrick taught me to never give up on my education. Although it's been a decade since you have been gone, your memories will continue to live on. I would also like to thank my mother Mary Asher-Fitzpatrick & husband Nathan Wallace, my biggest fan, of whom without their support my success may not have been possible.

EPIGRAPH

Don’t hold yourself back based on who you think you are.

~Roseanna Wallace

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LIST OF ABBREVIATIONS

ad libitum	as much or as often as necessary or desired
AMPK	Adenosine Monophosphate-Activated Protein Kinase
ANOVA	Analysis of variance
AUC	Area Under The Curve
CD	Control Diet
CO_2	Carbon Dioxide
C57BL/6	C57 black 6 mice
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
GTT	glucose tolerance test
HFD	High-Fat Diet
IACUC	Institutional Animal Care and Use Committee
KO	Knockout
LFC	lateral femoral condyle
LTP	lateral tibial plateau
MFC	medial femoral condyle
MSD	Meso Scale Discovery
MTP	medial tibial plateau
OA	osteoarthritis
OARSI scoring	osteoarthritis cartilage histopathology assessment system
PGC1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
ROS	reactive oxygen species
S.D.	standard deviation
S.E.M.	standard error of the mean
SIRT1	sirtuin 1
SREBP	sterol regulatory element-binding protein
TNF-α	tumor necrosis factor alpha
VA	Veterans Affairs
WT	Wild Type

LIST OF SYMBOLS

α	alpha
β	beta
γ	gamma
±	plus or minus

LIST OF FIGURES

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This study is being prepared for publication upon addition of more data. There are multiple lab members that I would like to acknowledge who contributed to the massive amount of work that this project entailed. I acknowledge Nhi Ngoc Quynh Nguyen, Tiffany C. Yan, Dr. Paulo V.G. Alabarse & Dr. Sushil K. Mahata for their contribution to the study and their sleepless nights in addition to months and years of work of whom without them there would be no paper.

ABSTRACT OF THE THESIS

The effect of deficiency of AMP-activated protein kinase (AMPK) in osteoarthritis development associated with obesity

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Professor Ru Bryan, Chair
Professor Nan Hao, Co-Chair

Osteoarthritis (OA) is the most common joint disorder, and obesity is a risk factor for OA development. However, how metabolic alterations associated with obesity contributing to OA is unclear. Because dysregulation of adenosine monophosphate-activated protein kinase (AMPK), a master regulator

of energy balance, has been linked to both obesity and OA, we investigate the role of AMPK in obesity-induced OA. To do so, we subjected mice with knockout (KO) of AMPKα1, the predominant catalytic subunit of AMPK, and wild type (WT) control mice at 3 months of age to a long-term (40 weeks) high-fat diet (HFD) and control diet (CD). We then assessed metabolic changes and cartilage degeneration that is a hallmark of OA. We found that AMPKα1 KO compared to WT mice after a long-term HFD displayed profound metabolic alterations such as substantial increase in body weight, fasting blood glucose levels and severe glucose intolerance. These were likely attributed to that AMPKα1 KO mice had reduced ability of producing insulin and development of leptin resistance in response to HFD, supported by significantly lower plasma levels of insulin and leptin. AMPKα1 KO compared to WT mice after HFD also exhibited systemic inflammation, evidenced by marked induction of plasma levels of TNFα, an inflammatory marker. Importantly, AMPKα1 KO compared to WT mice after HFD presented OA pathology, because mild but significant cartilage degeneration were observed. Taken together, these results suggest that dysregulation of AMPK could contribute to OA development associated with obesity.

Introduction

Osteoarthritis (OA) is the most common joint disorder in the United States (1). It is a disease of the whole synovial joint including the core feature progressive cartilage degeneration, synovial inflammation (synovitis), osteophyte formation, and subchondral bone sclerosis, leading to pain and life impacting disability (1). Aging, obesity, and joint injury are the main risk factors for OA development (2). There are no current effective treatments and no disease modifying therapies available to slow OA progression. Most of the treatments are targeted at improving the symptoms such as pain relief (3). As the U.S. population ages, the number of patients with OA is projected to reach 60 million by the year 2030 (4).

Role of AMP-activated protein kinase (AMPK) in cartilage homeostasis and OA

Effective regulation of cellular energy metabolism is essential for tissue homeostasis. Articular cartilage is the smooth white avascular tissue assisting with frictionless movement and dissipating stress between the joints (5). Chondrocytes, the only cells embedded in articular cartilage, are responsible for maintaining extracellular matrix (ECM) (6). ECM homeostasis is maintained by a balance between rates of synthesis and degradation (4). Overactive chondrocyte catabolic responses lead to degradation of the cartilage (7). In the pathogenesis of OA, failure of ECM homeostasis is one of the earliest mechanisms that leads to cartilage degradation (4).

Adenosine monophosphate-activated protein kinase (AMPK), a highly conserved serine/threonine kinase, is a master regulator of cellular energy homeostasis (8). AMPK is a heterotrimeric complex consisting of multiple subunits each having 2-3 isoforms; catalytic alpha subunits (α1, α2), regulatory beta subunits (β1, β2) and gamma subunits (γ1, γ2, γ3). Phosphorylation of a conserved threonine 172 (Thr172) within the catalytic domain of both the α1 and α2 sub- units (which are 90% homologous in their catalytic cores) is critical for AMPK activity (9). AMPK is activated by an increase of AMP/ATP ratio through phosphorylation by upstream kinases such as by Liver Kinase B1 (LKB1) (10). Upon activation, AMPK phosphorylates multiple downstream substrates allowing for the inhibition of anabolic pathways that consume ATP (eg. fatty acid and protein synthesis) and activation of catabolic pathways

that produce ATP (eg. fatty acid oxidation and glucose transport) to help cells adjust to changing energy demands (8).

Articular chondrocytes express α1, α2, β1, β2 and γ1 isoforms of AMPK subunits, with α1 as the pre- dominantly expressed and functionally active AMPKα isoform (11). Decreased phosphorylation of AMPKα is a core event compromising OA chondrocyte mitochondrial biogenesis and function. Glycolysis and mitochondrial oxidative phosphorylation (OXPHOS) provide metabolic energy in articular cartilage (12). Chondrocytes cope with a nutritionally challenging avascular and hypoxic environment with changing metabolic demands in response to aging, inflammation and biomechanical stress (13). It was discovered that phosphorylation of AMPKα Thr172 (indication of AMPK activity) is constitutively present in normal articular chondrocytes/cartilage, but is decreased in human knee OA chondrocytes/cartilage, in mouse knee OA cartilage, and in aged mouse knee cartilage indicating that loss of AMPK signaling may be a key contributor to OA (11). Reduced capacity of AMPK activation in articular chondrocytes limits energy availability for cellular maintenance, triggers significant cell stress by inducing mitochondrial dysfunction, oxidative stress and inflammation, resulting in compromising cell survival and tissue function (11).

Previous studies have demonstrated that aging, inflammation and biomechanical injury can decrease phosphorylation of AMPKα Thr172 in articular chondrocytes, which disrupts the balance between chondrocyte catabolic and anabolic function. Sustained AMPK activity in chondrocytes appears to be critical for cartilage homeostasis (14). Conditional chondrocyte-specific AMPKα1/α2 knockout (KO) mice have accelerated OA development in a post-traumatic OA model (15). C57BL/6 mice developed mild and moderate OA at 18 and 24 months of age, respectively. However, this age-related OA phenotype is limited in C57BL/6 mice receiving treatment of berberine, a natural plant product that activates AMPK (16).

Association of obesity with dysregulation of AMPK

Obesity is a state of energy imbalance as a result of excessive energy intake (overnutrition) and inadequate energy expenditure (17). One of the main characteristics is as a state of chronic low-grade

inflammation with progressive pro-inflammatory macrophage infiltration into adipose tissue (18). During weight gain, there is an increase in size of fat cells (adipocytes) (19). The amount of adipocytes is correlated with visceral adiposity and expression of the hormones insulin and leptin (20). In the state of overnutrition, AMPK is impaired whereas reducing calorie intake has been shown to increase AMPK activity (11). AMPK plays a role in lipid metabolism via phosphorylation and inhibition of sterol regulatory element-binding protein (SREBP), the master regulator of lipogenesis (21). Pro-inflammatory adipokines released by adipocytes and chemokines released by macrophages are key mediators in the cross talk between adipocytes and adipose tissue macrophages (18). AMPK has the ability to regulate adipokines such as leptin and adiponectin for food intake, weight gain, glucose, and lipid metabolism (22). It has been shown that inflammatory stimuli and a fatty acid rich-diet decreased the expression and activity of AMPKα in mouse adipose tissue and macrophages induced increased expression of TNF-α (23).

Obesity and OA development

Worldwide, an estimated 1.4 billion adults are obese (overweight), a number of which has doubled in the past 30 years (24). Obesity has become one of the major risk factors for OA development (24). The amount of people with OA will likely increase due to the aging population and the obesity epidemic. Obesity-induced metabolic disturbances cause low-grade chronic inflammation that can result in the development of metabolic syndrome and diabetes, which are often associated with OA (11). For example, 59% of OA patients had metabolic syndrome (abdominal adiposity, diabetes mellitus, insulin resistance, high cholesterol, high blood pressure) compared to 23% of the general population. Obesity-related metabolic factors induce expression of proinflammatory factors & degradative enzymes leading to inhibition of cartilage matrix synthesis & stimulation of cartilage matrix degradation and subchondral bone remodeling which contributes to OA development (25).

Poor lifestyle choices and diet can lead to weight gain and obesity. High-fat diets may stimulate joint inflammation and OA through activation of innate immune pathways. In an obese state, AMPK signaling is dysregulated triggering significant chondrocyte stress by inducing mitochondrial dysfunction,

oxidative stress and inflammation, all of which compromise cell survival and tissue integrity leading to OA development and progression (14). Given that both obesity and OA are associated with chronic low-grade inflammation, it is likely that dysregulation of AMPK is the missing link between obesity and OA development. It is known that C57BL/6 mice on high-fat diet increases the incidence of knee OA by 2-fold (14). In this study, we tested the hypothesis that mice deficient in AMPKα1 after development of obesity via high-fat diet increase the chance of development of OA compared with wild type control mice.

Materials & Methods

Animals

All animal studies were done in compliance with an institutionally reviewed and approved protocol by Institutional Animal Care and Use Committee (IACUC) at the VA San Diego. AMPKα1 global knockout (KO) mice on the C57BL/6 and 129 background were kindly provided by Dr. Benoit Viollet (INSERM U1016, France). Mice were maintained under the standard protocol and humanely euthanized at the end of studies via CO_2 chamber. AMPKα1 KO and the wild type (WT)(C57BL/6 and 129) mice were bred in house and kept on a 12hr light/dark cycle and fed food and water *ad libitum*. Because estrogen is well known to have beneficial effects on metabolism, only male mice were used for the study to avid introducing a bias.

Assessment of Metabolic Changes

Body weight

Starting at twelve weeks of age, mice were placed on a high fat diet (60% kcal from fat from Research Diet, Inc.) and the control diet (10% kcal from fat from Research Diet, Inc.) for 40 weeks. The high fat diet (HFD) consisted of (g/773.85 g): casein (200.0), L-cystine (3.0), maltodextrin 10 (125.0), sucrose (68.80), cellulose (50.0), soybean oil (25.0), lard (245.0), mineral mix (10.0), dicalcium phosphate (13.0), calcium carbonate (5.5), potassium citrate (16.5), vitamin mix (10.0), choline bitartrate (2.0), and FD&C blue dye (0.05). The energy densities of the HFD is 5.24 kcal/g. The control diet (CD) consisted of (g/1055.05 g): casein (200.0), L-cystine (3.0), corn starch (506.2), maltodextrin 10 (125.0),

sucrose (68.80), cellulose (50.0), soybean oil (25.0), lard (20.0), mineral mix (10.0), dicalcium phosphate (13.0), calcium carbonate (5.5), potassium citrate (16.5), vitamin mix (10.0), choline bitartrate (2.0), FD&C yellow dye (0.04), and FD&C blue dye (0.01). The energy densities of the CD is 3.85 kcal/g. Animal body weight (grams) was measured at baseline, bi-weekly until 20 weeks, and then bi-monthly.

Glucose Tolerance Test (GTT)

Mice were fasted overnight for eight hours. A baseline blood glucose value was taken, then glucose [1mg/kg] was administered via intraperitoneal injection. Blood was drawn from the tip of the tail and measured with blood glucose test strips and glucometer (Easy Step). Blood glucose values were taken at 15, 30, 60, 90 and 120 minute time points after glucose injection.

Cytokine & hormone measurements

Animals were sacrificed per protocol and blood was immediately collected via cardiac puncture. Plasma was separated from whole blood via centrifugation and stored at -80C until analysis. The levels of insulin, leptin, and TNFα were measured by ELISA using a customized U-PLEX assay (Meso Scale Discovery).

OA Assessment

Processing mouse knee joints

After mice were sacrificed, knee joints were harvested by cutting the femur and tibia/fibula 3 cm above and below the joint. They were then fixed in Z fix (Anatech LTD) for 1 week, decalcified (Thermo Scientific) for 1 week and dehydrated for one week with 70% ethanol (Decon Labs, Inc.), and embedded in paraffin wax. Serial coronal plane sections of 5 um thickness were obtained using leica RM 2165 rotary microtome. Sections were mounted on Diamond White Glass Pink Frosted slides (Globe Scientific, Inc.) and allowed to dry at room temperature overnight.

OA scoring

Ten to twelve slides per knee (spaced between 30 uM) were subjected to fast green to stain bone green and safranin-O (Sigma-Aldrich) to stain cartilage red in proportion to its proteoglycan content in order to utilize the scoring paradigm as described below (26). Sections were dehydrated in a graded series

of ethanol (Koptec), cleared in propar (Anatech LTD) and mounted with Permount (Fisher Chemical) and microscope coverglass (Globe Scientific). Slides were dried at room temperature for a few days. Images of sections were taken using a digital microscope (Keyence) under 4x and 10x objectives. Severity of OA was assessed using the OARSI scoring system (26) and was applied to all four quadrants of the joint: medial femoral condyle (MFC), medial tibial plateau (MTP), lateral femoral condyle (LFC), lateral tibial plateau (LTP). A score of 0 represents normal cartilage, 0.5 = loss of PG with an intact surface, 1 = superficial fibrillation without loss of cartilage, 2 = vertical clefts and loss of surface lamina (any % or joint surface area), 3 = vertical clefts/erosion to the calcified layer lesion for 1-25% of the quadrant width, 4 = lesion reaches the calcified cartilage for 25-50% of the quadrant width, 5 = lesion reaches the calcified cartilage for 50-75% of the quadrant width, 6 = lesion reaches the calcified cartilage for >75% of the quadrant width. The OA severity is expressed as summed scores which are combined for the entire joint.

Statistical Analysis

All data were expressed as mean ± S.D. or mean ± S.E.M. Statistical analyses were performed by a two-way ANOVA with Tukey post-hoc test using GraphPad PRISM 8.

Results

Assessment of metabolic perturbations in both WT and AMPKα1 KO mice after 40 weeks' HFD

Since mice on HFD can become obese, we firstly evaluated body weights of and aged matched male and female WT and AMPKα1 KO mice at both baseline and after 40 weeks on both HFD and CD. As shown in Figure 1, as expected under high fat diet conditions, WT mice gained a significant amount of weight when compared to the CD (A and C). Notably, AMPKα1 KO mice also gained a significant amount of weight (A and C). However, there was no significant difference in mean body weight gained between AMPKα1 KO and wild type mice (Figure 1B). Figure 1, in part is being prepared for submission for publication of the material upon addition of more data. Nguyen, Nhi Ngoc Quynh; Yan, Tiffany C; Liu-Bryan, Ru. The thesis author was one of the primary investigators and co-authors of this material.

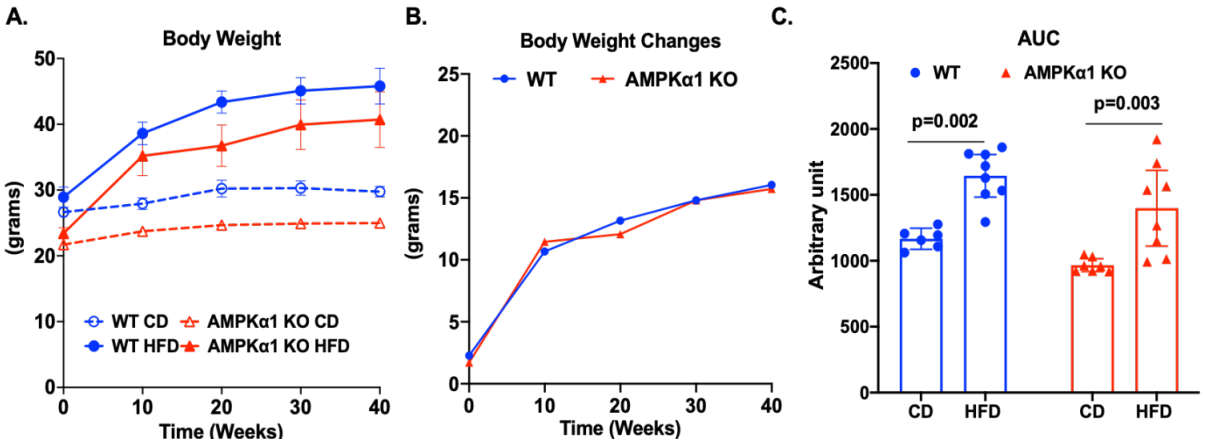

Figure 1: Both WT and AMPKα1 KO mice gained similar amounts of body weight after a long-term of HFD. Aged matched WT and AMPKα1 KO mice were fed on both high-fat diet (HFD) and control diet (CD) for 40 weeks. The body weight of the mice was monitored periodically (A) and the entire period of body weight gain were presented as area under curve (AUC) (C). The absolute mean body weight gain was presented in (B). WT CD (n=6), WT HFD (n=8), AMPKα1 KO CD (n=7), and AMPKα1 KO HFD (n=8). Two-way ANOVA plus Tukey comparison test was used for the statistical analysis.

To assess metabolic perturbations under a high fat diet, we next measured baseline glucose levels after fasting and performed glucose tolerance test (GTT). As seen in Figure 2A, the fasting glucose levels were significantly increased in both WT and AMPKα1 KO mice on HFD (p=0.02 and p=0.01, respectively). In addition, AMPKα1 KO mice exhibited substantially higher fast glucose levels, compared to WT mice under HFD condition (p=0.001). Interestingly, AMPKα1 KO mice also showed significant higher fast glucose levels than that in WT mice even under CD (p=0.04). Figure 2, in part is being prepared for submission for publication of the material upon addition of more data. Nguyen, Nhi Ngoc Quynh; Yan, Tiffany C; Mahata, Sushil K; Alabarse, Paulo V.G; Liu-Bryan, Ru. The thesis author was one of the primary investigators and co-authors of this material.

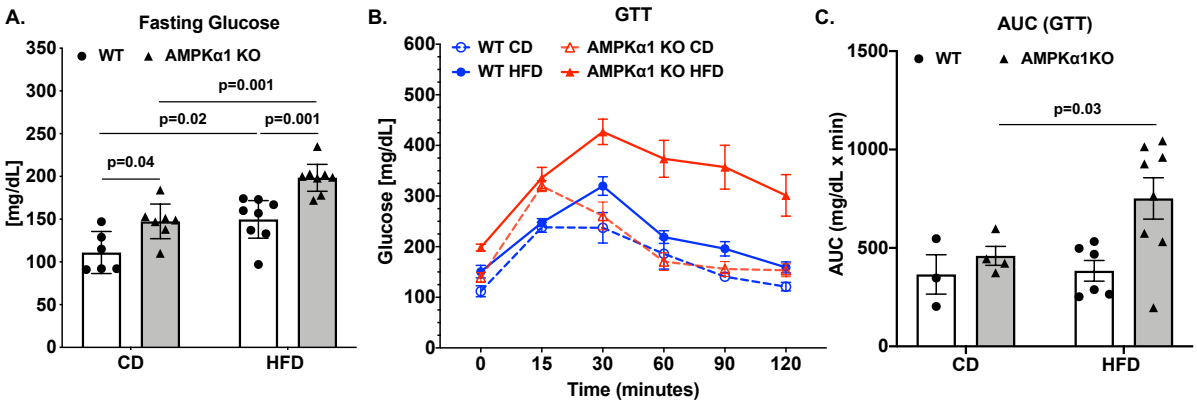

Figure 2: AMPKα1 Knockout male mice exhibited prominent glucose intolerance. Aged matched WT and AMPKα1 KO mice were fed on both high-fat diet (HFD) and control diet (CD). At 40 weeks after diet was initiated, the mice were subjected to 8 hours fasting. The baseline blood glucose (fasting glucose) levels were determined (A) and Glucose Tolerance Test (GTT) was performed (B) and presented as AUC (C). WT CD (n=3), WT HFD (n=6), AMPKα1KO CD (n=4), and AMPKα1KO HFD (n=8). Two-way ANOVA plus Tukey comparison test was used for the statistical analysis.

We also measured plasma insulin, leptin and TNFα levels. As depicted in Figure 3, insulin levels were markedly increased in WT but not AMPKα1 KO mice fed on HFD, compared to CD. Despite leptin was greatly increased in both WT and AMPKα1 KO mice on HFD compared to CD, the levels of leptin in AMPKα1 KO mice were significantly lower than that of WT mice. Figure 3, in part is being prepared for submission for publication of the material upon addition of more data. Nguyen, Nhi Ngoc Quynh; Liu-Bryan, Ru. The thesis author was one of the primary investigators and co-authors of this material.

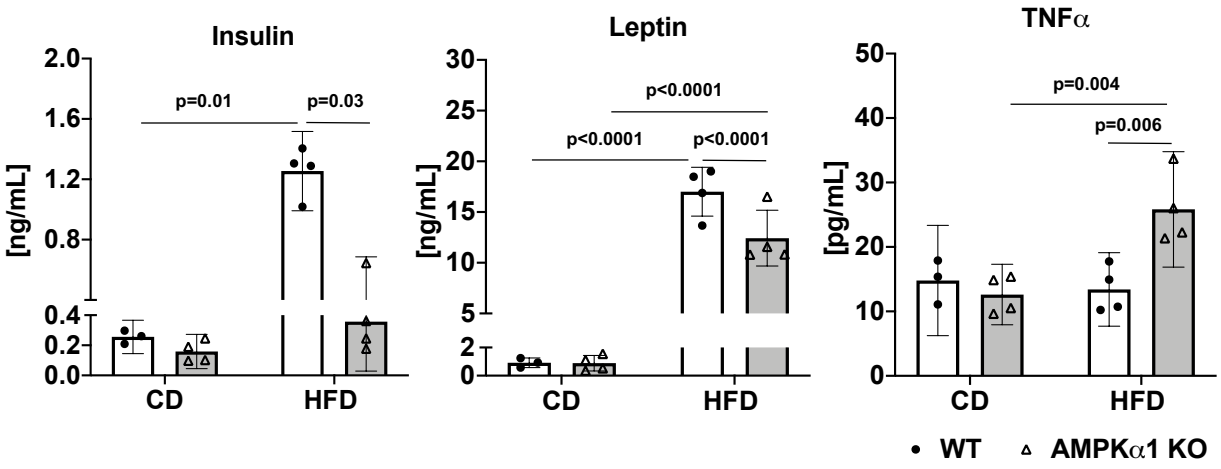

Figure 3: Differential metabolic alterations between WT and AMPKα1 KO mice after a long-term HFD. Aged matched WT and AMPKα1 KO mice were fed on both high-fat diet (HFD) and control diet (CD) for 40 weeks. The plasma levels of insulin, leptin and inflammatory cytokine TNFα were assessed by MSD ELISA analysis. WT CD (n=3), WT HFD (n=4), AMPKα1KO CD (n=4), and AMPKα1KO HFD (n=4). Two-way ANOVA was used for the statistical analysis.

Assessment of OA in both WT and AMPKα1 KO mice after 40 weeks' HFD

The OARSI score system as described in Materials & Methods was used to evaluate cartilage degradation. As depicted in figure 4, AMPKα1 KO, but not WT mice display a significant cartilage degradation (indicated by black arrows in A) after a long-term HFD. In comparison, little cartilage damage was observed in both WT and AMPKα1 KO mice after a long-term CD. Figure 4, in part is being prepared for submission for publication of the material upon addition of more data. Liu-Bryan, Ru. The thesis author was one of the primary investigators and co-authors of this material.

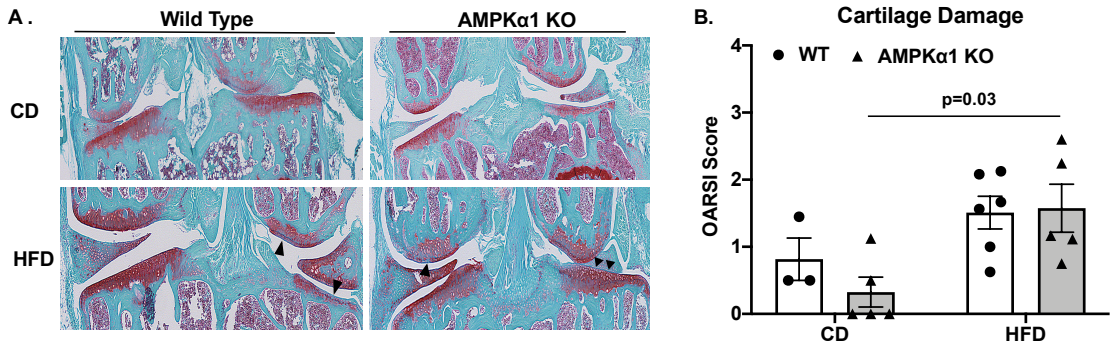

Figure 4: Assessment of mouse knee OA. Aged matched WT and AMPKα1 KO mice were fed on both high-fat diet (HFD) and control diet (CD) for 40 weeks. Mouse knees were collected, fixed, decalcified, sectioned and histologically stained with safranin-O and fast green (A) as described in Material and Methods (B) and Cartilage damage was examined using OARSI score system was determined using the method described in Materials and Methods. WT CD (n=3), WT HFD (n=6), AMPKα1 KO CD (n=5), and AMPKα1 KO HFD (n=5). Images in A are representative of WT and AMPKα1 KO mice on both HFD and CD. Two-way ANOVA was used for the statistical analysis.

Discussion

In this study, we investigated if AMPK is involved in regulation of obesity-induced OA using mouse model of HFD-induced obesity. We found that AMPKα1 KO mice gained similar amount of body weight to WT mice after a long-term (40 weeks) HFD. However, AMPKα1 KO mice on HFD had profound glucose intolerance compared to WT mice, which was likely due to insufficient insulin and leptin resistance as AMPKα1 KO mice on HFD exhibited significantly lower of plasma insulin and leptin levels. Systemic inflammation was also observed in AMPKα1 KO mice after HFD, as plasma TNFα levels were significantly higher in AMPKα1 KO compared to WT mice. OA assessment of these mice revealed that AMPKα1 KO mice on HFD displayed mild but significant cartilage damage, which was not seen in WT mice.

As expected, mice fed a long-term HFD became obese (27). Both WT and AMPKα1 KO mice were put on the HFD at similar age (about 3 months old). Despite that AMPKα1 KO had a slightly lower body weight than WT, they gained similar amount of weight after HFD. AMPK is involved in the regulation of bone metabolism (28). Previous studies showed that AMPKα1 KO have delayed bone

development, supported by that AMPKα1 KO have reduced bone mass in the early stage of development but it is possible that they can catch up to the WT mice when they reach to the skeletal mature age (29).

AMPKα1 plays an important role in regulation of glucose homeostasis (30). Although AMPKα1 KO mice gain a similar amount of weight when compared to WT under HFD, AMPKα1 KO mice exhibit a severe glucose intolerance. Unexpectedly, WT mice on HFD did not show a decreased glucose tolerance. This may be resulted from overnight fasting for 8 hours, as mice have high metabolic rate and consume most of their calories at night (31). In addition, these mice were on mixed genetic background (C57BL6/129), which may not develop consistently glucose intolerance as seen in pure C57BL/6 mice after HFD (32). C57BL/6 mice are prone to dietary obesity and metabolic disturbances related to obesity however recent studies have indicated a significant amount of phenotypic variation in response to HFD (33). Leptin is known to be upregulated in an obese state (34). Indeed, plasma leptin was significantly induced by HFD in both WT and AMPKα1 KO. Insulin plays an important role in regulation of blood glucose. We observed that plasma insulin levels were significantly increased only in WT but not in AMPKα1 KO mice after a long-term HFD. This may at least in part explain glucose intolerance in AMPKα1 KO mice under HFD condition. Obesity is associated with chronic inflammation which is marked by increase in inflammatory cytokines such as TNFα (35). Significant induction of plasma TNFα levels was seen in AMPKα1 KO mice after HFD.

AMPKα1 KO mice after long-term HFD mice exhibited mild OA phenotype (cartilage degeneration). This was correlated with the systemic metabolic perturbations observed. It is reported that leptin expression is increased in articular tissue such as cartilage, osteophytes and subchondral bone (34). Since osteophyte formation and subchondral bone thickening are also key features of OA, it would be interesting to assess and compare the impact of long-term HFD on these features in WT and AMPKα1 KO mice. Activation of AMPK can limit oxidative stress and inflammatory processes in chondrocytes (11). When chondrocytes are exposed to proinflammatory cytokines and nutrient stress, mitochondrial function is impaired. This can lead to accumulation of reactive oxygen species (ROS), inhibition of AMPK activity and downregulation of downstream signaling sirtuin 1 (SIRT1) and peroxisome

proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), the master regulator of mitochondrial biogenesis (6). Whether HFD-induced cartilage damage in AMPKα1 KO mice is due to mitochondrial dysfunction through SIRT1 and PGC-1α signaling remains to be determined.

This study has some limitations. One limitation is that the numbers of mice were low. HFD-induced obesity can lead to metabolic syndrome and multitude of different pathologies shortening the lifespan of the mice. Although we started with a good number of mice, not all the mice make it to the end of the study, likely due to complications related to long-term effect of metabolic alterations. Another limitation is that only male mice were used for this study, because we did not want to introduce a bias of using female mice due to cyclic intra-individual variability across the estrous cycle in females. Estrogen is shown to have beneficial effects on metabolism (8) and a chondroprotective effect in cartilage-specific deficient AMPKα1/α2 KO female mice (36). Given that the incidence of OA increases in women after menopause, it would be important to explore how AMPK is regulated in obesity-induced OA development in female mice.

In conclusion, mice deficient in AMPKα1 have profound metabolic disturbances (obesity, glucose intolerance, insulin and leptin resistance) and exhibited mild OA phenotype after a long-term HFD, suggesting dysregulation of AMPK contributes to obesity-induced OA development. Since dysregulation of AMPK is also shown to associate with injury-induced and age-related spontaneous OA; AMPK as a therapeutic target for OA could have a great potential.

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