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IL-4 Western Blot Analysis of High Fat Diet and Control Diet Sprague Dawley Rats

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ABSTRACT

The Lowry Protein Assay is a fundamental process in the ultimate procedure of Western Blotting; it is used to determine the protein content of a solution, which is essential in calculating the necessary concentration of antibodies. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels were prepared with a 12% resolving gel and 5% stacking gel. After running the SDS-PAGE, we performed a western blot analysis on our samples. Western blot results showed that there was a significant difference between IL-4 expression in our two different animal experimental groups. Animals with a maternal high fat diet showed increased expression of IL-4, but animals with a maternal control diet showed lower expressions of IL-4. Methylated DNA immunoprecipitation also confirmed our results and showed a region of hypermethylation upstream of the IL-4 gene in the control group.

INTRODUCTION

Obesity is a relevant challenge for the global population. Obesity affects individuals for a variety of reasons and has many symptoms, including stroke, type 2 diabetes, and various cancers (Wang, 2007; Poirier, 2006; Rexrode, 1997; Larsson, 1981; Bostick, 1994; Garland, 1985). In the United States of America, obesity substantially lends itself to the overall deaths as a consequence of the aforementioned symptoms (McGinnis, 1993). According to the national data, the obesity in the United States is on the increase in adults and children (Lyznicki, 2001). Obesity can occur if there is an excess in food consumption, accretion of fatty intake or abridgement of correct nutrition intake, reduction of physical activity, or a consequence of a mother's prenatal diet.

When a mother is pregnant, the habits she expresses tend to be stressed in the offspring. For instance, if a mother adopts a healthy diet, the child may have a small chance of developing a large appetite. If a mother has a high dietary intake, the child may have a larger appetite and be more vulnerable to obesity and its symptoms (Brion, 2010).

The design of this experiment included the analysis of a western blot for Interleukin -4 (IL-4), an antibody that has anti-inflammation affects, and its relation to rat livers. A Lowry Protein Assay will be conducted to find the concentration of protein in solutions, and using the data, samples will be created and utilized for the western blot (Ferrero, 2013). The western blot will reveal how much IL-4 is in the high fat (HFC) and control (BC) diet samples from the livers of Sprague-Dawley rats. I hypothesize that if we take liver samples from the HFC rats, we will see an increased expression of IL-4.

LITERATURE REVIEW

The United States is one of the leading country for obesity, and obesity is extremely prevalent in the United

States. In the past 40 years, obesity has increased by more than 50% (Ogden, 2014. NHLBI, 1998). According to the Center for Disease Control and Prevention (CDC), obesity in the United States is increasing. From a study in 2011, the fifty states had individual obesity percentages. In 2012, the same test revealed that some of the states' had become more susceptible to obesity (CDC, 2012). Obesity has several possible origins, including increased consumption, decreased physical activity, and proneness to health risks induced by maternal prenatal patterns (Brion, 2010). Maternal studies indicate that prenatal diets affect offspring after birth. Abnormal gene expression is present when nutrition patterns are atypical, as suggested by DNA methylation (Lillycrop, 2005).

According to studies, IL-4 is an anti-inflammatory cytokine. It has affects that can alleviate the inflammation symptoms presented by obesity (Zhao, 2006; Marie, 1996). Due to its qualities and multi-purpose design, actin is a ubiquitous control (Johnson, 2012). Because of its usefulness, actin fits the purpose of Western Blots when they are being analyzed. Comparing it to the other protein gives vital visual and numerical data.

METHODOLOGY

Lowry Protein Assay

Collect eight 1.5 mL tubes and label them 0, 5, 10, 20, 30, 40, 50, 60. Use a pipette to add diluted BSA to each of the eight tubes in the uL amount labeled on tube. Add 10 uL of each sample to the sample tubes. Use an electronic pipette to add 1 mL TCA to each tube. Place the tubes in 40 degrees Celsius for 10 minutes. Centrifuge the tubes in the 40 degree Celsius temperature for 20 minutes. Gently pour off supernatant. Speed dry the samples for up to 2 hours to centralize the protein. Add 100 uL of o.2505/0.2 NaOH to each tube and vortex for a few seconds. Add 650 mL of Lowry Cu Reagent to each tube and vortex for a few seconds. Incubate the samples at room temperature for 10 minutes. Add 60uL of 1:1 ddH20 and Folin-Ciocalteu Reagent. Incubate the samples at room temperature for 10 minutes. Load 200 uL of each sample on a flat 96 well plate. Load the plate into the spectrophotometer and read absorbance at 630nm. The spectrophotometer will give you measurements that will be necessary to prepare the samples for the Western Blot.

SDS-PAGE

Collect the materials necessary for the apparatus and the gels. We will use a 50mL tube to prepare the bottom layer and a 15mL tube to prepare the top layer. The gels are composed of 2 layers. The bottom layer, the 12% resolving gel, is added to the apparatus first. To create it, aliquot 3.3 mL of ddH2O, 4 mL of 30% A + B, 2.5 mL 1.5 M Tris-HCl (pH 8.8), 100 uL of 10% SDS, 100 uL of 10% APS, and 4 uL of TEMED in the 50 mL tube. The TEMED will catalyze the crosslinking. After the TEMED is added, close the tube and shake it to mix the ingredients. Use a pipette to distribute the resolving gel into about ¾ of both compartments of the apparatus. Add ddH2O to both compartments to level the resolving gel. While the resolving gel solidifies, prepare the stacking gel. Add 2.25 mL of ddH2O, 670 uL 30% A + B, 1 mL 0.5 M Tris-HCl (pH 6.8), 40 uL 10% SDS, 40 uL and 10% APS to the 15 mL tube. Do not add the TEMED to the stacking gel until a very fine line is visible. This line indicates the resolving gel's solidification. Dump the top layer of water. Add 4 uL of TEMED to the stacking gel and immediately add the contents to the apparatus. Place a comb in both of the compartments to sculpt wells. When the stacking gel solidifies, place the gels in the gel electrophoresis apparatus. Prepare the running buffer for the apparatus. To do so, place a 3 L pitcher on the stir machine and drop the stir bar magnet in it. Measure 90 g of the Tris-Base, 432 g of Glycine, and 30 g of SDS. Add about 1.5 to 2 L of ddH2O to the pitcher. Start the stir machine and slowly add the reagents. Stir for 10 minutes on a medium speed. Pour everything from the pitcher into the cylinder. Only fill the cylinder to the 2 L mark. Pour the contents of the cylinder into the running buffer pitcher. If there are left over contents in the pitcher, pour that into the cylinder again. Add ddH2O into the cylinder until it reaches 1 L. Pour the contents into the running buffer pitcher. Pour the running buffer into the gel electrophoresis apparatus until it reaches the top of the container. Use a pipette to inject 10 uL of the Standard to the designated wells and 25 uL of each sample in the designated wells. Set the gel electrophoresis at 80 volts for 30 minutes as Set 1 and at 110 volts for 150 minutes as Set 2.

Transfer

Prepare the transfer device by pouring transfer buffer into the container. Create the transfer buffer by mixing 150 mL of Towbin x10 with 300 mL of methanol and 1050 mL of distilled water (dH2O). Place the red plate of the device above the black plate. Lay two sponges horizontally across the black plate. Place two filter sheets vertically across the sponge. Collect two membranes and gently write distinct markings, such as your initials and/or date, on one side. Soak the membrane sheets in methanol for five seconds each. Place one membrane on each filter sheet with the side with the marking face up. Remove the gels from the electrophoresis apparatus and place them on each membrane. Place one filter page on each gel. Lay a sponge across the filter pages. Close the open device by bringing the red plate on top of the last sponge. Put this device in the second gel electrophoresis apparatus and match the red plate with the apparatus' red plate and the device's black plate with the apparatus' black plate. Set the cycle for 1 hour at constant 0.3 amps.

IL-4 Western Blot

When the cycle is complete, extract the membranes and discard the gel into the biohazard. Prepare a 5% milk mixture by diluting milk to 5% of TBST/.1% Tween 20. Prepare the TBST/.1% Tween 20 by measuring 300 mL of 10xTBS into a cylinder and add to a pitcher. Measure out 2 L of ddH2O into the cylinder and add it to the pitcher until the pitcher reads 2 L. Measure 3 mL of Tween 20 with an electronic pipette with a 5 mL tip into the pitcher. Pipette up and down for 1 minute to get the residual Tween 20 out of the tip. Stir for 10 minutes on a medium speed. Pour the pitcher's contents into the cylinder up to the 2 L mark. Pour the contents of the cylinder into the TBST/.1% bottle. Add any remaining liquid from the pitcher into the cylinder. Add ddH2O into the cylinder until it reach 1 L. Pour the contents into the TBST/.1% bottle. Pour some of the milk into two containers. Place one membrane, words face up, into each milk container. Put the two containers onto the orbital shaker and block them for 1 hour. Incubate the primary for 14 hours at 1:1000 5% TBST/.1% Tween 20. Dilute the TBST/.1% Tween 20 to 3%. Wash it for 30 minutes. Incubate the secondary for 1 hour at a 1:5000 scale with 3% TBST/.1% Tween 20. Wash the membranes for 30 minutes in TBST/.1% Tween 20 diluted to 1%. Add 450 uL of SuperSignal to membrane and take a picture.

Actin Western Blot

Wash the membrane in pure TBST/.1% Tween 20 (no milk added) for 30 minutes. Block it in 3% TBST/.1% Tween 20 for 1 hour. Incubate the primary with 3% TBST/.1% Tween 20 for 3 hours. Wash it in 1% TBST/.1% Tween 20 for 30 minutes. Incubate the secondary at a 1:5000 scale with 1% TBST/.1% Tween 20 for 1 hour. Wash it in 1% TBST/.1% Tween 20 for 30 minutes. When the wash is over, add 450 uL of SuperSignal to each membrane and take a picture.

Statistical Analysis

The calculations that succeeded the measuring of the Western Blot's intensities were entered in a two sided t-test at alpha equal to .05.

FINDINGS

Figure 1 indicates the BSA standard from the Lowry Protein Assay. The R^2 value is 0.996. The closer the R^2 value is to 0.1, the more valid the data is.

Figure 1:
Lowry Assay BSA standard measurements.

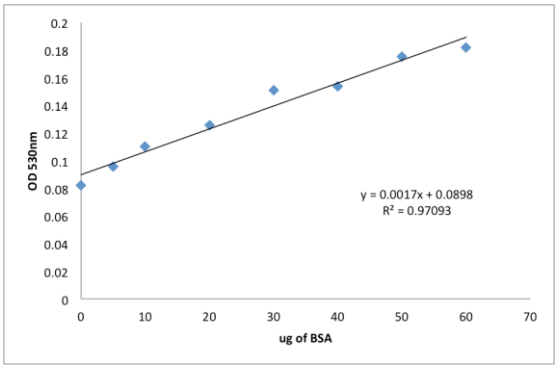
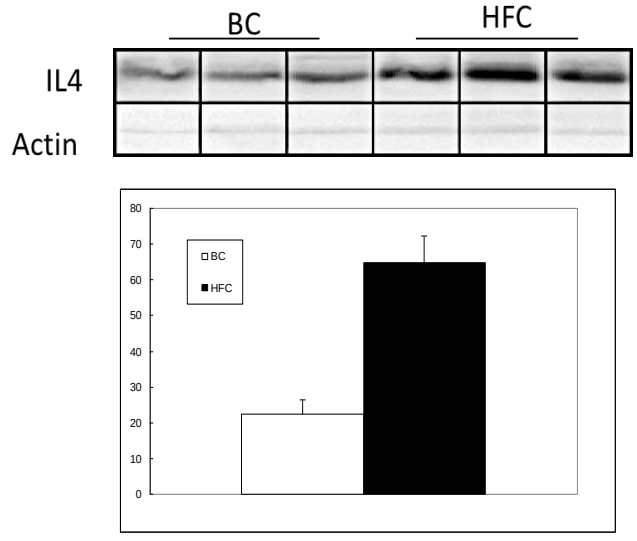


Figure 2 indicates a Western Blot of IL-4 compared to Actin. The row of IL-4 shows the concentration of IL-4 viewed through the camera. The HFC portion is much darker than that of the BC, suggesting HFC has a higher concentration of IL-4 proteins.

Figure 2:
Protein concentration determined through western blot analysis.



CONCLUSIONS

In the experiment, rat livers were ground up and inserted into sample tubes to be tested later for protein concentration. At the end of the experiment, it was found that the IL-4 protein had a substantial presence in the HFC diet livers while the protein in the BC diet was very minimal. The presence of the IL-4 proteins in the HFC samples was astounding because they were indeed more than that of the BC samples. The Actin remained in constant concentration and intensity, lending validity and standardization can h to the legitimization of the IL-4 results. This is similar to an experiment involving DNA methylation of a Cox-2. The more the Cox-2 was methylated, the less was recognizable. The less methylation that occurred, the more susceptible Cox-2 was to recognition. The BC diet rats had more methylation, thus less IL-4 proteins read. However, the HFC diet rats received less methylation, which is supported by the data that suggests a relatively tremendous concentration of the protein in the fatty livers.

Obesity causes many functional errors in the body that must have the appropriate response. When the liver is inflamed as a consequence of obesity, the concentration of IL-4 proteins aggregates possibly because IL-4 studies indicate that it is an anti-inflammatory cytokine. When the rat livers were tested for protein concentration, livers from rats with high fat diets and control diets were used. The high fat diet livers were hypothesized to contain more IL-4 because of its theorized effect on inflammation as a result of obesity. The hypothesis was successfully supported by the data because in figure 2 the HFC diet's IL-4 concentration was accentuated.

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