Biglycan Deletion Alters Adiponectin Expression in Murine Adipose Tissue and 3T3-L1 Adipocytes

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Abstract

Obesity promotes increased secretion of a number of inflammatory factors from adipose tissue. These factors include cytokines and very lately, extracellular matrix components (ECM). Biglycan, a small leucine rich proteoglycan ECM protein, is up-regulated in obesity and has recently been recognized as a pro-inflammatory molecule. However, it is unknown whether biglycan contributes to adipose tissue dysfunction. In the present study, we characterized biglycan expression in various adipose depots in wild-type mice fed a low fat diet (LFD) or obesity-inducing high fat diet (HFD). High fat feeding induced biglycan mRNA expression in multiple adipose depots. Adiponectin is an adipokine with anti-inflammatory and insulin sensitizing effects. Due to the importance of adiponectin, we examined the effect of biglycan on adiponectin expression. Comparison of adiponectin expression in biglycan knockout (bgn−/−) and wild-type (bgn+/+) reveals higher adiponectin mRNA and protein in epididymal white adipose tissue in bgn−/− mice, as well higher serum concentration of adiponectin, and lower serum insulin concentration. On the contrary, knockdown of biglycan in 3T3-L1 adipocytes led to decreased expression and secretion of adiponectin. Furthermore, treatment of 3T3-L1 adipocytes with conditioned medium from biglycan treated macrophages resulted in an increase in adiponectin mRNA expression. These data suggest a link between biglycan and adiponectin expression. However, the difference in the pattern of regulation between in vivo and in vitro settings reveals the complexity of this relationship.

Introduction

Biglycan is a class I member of the small leucine rich proteoglycan (SLRP) family [1] and a component of the extracellular matrix (ECM). Biglycan is associated with bone formation, collagen interaction, and TGF-β signaling [2–4]. Biglycan is also able to signal through toll-like receptors in adipokines. Adiponectin is an adipokine that is anti-diabetic and promotor of fatty acid oxidation. Unlike other adipokines, adiponectin decreases with obesity [14–17]. Due to the importance of adiponectin as an adipokine, we wanted to examine the effect of biglycan on adiponectin production. The purpose of this study was first to characterize biglycan expression in various adipose depots in mice fed either low or high fat diet. Next, we examined a possible link between biglycan and adiponectin in different systems. We determined adipose tissue and serum adiponectin levels in biglycan knockout mice and in 3T3-L1 adipocytes with siRNA suppressed biglycan expression. We further examined the effect of biglycan on crosstalk between macrophages and adipocytes using macrophage conditioned medium from biglycan treated RAW 264.7 macrophages. Results of these studies show that adiponectin expression is higher in biglycan knockout mice compared to wild type mice. However, the in vitro studies indicate that biglycan may promote adiponectin production. Knockdown of biglycan in 3T3-L1 adipocytes resulted in reduced adiponectin expression, and addition of conditioned medium from biglycan treated macrophages induced adiponectin expression in 3T3-L1 adipocytes. These results show the complexity of the relationship between biglycan and adiponectin expression.

Materials and Methods

Animal Use

All animal care and use protocols in this study were approved by the Purdue Animal Care and Use Committee (PACUC). Animals were held under controlled environment at the Purdue small animal housing facility and all efforts were made to minimize discomfort. Heterozygous biglycan females on a C57BL/6J

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background were obtained from the Mutant Mouse Regional Resource Centers (Columbia, MO, USA) and were crossed onto wild-type C57BL/6j male mice. The progeny were backcrossed to yield wild-type (bgn+/0) and knockout (bgn−/−) male mice. The biglycan gene is located on the X chromosome; hence, male wild-type mice are bgn+/0 and male biglycan null mice are bgn−/−. This nomenclature is consistent with the original report characterizing these mice, as well as subsequent reports [18–20]. Mice were housed 2–3 to a cage. Animals were genotyped as previously described [21]. At eight weeks of age, bgn+/0 and bgn−/− mice were fed either a low fat diet (LFD, 10% kcal fat, #D12450B, Research Diets, New Brunswick, NJ USA) or a high fat diet (HFD, #D12492 Research Diets, New Brunswick, NJ USA) ad libitum for 10 weeks (n = 9–12 per treatment group) after which they were euthanized by CO2 asphyxiation for blood and tissue collection.

BMI Calculations
Mice were weighed and measured from the tip of the nose to the start of the tail prior to sacrifice. Body mass index (BMI) measurements were calculated using the following equation for mice: BMI = g/cm² [22,23].

Fasting Glucose and Insulin Measurements
Nine weeks into the diet, mice were fasted for 6 hours and fasting blood was collected. Fasting glucose was measured using a Freestyle® glucometer system (Abbott, Illinois, USA). Fasting blood was analyzed for serum insulin levels using an insulin enzyme-linked immunosorbent assay (ELISA) (Crystal Chem, Illinois, USA). The following cited in other mouse studies calculation was used to determine the homeostasis model of assessment of insulin resistance (HOMA-IR) index: [fasting plasma insulin (mU/l) x fasting glucose (mmol/l)]/22.5 [24,25].

Primary Cell Collection
Wild-type male C57BL/J mice, aged 4–5 months, were fed a high fat diet ad libitum for 2 weeks (average weight±SE = 44.56±1.67 g). Mice were fasted for 3 hours and then sacrificed. Epididymal adipose tissue from 4 mice were pooled into buffered saline (0.15 M NaCl, 10 mM HEPES, pH 7.4) for each replicate. The adipose tissue was then minced, rinsed with saline, then transferred to conical tubes containing collagenase type I at a concentration of 100 U/ml in Krebs-Ringer bicarbonate buffer cocktail (10 mM NaHCO₃, 10 mM HEPES, 5 mM D-glucose, 120 mM NaCl, 4.6 mM KCl, 1.25 mM CaCl₂, 1.20 mM MgSO₄, 1.20 mM KH₂PO₄, 6% BSA). The collagenase digestion mixture was incubated for 40 min at 37°C with gentle shaking. Cells were separated from large tissue by filtration through a 290 μm screen. The resulting solution was allowed to rest, allowing adipocytes to float to the top. The lower phase was centrifuged to collect stromal vascular cells. Cells were collected into Trizol (Invitrogen, Carlsbad, CA, USA) for RNA extraction.

3T3-L1 Cell Culture
3T3-L1 cells (ATCC, Manassas, VA) were differentiated as previously described [26]. Briefly, cells were grown at 5% CO₂ at 37°C in Dulbecco’s modified eagle media (DMEM) with 10% bovine calf serum supplemented with 1% penicillin-streptomycin mixture until cells reached 50% confluence. 3T3-L1 cells were then incubated in treatment media (DMEM, 0.1% bovine calf serum, 1% penicillin-streptomycin) with or without lipopolysaccharide (100 ng/ml, Sigma-Aldrich, St. Louis, MO, USA) or biglycan (10 μg/ml, bovine origin, Sigma-Aldrich, St. Louis, MO, USA) for 24 hours. Designated treatment groups were treated with LPS (100 ng/ml) in treatment media for 2 hours, after which cells were washed twice in phosphate buffered saline and then treated with or without biglycan (10 μg/ml) in treatment media for 22 hours. After treatments, media was harvested from the RAW 264.7 cells and spun for 3000 g for 5 min. Supernatant from macrophage conditioned media was then added to 3T3-L1 adipocytes 8 days post-differentiation. Adipocytes were incubated in the macrophage conditioned media for 24 hours, after which RNA was harvested for RT-PCR. All conditions were carried out at 5% CO₂ at 37°C.

Gene Expression Analysis
Total RNA was extracted from tissues homogenized in Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. RNA concentrations were determined using a Nanodrop reader (Thermo Scientific, Waltham, MA, USA). RNA samples were subjected to gel electrophoresis on a 0.8% agarose gel to check for degradation and genomic DNA contamination. We assessed the expression of select genes through RT-PCR. RNA samples were reverse transcribed using the Reverse Transcription system by Promega (Madison, WI, USA). PCR was performed on the Bio-Rad iCycler. The PCR reaction mix consisted of 0.5 ug of cDNA, 0.075 nmol of each of the primers, 1X of 2X RT-PCR Master Mix (SAbiosciences, Frederick, MD, USA); nuclease treated water (Ambion, Grand Island, NY, USA) and 1μl of Lipofectamine RNAiMax (Invitrogen, Carlsbad, CA, USA) at room temperature for 20 min. Cells were incubated at 5% CO₂ at 37°C for 48 hours, after which the medium was replaced with DMEM containing 10% fetal bovine serum for an additional 48 hours. Medium and cell samples were harvested for analysis.

siRNA Tranfection of Differentiated 3T3-L1 Cells
To perform the siRNA transfections, 3T3-L1 cells that were 6 days post-differentiation were trypsinized and plated onto siRNA/lipid complexes on 24-well plates at a concentration of 60,000 cells/well in DMEM with 10% fetal bovine serum without antibiotics. siRNA/lipid complexes were formed in wells by incubating 100 μl of OptiMEM (Invitrogen, Carlsbad, CA, USA) and either 6 pmol of biglycan target siRNA (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or scrambled siRNA (Ambion, Grand Island, NY, USA) and 1μl of Lipofectamine RNAiMax (Invitrogen, Carlsbad, CA, USA) at room temperature for 20 min. Cells were incubated at 5% CO₂ at 37°C for 48 hours, after which the medium was replaced with DMEM containing 10% fetal bovine serum for another 48 hours. Medium and cell samples were harvested for analysis.
Biglycan Deletion Regulates Adiponectin Expression

Tissue sections were fixed in 10% neutral buffered formalin and embedded in paraffin wax. 8 μm sections were mounted onto slides and deparaffinized in changes of xylene, 100% ethanol, 95% ethanol and 70% ethanol. Antigen retrieval was performed in sodium citrate buffer (10 mM sodium citrate, 0.05% tween 20, pH 6.0) heated to 95°C for 3 minutes. Sections were blocked in 50 mM Tris buffered saline pH 7.4 (TBS) containing 10% normal donkey serum and 1% BSA at room temperature for 2 hours. After blocking, sections were incubated in primary goat anti-biglycan antibody (Abcam, Cambridge, MA, USA) diluted in TBS containing 1% BSA overnight at 4°C. To control autofluorescence, sections were washed three times in TBS containing 0.025% Triton-X 100 then incubated in a solution of 0.1% sudan black dye in 70% ethanol for 30 minutes at room temperature following primary antibody incubation. Sections were then incubated in Alexa Fluor® 488 donkey anti-goat antibody (Invitrogen, Carlsbad, CA, USA) diluted in TBS 1% BSA for 1 hour in the dark at room temperature. Fluorescent images were captured with a Coolscan HQ CCD camera (Photometrics, Tuscon, AZ, USA) driven by IP Lab software (Scantangles Inc, Ontario, NY, USA) using a Leica DM6000 microscope (Leica, Buffalo Grove, IL, USA). Background was subtracted from the figures using the rolling ball algorithm (pixel = 50) in Image J (National Institutes of Health, Bethesda, MA, USA). Image and sample processing was performed in tandem. All contrast and brightness adjustments were performed in parallel.

Immunohistochemistry

Tissues were homogenized in radio-immunoprecipitation assay buffer and centrifuged at 10,000 g to generate protein samples. Protein concentrations were determined through bicinchoninic acid assay (Thermo Scientific, Rockford, IL, USA). Equal protein amounts were resolved on 10% SDS polyacrylamide gels. Proteins were electrothermically transferred onto nitrocellulose membranes for blotting. Successful transfer was assayed through Ponceau S staining. The following primary antibodies were used: anti-adiponectin (Biovision, Mountain View, CA, USA), anti-β-actin (Cell Signaling Technology, Danvers, MA, USA), anti-biglycan (Abcam, Cambridge, MA, USA), anti-Hsp90 (Cell Signaling Technology, Danvers, MA, USA). Membranes were developed using the Immobilon chemiluminescent HRP substrate kit (Millipore, Billerica, MA, USA).

Serum Adiponectin Measurements

To measure serum adiponectin measurements, we used a Quantikine® adiponectin ELISA kit (R&D Systems, Minnesota, USA) which measures full-length mouse adiponectin.

LDH assay

Medium from cell culture was measured using the lactate dehydrogenase (LDH) diaphorase kit from Cayman Chemical (Ann Arbor, Michigan, USA).

Western Blotting

Statistics

All statistics were performed using SAS software (SAS institute, Cary, NC, USA).

Data was analyzed using ANOVA through the proc MIXED procedure followed by separation of means by Tukey analysis. If data residuals were non-normal, data was transformed using the Box-Cox procedure. Student’s t-test was used where specified. P-values less than 0.05 were deemed significant.

Results

Distribution of Biglycan in Mouse Adipose Tissue

Figure 1A shows the gene expression of biglycan in LFD and HFD fed wild-type mice (n = 9–10) in multiple adipose depots, liver and gastrocnemius muscle. Biglycan expression was increased in all adipose depots under HFD. However, the increase in biglycan was significant in only the brown, mesenteric, and epididymal fat pads. No statistical differences were detected in the liver or gastrocnemius muscle. Immunohistochemistry was performed to determine the distribution of biglycan in epididymal white adipose tissue (EWAT) under LFD and HFD bgn+/0 mice (fig. 1B). Under LFD, biglycan signal in bgn+/0 mice was indistinguishable from background found in bgn−/− mice. However, under HFD, strong biglycan signal appeared around pericellular areas which are rich in extracellular matrix proteins. Western blot of the core biglycan protein in EWAT confirmed higher amount of biglycan in HFD fed mice (fig. 1C). To determine which cells within the adipose tissue are contributing to biglycan content, we measured biglycan transcript levels in primary adipocytes and stromal vascular cells (SVC) from EWAT of HFD fed wild-type mice. PPARγ and CD68 expression levels were used to confirm cell types (fig. 1D). Transcript levels of biglycan were not significantly different between primary adipocytes and SVC cells.

To determine the adipsity of bgn+/0 and bgn−/− mice, first we measured body weight and noted that bgn−/− mice had significantly higher body weights than bgn+/0 mice (Table 1). However, bgn−/− mice had higher levels of monomeric biglycan at 2 months of age which may impact body weight [18]. When measuring the length of the mice, we found that the bgn−/− mice were shorter in length than the bgn+/0 mice (fig. 1E). To determine the adiposity of bgn−/0 mice had an altered growth rates after 3 months of age which may impact body weight [18]. When measuring the length of the mice, we found that the bgn−/− mice were shorter in length than the bgn+/0 mice. Because the mice were 18 weeks of age at the time of sacrifice, differences in growth rates may have arisen between the genotypes, leading to different body weights between genotypes. To normalize adiposity between genotypes, other measures of adiposity were considered. BMI was not significantly different between the bgn−/− and bgn+/0 mice. Additionally, weights of excised adipose depots were expressed as a percentage of total body weight. As expected, high fat diet resulted in increased adipose depot percentages (Table 1). We also observed a significant interaction between genotype and diet in EWAT percentage, where HFD-fed bgn−/− mice had an increased percentage of EWAT when compared to HFD-fed bgn+/0 mice, suggesting an increased capacity for EWAT expansion when bgn−/− are HFD-fed. However, there was no genotype effect on the percentages of EWAT, SWAT or RWAT, suggesting similar adiposities between bgn+/0 and bgn−/− mice (Table 1).

Adiponectin transcript levels were measured in the EWAT of bgn+/0 and bgn−/− mice to determine whether the absence of biglycan affects adiponectin expression. Bgn−/− mice, regardless of diet, exhibited increased adiponectin transcript levels in EWAT when compared to bgn+/0 mice (fig. 2A). Next we compared adiponectin protein in EWAT of HFD fed mice (fig. 2B). Bgn−/− mice had higher levels of monomeric biglycan.
Figure 1. Biglycan expression in adipose tissue. Biglycan mRNA expression in adipose tissue of C57BL6/J wild-type mice fed either LFD or HFD (A). Biglycan expression is elevated in all the adipose depots of HFD mice; however, only mesenteric, brown, and epididymal adipose depots had a significantly higher level. Student's t-test were performed between LFD and HFD samples (*p<0.05). Sample sizes: brown fat (n = 6), mesenteric
Biglycan Deletion Regulates Adiponectin Expression

when compared to bgn+/0 mice. Circulating levels of adiponectin were also overall increased in the bgn−/− mice when compared to bgn+/0 mice (fig 2C). We did not detect a difference in circulating adiponectin between diets in either the bgn−/− or bgn+/0 mice. This result is consistent with other reports that show no decrease in serum adiponectin with long periods of HFD feeding despite changes in adiponectin transcript and protein as well as adiposity [27–29]. Because circulating adiponectin is higher in the bgn−/− mice, we hypothesized that bgn−/− and bgn+/0 mice may have differing levels of insulin sensitivity. In order to measure insulin sensitivity, we measured fasting insulin, fasting glucose and calculated HOMA-IR. Both fasting insulin and HOMA-IR were significantly affected by diet (p<0.001 for insulin and HOMA-IR) (fig. 3). Fasting insulin was overall significantly decreased in the bgn−/− mice, and HOMA-IR showed a trend (p=0.069) towards greater insulin sensitivity (lower HOMA-IR values) in bgn−/− mice.

Table 1. Mouse characteristics.

|          | bgn+/0 | bgn−/− | p-value |
|----------|--------|--------|---------|
| Body weight (g) | 28.487 ± 1.061 | 34.046 ± 1.546 | <0.001 |
| Length (cm) | 8.555 ± 0.164 | 8.930 ± 0.209 | <0.001 |
| BMI (g/cm²) | 0.389 ± 0.014 | 0.389 ± 0.015 | <0.001 |
| EWAT (g) | 0.681 ± 0.090 | 0.958 ± 0.156 | <0.001 |
| SWAT (g) | 0.444 ± 0.048 | 0.674 ± 0.210 | <0.001 |
| SWAT % | 1.545 ± 0.153 | 2.001 ± 0.283 | <0.001 |
| RWAT (g) | 0.231 ± 0.029 | 0.359 ± 0.083 | <0.001 |
| RWAT % | 0.795 ± 0.093 | 0.994 ± 0.190 | <0.001 |
| Gastrocnemius (g) | 0.465 ± 0.072 | 0.558 ± 0.071 | <0.001 |
| Gastrocnemius % | 1.619 ± 0.238 | 1.384 ± 0.203 | <0.001 |
| Liver (g) | 1.186 ± 0.163 | 1.235 ± 0.057 | <0.001 |
| Liver % | 4.132 ± 0.435 | 3.824 ± 0.343 | <0.001 |

|          | genotype | diet | gene × diet |
|----------|----------|------|-------------|
| Body weight (g) | 0.001 | <0.001 | 0.972 |
| Length (cm) | <0.001 | 0.001 | 0.304 |
| BMI (g/cm²) | 0.827 | <0.001 | 0.838 |
| EWAT (g) | 0.857 | <0.001 | 0.016 |
| SWAT (g) | 0.012 | <0.001 | 0.283 |
| SWAT % | 0.096 | <0.001 | 0.756 |
| RWAT (g) | 0.008 | <0.001 | 0.279 |
| RWAT % | 0.144 | <0.001 | 0.872 |
| Gastrocnemius (g) | 0.607 | 0.670 | 0.289 |
| Gastrocnemius % | 0.196 | 0.005 | 0.474 |
| Liver (g) | 0.176 | 0.013 | 0.243 |
| Liver % | 0.752 | 0.538 | 0.402 |

Lengths of individual mice were measured from the nose to anus. Organ weights were divided by total body weight to express organs as a percentage of body weight. Results are represented as mean ± SE. EWAT = epididymal white adipose tissue, SWAT = subcutaneous white adipose tissue, RWAT = retroperitoneal white adipose tissue. When a significant diet by genotype interaction was present, means were separated by Tukey analysis and superscript letters are used to indicate significantly different means. P-values less than 0.05 are deemed significant.

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through RT-PCR (data not shown). We treated RAW264.7 macrophages with combinations of lipopolysaccharide (LPS) and biglycan as listed in figure 5A. Because RAW264.7 macrophages are naïve, RAW264.7 cells were first pre-treated with LPS for 2 hours for activation before treatment with biglycan. Activation of macrophages with LPS did not induce biglycan expression (data not shown). When 3T3-L1 differentiated adipocytes were treated with macrophage conditioned media (MCM), MCM from biglycan treated macrophages induced higher adiponectin expression than in 3T3-L1 adipocytes treated with MCM without biglycan (figure 5B). In addition, treatment of 3T3-L1 adipocytes with MCM from RAW264.7 macrophages that had been treated with LPS and biglycan resulted in higher adiponectin mRNA when compared to adipocytes treated with MCM from macrophages that were treated with LPS alone (figure 5B). However, direct treatment of 3T3-L1 adipocytes with biglycan had no effect on adiponectin expression (figure 5C). These data indicate that biglycan can indirectly act through macrophage conditioned medium to influence adiponectin expression, implicating a potential role for biglycan in the crosstalk between macrophages and adipocytes. Next, we measured transcript levels of TNFα, IL-6, and IL-1β in the treated RAW 264.7 cells to determine if the adiponectin response was due to changes in inflammatory cytokines coming from the RAW 264.7 cells. However, as shown in figure 5D, there were no significant changes in TNFα, IL-6, or IL-1β mRNA levels in the RAW 264.7 macrophages due to biglycan treatment.

**Discussion**

Extracellular matrix (ECM) components display dynamic expression during obesity. The ECM is remodeled during adipose...
Figure 4. Knockdown of biglycan in 3T3-L1 adipocytes. A) Biglycan expression from 3T3-L1 mature adipocytes treated with siRNA against biglycan ("si biglycan") or nontargeting siRNA ("scrambled"). Results are from RT-PCR and western blot for core biglycan protein (representative blot). (n = 3 replicates) B) Adiponectin mRNA and secreted measurements from siRNA treated 3T3-L1 adipocytes. (n = 3 replicates) C) PPARγ and FAS expression measured by RT-PCR. (n = 3 replicates) D) Concentration of LDH in the medium of siRNA treated 3T3-L1 adipocytes. For all graphs in figure 4, results are expressed as mean ± SE, *p < 0.05 target vs. scrambled. 

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A

Treatment
control
control, biglycan
LPS all
LPS, biglycan
LPS, wash

0 hrs
2 hrs
24 hrs, harvest media

RAW 264.7
treatment media
biglycan 10μg/ml
LPS 100ng/ml
2x wash, biglycan (10μg/ml)
2x wash, treatment media

B

3T3-L1 adipocytes treated with MCM

| adipoletin/18S mRNA | MCM control | MCM biglycan | MCM LPS, all | MCM LPS, biglycan | MCM LPS, wash |
|---------------------|-------------|--------------|--------------|------------------|---------------|
| C                   | B           | B            | BC           | B                | B C           |


C

3T3-L1 adipocytes treated with biglycan

| adipogenin/18S mRNA | Control | biglycan 10μg/ml |
|---------------------|---------|------------------|
| n.s.                |         |                  |


D

RAW 264.7 macrophages treated for 24 hours

| Relative expression | control | biglycan | LPS, all | LPS, biglycan | LPS, wash |
|---------------------|---------|----------|----------|---------------|-----------|
| TNFα                | B B B   | B B B A  | A A      | A B B B       | B B B     |
| IL-6                | B B B   | B B B A  | A A      | A B B B       | B B B     |
| IL-1β               | B B B   | B B B A  | A A      | A B B B       | B B B     |
We show that while biglycan knockout mice on HFD relative to controls implicates a negative mechanism that suppress adiponectin expression. Several studies have linked increased oxidative stress and inflammation to reduced adiponectin expression. Since activation of PPARγ is associated with increased adiponectin expression, inhibition of PPARγ by nuclear factor kappa B (NFκB) during obesity could be a potential link between obesity and reduced adiponectin expression. We have provided evidence that biglycan knockout mice have reduced adipose tissue inflammation indicated by lower expression of inflammatory markers such as IL-6, TNFα and CD68. Thus the lack of biglycan in the knockout mice will prevent the inhibitory effect of inflammation on adiponectin expression, hence the higher adiponectin expression in the bgn−/− mice. On the other hand, suppression of biglycan in vitro in 3T3-L1 cells may send a yet unknown signal into the cell that suppresses adiponectin expression. Since biglycan and adiponectin interact leading to sequestration of adiponectin, the lack of biglycan might indicate that less adiponectin is needed for the same level of available adiponectin for bioactivity. Additionally, culture of cells in vitro on plastic does not perfectly replicate the in vivo conditions of adipocytes in adipose tissue due to the absence of other cellular and non-cellular tissue components. Furthermore, knock down of adiponectin in 3T3-L1 adipocytes did not lead to an alteration in the inflammatory state of the cells, marking another major difference between biglycan absence in the in vivo and in vitro models. Instead, the expected disruption of collagen matrix formation in the 2-D culture condition in the absence of biglycan on plastic surface could affect the integrity of the extracellular matrix, and perhaps extracellular matrix characteristics that may be necessary for adiponectin expression. The induction of adiponectin in adipocytes treated with MCM from biglycan and biglycan and LPS treated macrophages may suggest that these treatments lead to production of yet unidentified factors that induce adipocytes to increase adiponectin expression. What remains constant through both the in vivo and in vitro results is that the absence of biglycan can impact adiponectin expression, implicating a mechanism where adipocytes can sense biglycan abundance to regulate adiponectin production.
In summary, our findings show an increase in biglycan expression in adipose tissue during obesity. We also observed a modest increase in adiponectin in bgn−/− mice; however, transient knockdown of biglycan in 3T3-L1 cells resulted in decreased adiponectin expression. These studies elucidate a complex mechanism by which adipocytes are able to sense biglycan presence in both in vivo and in vitro settings to regulate adiponectin expression.

Further work will be needed to clarify the true nature of this relationship.

**Author Contributions**

Conceived and designed the experiments: KA MW. Performed the experiments: MW. Analyzed the data: KA MW. Contributed reagents/materials/analysis tools: KA MW. Wrote the paper: KA MW.