DsbA-L protein levels in white adipose tissue in an obesity model.

Madison E. Kerley

University of Louisville

Follow this and additional works at: https://ir.library.louisville.edu/honors

Part of the Biology Commons

Recommended Citation
Retrieved from https://ir.library.louisville.edu/honors/172

This Senior Honors Thesis is brought to you for free and open access by the College of Arts & Sciences at ThinkIR: The University of Louisville's Institutional Repository. It has been accepted for inclusion in College of Arts & Sciences Senior Honors Theses by an authorized administrator of ThinkIR: The University of Louisville's Institutional Repository. This title appears here courtesy of the author, who has retained all other copyrights. For more information, please contact thinkir@louisville.edu.
DSBA-L PROTEIN LEVELS IN WHITE ADIPOSE TISSUE IN AN OBESITY MODEL

By

Madison Kerley

Submitted
in Partial Fulfillment of the Requirements for Graduation summa cum laude
and for Graduation with Honors from the Department of Biology

University of Louisville
Louisville, Kentucky

May, 2018
DsbA-L Protein Levels in White Adipose Tissue in an Obesity Model

By

Madison Kerley

A Thesis Approved on

March 26, 2018

by the Following Thesis Committee:

________________________________________

Dr. Joseph Steffen

________________________________________

Dr. Michael Menze

________________________________________

Dr. Eugene Mueller
Abstract

DSBA-L PROTEIN LEVELS IN WHITE ADIPOSE TISSUE IN AN OBESITY MODEL

Madison Kerley
March 26, 2018

Adiponectin is an adipose tissue-secreted protein found in three isoforms. Disulfide bond A oxidoreductase-like protein (DsbA-L) is thought to assist with protomeric disulfide bonding to generate the HMW (high molecular weight) isoform, which regulates insulin sensitivity. Both adiponectin and DsbA-L were previously found to negatively correlate with obesity.

Immunoblots were conducted on adipose tissue samples from male mice fed a high or low-fat diet for 6, 10, or 16 weeks. Immunoblots from high-fat diet-fed mice revealed double bands for DsbA-L. High and low molecular weight bands were analyzed together, revealing significantly higher relative band densities with 10 and 16-week high-fat diet feedings compared to 6-week high-fat diet feedings, as well as with 10-week low-fat diet feedings compared to 6-week low-fat diet feedings. 6-week high-fat diet feedings showed higher relative band density than 6-week low-fat diet feedings. Results indicate significant DsbA-L expression upregulation with increased exposure time to a high-fat diet.
LIST OF TABLES

TABLE 1: Information on Samples Utilized .........................................................9

TABLE 2: Factorial ANOVA of All High-Fat and Low-Fat Diet-Fed Mice..............11
LIST OF FIGURES

PAGE

FIGURE 1: Diagram highlighting the complex multimerization of adiponectin and the proposed role of DsbA-L in the process…………………………………………………………2

FIGURE 2: Bradford Protein Assay standard curve ……………………………………………………9

FIGURES 3A and B: Results of two immunoblots on 6, 10, and 16-week high-fat and low-fat diet-exposed samples………………………………………………………………………10

FIGURE 4: Graphical representation of relative band densities of DsbA-L in mice exposed to either a high-fat or low-fat diet for 6, 10, or 16 weeks…………………………………12

FIGURE 5: Comparison of the relative band densities of the higher molecular weight DsbA-L bands seen in the high-fat diet exposure over time …………………………………………13
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>v</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>HYPOTHESES</td>
<td>5</td>
</tr>
<tr>
<td>METHODS</td>
<td>6</td>
</tr>
<tr>
<td>RESULTS</td>
<td>9</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>14</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>20</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>21</td>
</tr>
</tbody>
</table>
INTRODUCTION

Adiponectin is a secreted protein hormone originating from the endoplasmic reticulum of white adipocytes that has been found to influence insulin sensitivity in liver and muscle cells, among other effects (1, 2). Its protomeric form consists of a globular C-terminal domain and a collagen-like N-terminal domain. Adiponectin can be found circulating in one of three multimeric isoforms: a trimer (low molecular weight, LMW), a hexamer (middle molecular weight, MMW), and an isoform containing up to 18 protomers (high molecular weight, HMW) (1, 2). The circulating levels of the HMW form regulate insulin sensitivity in target tissues (3, 4).

A study originally published by Arita et al (1999) and validated many times since showed that adiponectin levels negatively correlate with body mass index (BMI) (5, 6). BMI is a weight-to-body surface area ratio calculated using the individual’s weight (kg) divided by the height (in meters squared); BMI is frequently used to categorize obesity levels (7). A BMI of <25 kg/m² is considered to be within a normal range, with a BMI of 25-30 kg/m² being classified as overweight and a BMI of ≥30 kg/m² being classified as obese (7). However, an increase in adiposity has been shown to initially correlate to increase adiponectin levels before chronic obesity causes them to eventually decline (8).
Blood levels of the HMW form appear to correlate negatively with testosterone levels (9). This gender-based variation in adiponectin levels occurs in conjunction with puberty, with males having significantly lower adiponectin levels during and after puberty compared to females (10). Furthermore, high-fat feeding of mice significantly lowers the adiponectin mRNA levels in epididymal fat (11). The lowering of adiponectin levels in males appears to be through testosterone inhibition of adiponectin secretion from adipocytes (9).

![Diagram](image)

**Figure 1.** Diagram highlighting the complex multimerization of adiponectin and the proposed role of DsbA-L in the process (12).

A 2008 review detailed several adiponectin-interacting proteins that act as regulators of adiponectin multimerization (Figure 1) (12). Many of these, such as Ero1-La (endoplasmic reticulum oxidoreductase 1 alpha), PDI (protein disulfide isomerase), and DsbA-L (disulfide bond A oxidoreductase-like protein), are enzymes involved in the process. However, each of their roles has yet to be fully explored. DsbA-L is currently the only protein thought to be involved in assisting the conversion from hexameric
(MMW) to multimeric (HMW) adiponectin (12). Both ERp44 (endoplasmic reticulum protein 44) and GGA1 (golgi associated, gamma adaptin ear containing, ARF binding protein 1) appear to be involved in preparing adiponectin to leave the cell (12).

The focus of this study, DsbA-L, is a glutathione S-transferase-K1 (GST-K1) found to be largely expressed in white adipose tissue (2). DsbA-L has been found in the mitochondria, peroxisomes, and the endoplasmic reticulum (ER) of adipocytes (2, 8). DsbA-L has levels of expression that are negatively correlated with obesity, much like HMW adiponectin levels (13). The present study is an attempt to replicate this previous work in order to combine the findings with those of studies regarding the amount of HMW adiponectin and peroxisome proliferator-activated receptor gamma (PPAR-γ) levels found in high-fat vs low-fat diet fed mice.

Overexpressing DsbA-L in adipose tissue leads to an increase in circulating HMW adiponectin (13). However, the role that DsbA-L plays in adiponectin multimerization is not fully understood. It is possible that DsbA-L functions as a chaperone protein, assisting in the folding of adiponectin (2). Adiponectin protomers are presumed to associate through hydrophobic interactions in their globular domains to form trimers. Inter-trimer disulfide bonds are required for higher order multimerization (4). Near the N-terminus of protomeric adiponectin, there is a universally conserved cysteine residue. Substituting this residue with serine or alanine prevents adiponectin from undergoing multimerization (1). DsbA-L interacts with glutathione to create a thiolate ion, potentially in order to maintain an oxidizing environment for further reactions (4). Because of this, DsbA-L may play a role in linking adiponectin protomers into higher order structures before protein secretion. A recent study on transgenic mice lacking
DsbA-L suggests that the absence of this protein does not impair levels of adiponectin oligomers (8); it is possible that chaperone proteins with overlapping functions may compensate for an absence of DsbA-L (4).

Since obesity correlates with decreased adiponectin levels (5, 6) and DsbA-L has been found to be, at the very least, important for adiponectin multimerization, a study was designed to determine the effects of high-fat feeding (as a model for obesity) on DsbA-L expression. In this study, differences in DsbA-L expression levels will be determined in white adipose tissue obtained from mice fed either a high-fat or a low-fat diet. This data will ultimately be correlated with the isoforms of adiponectin present in white adipose tissue.

This study has the potential to extend the knowledge regarding the mechanism by which adiponectin levels change in obesity. Since DsbA-L has been determined to play a key role in adiponectin multimerization (14), this study could expand upon current knowledge on the effects of obesity and obesity-related disorders (i.e. Type-2 diabetes). This could allow for novel treatments that would affect adiponectin multimerization at its source instead of affecting the resulting responses (i.e. insulin sensitivity in tissues). The findings of this study will also be combined with studies regarding the amount of HMW adiponectin and peroxisome proliferator-activated receptor gamma (PPAR-γ) levels found in high-fat vs low-fat diet-fed mice to expand knowledge of adiponectin multimerization.
Hypotheses:

Since obesity has been demonstrated to reduce adiponectin levels (specifically the HMW isoform), and because DsbA-L is thought to be related to the regulation of adiponectin multimerization, it is hypothesized that there will be significantly lower levels of DsbA-L expression in the white adipose tissue of mice fed a high-fat diet as compared to those of mice fed a low-fat diet, assuming that DsbA-L is a critical regulatory factor. It is also anticipated that there will be a relationship between DsbA-L expression and time exposed to a high-fat diet, with longer diet exposure time correlating with lower levels of DsbA-L expression.
METHODS

*Animals.* In this study, 8-week old male C57BL/6J mice were donated from the Hill Research Laboratory at the University of Louisville (14). These mice had been fed either a high-fat (60% fat/20% carbohydrate/20% protein) or a low-fat (10% fat/70% carbohydrate/20% protein) diet from Research Diets, Inc. (New Brunswick, NJ).

*Sample Preparation.* For each group of mice, 3 white adipose tissue samples were obtained from the epididymal fat pad. These samples were homogenized in ice-cold lysis buffer consisting of 50 mM Heps (pH 7.6), 150 mM sodium chloride, 1% NP-40, 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM sodium fluoride, 1 mM potassium pyrophosphate, 1 mM sodium orthovanadate, 5 μg/mL Alfa Aesar Protease Inhibitor Cocktail I (Haverhill, MA), and 1 mM phenylmethylsulfonyl fluoride (15). The homogenate was centrifuged (10,000 × g, 4°C, 10 min), and the supernatant was stored at −80°C until used.

*Protein Assay.* After homogenization, protein concentrations were determined using the Bradford dye-binding method (16) with a protein assay kit from Bio-Rad (Hercules, CA). BSA served as the standard for comparison.

*Immunoblot.* Immunoblots were carried out in non-reducing 10% polyacrylamide gels utilizing the equivalent of 10 micrograms of protein from each sample along with
Laemmli buffer. Two reference ladders were also used: a biotinylated Protein Ladder from Cell Signaling Technology (Danvers, MA) and a Dual Color Precision Plus Protein Standard from Bio-Rad Laboratories (Hercules, CA). The gel was run at 120 V for 1-2 h, depending on how rapidly separation occurred. The proteins were transferred to a nitrocellulose membrane on ice at 100 V for 1 h before Ponceau staining in order to ensure complete protein transfer. Tris-buffered saline with Tween 20 was then used to wash Ponceau stain.

**Antibody Testing.** The membrane was cut into 3 sections for antibody testing, with a cut being made for testing the biotinylated ladder and a cut between the 25 and 37 kD markings (shown by the Dual Color Ladder) to separately test for the presence of β-actin (45 kD) and DsbA-L (25 kD). TBST was mixed in 5% w/v skim milk in order to block the membrane. The biotinylated ladder was then placed in TBST in 5% skim milk with anti-biotin HRP-linked antibody from Cell Signaling Technology. To test for the presence of DsbA-L, rabbit anti-mouse DsbA-L from Phosphosolutions (Aurora, CO) was used. The presence of β-actin was tested for utilizing β-actin antibody from Cell Signaling Technology. β-actin is a constitutive protein with levels not expected to vary between samples. The membranes and their respective antibodies were rotated at 4°C for 12-18 hours, after which they were each rinsed 5 times with TBST. Anti-rabbit IgG HRP-conjugated secondary antibody from Cell Signaling Technology was used as the secondary antibody for the DsbA-L and β-actin, along with TBST and skim milk. The secondary antibodies were left in contact with the membrane for 1 h at room temperature, after which they were washed 3 times with TBST.
Chemiluminescent Detection. Chemiluminescent detection was carried out using Lumiglo (Cell Signaling Technology) and radiography film. The film images were digitized and subject to analysis using Image-J software from the National Institute of Health.

Results were expressed as the ratio of the DsbA-L band density from each immunoblot to the band density of its respective 6 week HFD sample. Means of these ratios were determined and statistically analyzed by ANOVA and post-hoc one-tailed student t-tests.
RESULTS

Table 1

Information on Samples Utilized

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Sample Type</th>
<th>Weight (mg)</th>
<th>Protein Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HF 6 week</td>
<td>59.6</td>
<td>1.15</td>
</tr>
<tr>
<td>2</td>
<td>HF 6 week</td>
<td>92.6</td>
<td>0.627</td>
</tr>
<tr>
<td>3</td>
<td>LF 6 week</td>
<td>33.6</td>
<td>0.675</td>
</tr>
<tr>
<td>4</td>
<td>LF 6 week</td>
<td>44.8</td>
<td>0.697</td>
</tr>
<tr>
<td>5</td>
<td>HF 10 week</td>
<td>111.5</td>
<td>0.829</td>
</tr>
<tr>
<td>6</td>
<td>HF 10 week</td>
<td>109.5</td>
<td>0.700</td>
</tr>
<tr>
<td>7</td>
<td>LF 10 week</td>
<td>90.9</td>
<td>1.48</td>
</tr>
<tr>
<td>8</td>
<td>LF 10 week</td>
<td>60.5</td>
<td>1.01</td>
</tr>
<tr>
<td>9</td>
<td>HF 16 week</td>
<td>81.7</td>
<td>1.44</td>
</tr>
<tr>
<td>10</td>
<td>LF 16 week</td>
<td>116.8</td>
<td>0.936</td>
</tr>
<tr>
<td>11</td>
<td>LF 16 week</td>
<td>90.5</td>
<td>1.74</td>
</tr>
</tbody>
</table>

*Animals.* HF stands for high-fat diet and LF stands for low-fat diet. The mouse adipose tissue samples utilized ranged from 33.6-116.8 mg (see Table 1). A standard curve was generated using albumin as a standard (see Figure 2). The slope of this standard curve was used to calculate concentrations of protein in each white adipose tissue sample lysate (see Table 1).
**Figure 2.** Bradford Protein Assay standard curve. The dotted line represents the best fit line given by the equation.

**Immunoblot.**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3A and B.** Results of two immunoblots on 6, 10, and 16-week high-fat and low-fat diet-exposed samples. Lanes on both images are numbered and designated as follows: 1= 6-week high-fat diet, 2= 6-week low-fat diet, 3= 10-week high-fat diet, 4= biotinylated ladder, 5= dual color ladder, 6= 10-week low-fat diet, 7= 16-week high-fat diet, 8= 16-week low-fat diet.

Samples from each group were loaded into the wells for each gel run, with 1 each of the 6, 10, and 16-week high-fat and low-fat diet-fed mouse adipose tissue samples.
being used in each gel. Image-J software was utilized to quantify band densities for each immunoblot. The background noise-reduced bands are displayed in Figures 3A and B. In order to compare samples across the blots, all band density values were divided by the band density values obtained by using both the high and low molecular weight bands found in the 6-week high-fat diet exposure. This was made necessary by the inability to detect normalization bands from either β-actin or β-tubulin.

Since there were double bands detected in the high-fat diet condition, factorial ANOVAs were run to compare both high-fat diet bands to the low-fat diet bands, as well as to compare just the lower molecular weight bands. Later, the higher molecular weight bands from the high-fat diet exposure condition were compared to each other in a single-factor ANOVA to determine the relationship between diet exposure time and higher molecular weight band density.

Table 2
Factorial ANOVA of All High-Fat and Low-Fat Diet-Fed Mice

<table>
<thead>
<tr>
<th>ANOVA Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure</td>
<td>0.672</td>
<td>2</td>
<td>0.336</td>
<td>6.34</td>
<td>0.0331</td>
<td>5.14</td>
</tr>
<tr>
<td>Diet</td>
<td>0.687</td>
<td>1</td>
<td>0.687</td>
<td>13.0</td>
<td>0.0113</td>
<td>5.99</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.156</td>
<td>2</td>
<td>0.0778</td>
<td>1.47</td>
<td>0.303</td>
<td>5.14</td>
</tr>
<tr>
<td>Within</td>
<td>0.318</td>
<td>6</td>
<td>0.0530</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.83</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 summarizes the factorial ANOVA comparing relative band densities for both diet conditions under each time exposure, using the Image-J derived relative band densities for the high and low-fat diet exposed mice. Both the high and low molecular
weight bands were used to determine relative band density of the high-fat diet-exposure condition. DsbA-L expression differed significantly with both exposure time (p= 0.033) and type of diet (p= 0.011). The fact that the relative band densities were higher with high-fat diet exposure than with low-fat diet exposure to a statistically significant degree caused a rejection of the null hypothesis of no difference between samples, meaning that there was a difference between DsbA-L expression in high-fat diet-fed mice and low-fat diet-fed mice over time. There was no interaction effect between type of diet and time exposed to the diet.

Since a double band was observed only in the high-fat-diet-exposed mice, a second factorial ANOVA was run in which the relative band density of only the lower molecular weight bands of high-fat-diet-exposed mice were compared to the bands of the low-fat-diet-exposed mice. The band densities for the high-fat diet exposure condition and low-fat diet exposure condition over time were not found to be significantly different. This implied that the difference between the low-fat and high fat diet exposures solely originated from the added presence of the higher molecular weight band in the high-fat diet exposure condition.
**Figure 4.** Graphical representation of relative band densities of DsbA-L in mice exposed to either a high-fat or low-fat diet for 6, 10, or 16 weeks. Asterisks (*) represent statistically significant (P< 0.03) differences between high-fat diet conditions over time (6 and 10-week, as well as 6 and 16-week). Plus signs (+) represent statistically significant (P< 0.03) differences between high-fat and low-fat diet conditions within the same exposure time (6-week high and 6-week low-fat diet exposures). Vertical lines (|) represent statistically significant (P< 0.03) differences between low-fat diet conditions over time (6 and 10-week).

Figure 4 compares the average relative band densities of both high-fat and low-fat diet exposed mice at 6 weeks, 10 weeks, and 16 weeks utilizing both bands. The 6-week high-fat diet-fed samples were used as the standard, as they had been hypothesized to have a low band density. Post-hoc t-tests were carried out to determine where statistically significant differences occurred. There was a statistically significant difference between the 6 and 10-week diet exposures, both within high-fat (P= 0.020) and within low-fat diet (P= 0.024) exposures. There was also a statistically significant difference between 6 and 16-week diet exposure in the high-fat diet condition (P= 0.028), as well as between 6-week high-fat and 6-week low fat diet conditions (P= 0.008). Since these differences
were all significant, with the high-fat diet fed mice demonstrating a greater level of expression, the hypothesis that there would be significantly lower DsbA-L expression in the white adipose tissue of high-fat diet fed mice as compared to low-fat diet fed mice was rejected.

Since there was found to be little to no variation between the lower molecular weight DsbA-L bands of high-fat-diet-exposed and low-fat diet-exposed mice, a one-way ANOVA was carried out using the relative band densities from only the higher molecular weight DsbA-L bands of the high-fat diet exposed mice. This one-way ANOVA compared high fat-diet exposures for 6 weeks, 10 weeks, and 16 weeks. The 16-week diet condition was treated as the standard of comparison for the other two groups, since it had the least variability between samples.

![Upper Band Density vs Diet Exposure Time](image)

**Figure 5.** Comparison of the relative band densities of the higher molecular weight DsbA-L bands seen in the high-fat diet exposure over time.

There was not found to be a statistically significant difference (P= 0.236) between diet exposure time and relative band densities in the high-fat diet exposure condition. This caused an acceptance of the null hypothesis that longer high-fat diet exposure did not correlate with lower DsbA-L expression. However, Figure 5 demonstrates a
possibility that between 6 and 16 weeks of high-fat diet feedings, there could be an
increase in at least the higher molecular weight band of DsbA-L, consistent with a higher
expression of DsbA-L.
DISCUSSION

In this study, male mice were exposed to 6, 10, or 16 weeks of a high or low-fat diet. White adipose tissue samples were obtained from their epididymal fat pads, with weights ranging from 33.6-116.8 mg. Samples were homogenized in lysis buffer and a Bradford assay was carried out on each sample in order to determine the amount of sample necessary to run equivalent protein concentrations for each sample on an immunoblot.

It had been hypothesized that there would be significantly lower DsbA-L expression in the white adipose tissue of high-fat diet fed mice as compared to low-fat diet fed mice and that there would be a relationship between DsbA-L expression and time exposed to a high-fat diet, with longer exposure correlating with lower DsbA-L expression. The hypothesis was based upon multiple previous studies that found a correlation between obesity and lower levels of adiponectin (5, 6). However, higher levels of HMW adiponectin have been found to correlate with increased DsbA-L expression (13). Immunoblots demonstrated a difference between high and low-fat diet exposures. Only the samples from the high-fat diet condition exhibited two bands, with one being the expected DsbA-L band at 25 kDa and the other being an unexpected higher molecular weight band at 30 kDa. The low-fat diet exposure condition only displayed the
25 kDa band. This second band had not been observed in any previous studies carried out on DsbA-L.

Unfortunately, the β–actin normalization band did not appear. This prevented the DsbA-L results from being expressed relative to this potentially unchanging control protein. β–tubulin was also tried as a normalization control, but also did not appear. As a result, all DsbA-L bands were compared to the 6-week high-fat diet-fed samples in order to control for differences occurring between the immunoblots. The 6-week high-fat diet-fed samples were used because they had been hypothesized to have low expression levels.

A factorial ANOVA was carried out on both bands observed in the high-fat diet-fed group along with those from the low-fat diet-fed group to determine whether the relative band densities of the two treatments varied significantly and if there was a relationship between type of feeding and exposure time. There were statistically significant differences with regard to exposure time and diet type, causing a rejection of the null hypothesis that there would be no difference in DsbA-L expression in white adipose tissue between high-fat and low-fat diet feedings. There was no interaction effect between exposure time and diet type. The presence of the higher molecular weight band in the high-fat diet exposure was the cause of the variation in relative band densities between high and low-fat diet feedings.

Post-hoc t-tests revealed that there was a significant difference between relative band densities in mice from 6 to 10-weeks, both within high-fat (P= 0.020) and within low-fat diet (P= 0.024) conditions; the 10-week condition condition had higher average relative band densities then the 6-week in both cases. The difference between 6 and 16-
week high-fat diet exposure conditions was also statistically significant (P= 0.028), with the 16-week high-fat diet exposure condition having a higher average relative band density. Additionally, there was a statistically significant difference between 6-week high-fat and 6-week low-fat diet exposure conditions (P= 0.008), with the high-fat diet exposure condition resulting in a larger average relative band density. The hypothesis that that there would be significantly lower DsbA-L expression in the white adipose tissue of high-fat diet fed mice as compared to low-fat diet fed mice was ultimately rejected.

Additionally, the higher molecular weight bands from the high-fat diet exposure were compared in a one-way ANOVA. A statistically insignificant variation occurred between the relative band densities, which caused an acceptance of the null hypothesis that longer high-fat diet exposure did not correlate with lower DsbA-L expression. However, it should be noted that there does appear (in Figure 5) to be an increase in higher molecular weight band density over time in the high-fat diet exposure condition.

Previous research indicates that there is an initial increase in adiponectin levels before the development of chronic obesity (8, 17). However, further studies showed that these levels dropped after 10 weeks of high-fat diet exposure in mice (17). DsbA-L is a protein that has been determined to serve a role in the multimerization of adiponectin (13). DsbA-L has also been shown to be negatively correlated with chronic obesity, but before the present study, DsbA-L expression levels as a result of high-fat diet feedings had been relatively unexplored, except for a study by Liu et al in 2012 (13).

Although the double bands observed for the high-fat diet exposure condition on the immunoblot appear to indicate that there may be increased expression of DsbA-L with prolonged exposure to high-fat feeding, this may not be the case. The variation in
bands could be the result of a loss of enzyme functionality, potentially by some means of post-translational modification to DsbA-L, such as disulfide bonding, phosphorylation, carbonylation, glycosylation, or ubiquitination. The expected band appeared around 25 kDa, but the other band occurred at a higher molecular weight (around 30 kDa). This had not been observed in previous studies. Since it was observed in nearly all high-fat diet exposed samples, it is not thought that the second band resulted from contamination.

There are two exposed cysteine residues on DsbA-L, both the 27th and 176th amino acid residues in the sequence (18). It is possible that one of these residues could be interacting with other molecules (or the other cysteine) and forming disulfide bonds, which could cause issues with a cofactor binding to DsbA-L or could affect the interaction of DsbA-L and adiponectin. Since the difference between the higher and lower molecular weight bands for DsbA-L in the high-fat diet exposure was around 5 kDa, disulfide bonding would not account for all the molecular weight variation between the two bands. Although DsbA-L interacting with other DsbA-Ls or other proteins via disulfide bonds could potentially affect its interaction with adiponectin, it may not account for the locations of the double bands observed in this experiment.

Phosphorylation is a post-translational modification known to regulate the activity of proteins (19). Phosphorylation can occur on various residues throughout proteins, with one being serine. Serine phosphorylation of various proteins, like the insulin receptor substrate proteins, has been a proposed mechanism of insulin resistance in general, so it could possibly also have an effect on related processes like adiponectin multimerization (20). As for the functionality of the protein, phosphates attached to the serine residues
block the active site that adiponectin interacts with, which would inhibit the ability of adiponectin to multimerize.

Additionally, obesity is suggested to be related to an increased level of oxidative stress (2). Reactive oxygen species (ROS) may result from this stress; many of these are free radicals (22). ROS are also created when Ero1-La is activated (23). These free radicals can modify proteins (23). One free radical reaction is carbonylation, which is the addition of a carbonyl group to an existing protein (22). Insulin resistance in mice has been found to be related to increased ROS and carbonylation of proteins, among other things (22). Carbonyl groups, like phosphate groups, could be added to the serine residues, which could also render DsbA-L inactive (22).

In summation, there are lower levels of the high molecular weight (HMW) form of adiponectin in obesity. This has been hypothesized to result from decreased levels of DsbA-L. The present study demonstrates that in mice, DsbA-L is expressed more among mice fed high-fat diets than those fed low-fat diets. Not only this, but there also is an effect attributed to the amount of time the mice are fed the diet. It is possible that some post-translational modification is occurring to DsbA-L that causes an increase in its molecular weight along with at least partial inactivation of protein function.

**Study Limitations**

Only male mice were utilized in this experiment, and the adipose tissue was obtained from only the epididymal region. Since testosterone is negatively correlated with the HMW form of adiponectin, the results obtained may not be consistent with findings obtained from female mice. Additionally, there were relatively few tissue samples utilized in this experiment, since only two samples were used per group. The
adipose tissue used may have not been representative of all mice under the same conditions. 10-well gels were utilized, meaning that all the samples were not able to run at once. Therefore, there may have been subtle differences in the outcome from one gel run to another that resulted in a variation in results obtained.

Additionally, there is a possibility that the gels were run for an improper amount of time, as some samples did not move down the blot at the exact same speed as others. The two DsbA-L bands on the high-fat fed samples could have represented some sort of contamination. Furthermore, there was no measure of activity for the DsbA-L.

**Clinical Implications**

The findings of this study could lead to more research into DsbA-L and whether its functioning is hindered under prolonged exposure to a high-fat diet. This would be important in determining the mechanism by which HMW adiponectin levels decrease with prolonged high-fat diet exposure. Finding out how adiponectin levels decrease would have further implications for obesity-related conditions, such as insulin resistance. The effects of obesity can potentially be reversed or at least stopped if the process that halts or slows adiponectin multimerization can be reversed or stopped.
CONCLUSION

There is a significant difference in DsbA-L expression between high-fat and low-fat diet exposure, as well as among varying exposure times to the diets (6, 10, and 16 weeks). Further studies should investigate more samples of the high-fat diet exposure conditions over time and should also investigate female mice under the same conditions. Additionally, further studies should also examine covalent modifications of DsbA-L.
REFERENCES


doi:10.1152/ajpendo.00245.2

https://www.ebi.ac.uk/pdbe/entry/pdb/1yzx/protein/1.

biology-learning-center/protein-biology-resource-library/pierce-protein-
methods/phosphorylation.html.

phosphorylation of insulin receptor substrate-1 and increased expression of
p85alpha: the two sides of a coin. Diabetes, 55(8), 2392-2397. doi:10.2337/db06-
0391

21. Marseglia, L., Manti, S., D'Angelo, G., Nicotera, A., Parisi, E., Di Rosa, G., Gitto, E.,

22. Frohnert, B. I., Sinaiko, A. R., Serrot, F. J., Foncea, R. E., Moran, A., Ikramuddin, S.,
Choudry, U., Bernlohr, D. A. (2011). Increased adipose protein carbonylation in
human obesity. Obesity (Silver Spring), 19(9), 1735-1741.
doi:10.1038/oby.2011.115

disulphide bond formation and endoplasmic-reticulum-generated oxidative stress.
EMBO Rep, 7(3), 271-275. doi:10.1038/sj.embor.7400645