Effects of protein deficiency on organ size in *Mus musculus*

Abstract:

The amount of protein (standard animal chow and protein-deficient chow) accessible to mice was manipulated to determine if size of the pancreas, heart, and testes is affected by protein deficiency. Organs were harvested and measures of the organ mass/body mass, total protein, and DNA were determined and statistically tested for significance. In the no protein condition the pancreas mass/body mass ratio and pancreatic protein content were significantly lower than in control mice. In contrast, there were no statistically significant changes in organ mass/body mass ratio or protein content of the testes and heart during protein deficiency. Additionally, no significant differences were found in the amount of DNA in any of the three organs. The observation that total protein, but not total DNA, of the pancreas decreased during protein deficiency suggests that changes in cell size, and not cell number, are responsible for the decrease in pancreas weight during protein deficiency. Interestingly, the observation that testes size, DNA, and protein content are maintained indicates that in the face of the significant environmental stress of lack of dietary protein, reproductive capability is likely maintained with other vital life functions.

Introduction:

Organisms are constantly altering their diets in response to food ability, competition, predation, and a multitude of other factors. How do organisms physiologically change in response to variations in diet? Which physiological processes are affected when an Alaskan brown bear switches from a low protein diet of wild berries

and roots to a protein rich diet of salmon returning to spawn? Countless other examples exist in nature where organism experience annual, seasonal, and daily differences in food availability. The purpose of this study is to elucidate the effects of dietary protein deficiency on the size, and thus the function, of various organs in mice. The organ chosen for this study were the heart, pancreas, and testes, which play a crucial role in circulation, digestion, and reproduction. Adequate circulation is absolutely necessary for the transfer and removal of nutrients and wastes under any condition. Therefore, the prediction was made that regardless of dietary protein intake the mass (an indirect measure of organ function) of the heart would be maintained. In contrast, maintenance of the pancreas was expected to decrease during protein deficiency, since its major role in digesting dietary protein is non-essential under these conditions. The main focus of interest is the question of whether the mass of the testes of mice will be maintained when faced with protein deficiency. One could argue that reproduction is a non-essential life function and thus would be turned off when resources are in low supply. On the other hand, in terms of evolution the success of an organism is measured by its genetic contribution to the future. If environmental conditions appear unfavorable an organism may sacrifice its own health and biological needs in order to reproduce. A classic example of this occurs every year as salmon migrate upstream to their original riverbeds to spawn and soon after die. It is hypothesized that in mice facing protein deficiency mass of the testes will be maintained, since in nature mice have a rapid turnover rate and the success of the future generations may take priority over individual success. This issue can be more broadly applied to the field of ecology by observing how changes in diet effect organ growth. Prolonged periods of protein deficient diets may be a problem for organisms that inhabit temperate or artic

regions where certain nutrient rich foods are not available throughout various time of the year. It is also interesting to infer the impact of diet on reproductive life histories.

Methods and Materials:

Experimental Design: The 8 mice used in this experiment were on a 12/12 hour light/dark cycle and kept at room temperature during the duration of the study. 4 days prior to the tissue harvesting, ½ of the mice were randomly selected and were switched from the standard University of Michigan rodent chow (LabDiet; Richmond, VA) to a isocaloric, but protein deficient chow (Dyets; Bethlehem, PA) and were given free access to the chow until the completion of the study. At the completion of the study, the mice were killed using carbon dioxide and the target organs were harvested and frozen in liquid nitrogen.

Organ weight/body weight: Measures of the body mass of the mice were taken before the harvesting of tissue and measures of the organ weight were taken prior to tissue homogenization to give the organ wt/ body wt ratios.

Measures of DNA and Protein: Measures for total protein content in the organs was determined by homogenizing 100mg of tissue in 2ml of solution containing 0.1% Triton X-100 and 5mM $MgCl_2$ and immediately sonicated for 15secs to further disrupt the tissue. Following the sonication the total protein content was determined spectrophotometrically using BioRad protein dye. Measures of the total DNA content were measured using a luminescence spectrometer and a DNA quantification kit from Sigma-Aldritch.

Immunoblot Analysis (Western blotting): Equal amounts of protein were run on a SDS-PAGE (polyacrylamide gel electrophoresis) and afterwards transferred to a nitrocellose

membrane. The membrane was blotted with milk for 1 hr and rinsed to remove any non-specific binding. Subsequently, placed overnight into a solution containing antibodies for GAPDH or s6p (a ribosomal protein). The following day the membranes were rinsed and incubated with the secondary antibody for 1 hr, washed, and developed using enhanced chemoluminescence. Electronic images of the membranes were taken and used for comparison of control and protein deficient tissue.

Statistical Analysis: Data are expressed as means ± standard deviation and were analyzed using a 2-tailed t test in the program SPSS. *P* values <0.05 were considered significant.

Results:

In the pancreas the mean organ wt/ body wt ratio in the control condition was 7.51 ± 0.62 (mg/g), whereas in the protein deficient mice the mean organ wt/ body wt ratio was 5.53 ± 0.77 (mg/g) (Figure 1). Differences between the control and protein deficient mice were statistically significant and had a *p*-value of 0.007 (Table 1). In the heart the organ wt/ body wt ratios were 5.17 ± 0.24 (mg/g) in the control condition and 5.33 ± 0.31 (mg/g) (Figure 1). The heart wt/ body wt ratios in the control and protein deficient mice are very close and have a statistically insignificant *p*-value of 0.459 (Table 1). The ratios of organ wt/ body wt in the testes in the control and protein deficient mice were also statistically insignificant having a *p*-value of 0.572 (Table 1). In the control condition the organ wt/ body wt ratio was 6.57 ± 1.23 (mg/g), whereas the protein deficient mice had a slight larger ratio of 7.13 ± 1.41 (mg/g) (Figure 1).

To gain insight into the types of changes protein deficient organs undergo measurements of the total DNA (a rough estimate of cell numbers) were taken to determine if protein deficient organs contained less total cells and/or equal number, but

smaller cells. In the pancreas, the mean values for the control condition was 0.82 ± 0.10 (mg/tissue) and the mean value in the protein deficient condition was .80 ± 0.09 (mg/tissue) (Figure 2). The numbers are not statistically significant and have a *p*-value of 0.769 (Table 1). In the control condition of the heart a mean value of 0.26 ± 0.08 (mg/tissue) was measured and a mean value of 0.22 ± 0.05 (mg/tissue) was measured in the protein deficient mice (Figure 2). A statistically insignificant *p*-value of 0.404 was determined for the heart tissue (Table 1). The *p*-value of 0.637 determined in the testes was also statistically insignificant (Table 1). The measure of the DNA content in the control condition of the testes was found to be 0.51 ± 0.12 (mg/tissue) and a similar average value of 0.55 ± 0.09 (mg/tissue) was found in the protein deficient mice (Figure 2). In all three organs no significant change in DNA content was observed.

Measurements of the total protein in the different organs yielded results similar to those found in the organ wt/ body wt ratios. In the control condition of the pancreas a mean value of 38.43 ± 5.68 (mg/tissue) was observed and a mean value of 18.13 ± 1.96 (mg/tissue) was observed in the protein deficient subjects (Figure 3). A significant *p*-value of 0.001 was found between the control and protein deficient conditions of the pancreas (Table 1). In the heart a mean value of 76.00 ± 13.4 (mg/tissue) was measured in the control and a mean value of 68.63 ± 4.30 (mg/tissue) was found in the protein deficient condition (Figure 3). The similar mean values in the heart lead to an insignificant *p*-value of 0.336 (Table 1). In the testes a difference between the control mean value of 65.60 ± 20.94 (mg/tissue) and the protein deficient mean value of 45.85 ± 9.19 (mg/tissue) was noticed, but a *p*-value of 0.135 failed to reject the null hypothesis (Figure 3, Table 1).

In the immunoblot for the protein gapdh there are no clear differences in protein expression between the protein deficient and control mice (Figure 4). This is likely attributed to the fact that gapdh performs various housekeeping activities, and is thus conserved. In the immunoblot for the protein s6p, a ribosomal protein, it is clear that protein expression in the pancreas is less in protein deficient mice in comparison to the control mice (Figure 5).

Discussion:

Much research has been done looking at the effects of nutritional stress on the body. For example, in a study on California voles it was observed that their choice of diet varied in breeding and non-breeding seasons. After giving the voles feed with differing nutrient content, it was inferred that nutrition plays an important role in reproduction (Batzli, 1986). Researchers hypothesized that various nutrients play a key role in an organism's reproductive success. The voles used in this experiment were fed either a diet consisting of grass seeds or a laboratory chow. The chow and seeds fed to the voles differed in concentrations of calcium and sodium, but not protein (Batzli, 1986). Voles fed the low calcium and sodium grass seeds reproduced less than voles fed a standard laboratory chow (Batzli, 1986). This experiment led to the belief that reproduction may be affected by an organism's diet.

Another study looked at effects of nutritional stress on sperm production in moths. In the study, the sperm count of moths fed a low-protein diet was compared to the sperm count of moths fed a normal protein diet (Gage and Cook, 1994). The results indicated that diet played a significant role in spermatogenesis as lower sperm counts were found in moths fed a low protein diet. However, the size of the individual sperm

cells was unaffected (Gage and Cook, 1994). My results that testes size was maintained during protein deficiency do not fit the findings of this study, but the difference in results may be due to the use of different organisms with different reproductive strategies.

In the following study the relationship between survival and reproduction was looked at in zooplankton undergoing starvation. In the experiment it was found that in starvation conditions some species ceased reproduction and had higher survival rates, whereas some species maintained or increased energy allocation towards reproduction and had lower survival rates (Kirk, 1997). Furthermore, the results indicated that allocating energy production decreases resistance to starvation (Kirk, 1997). These findings suggest that if energy is devoted towards reproduction in times of low energy availability, individual fitness is decreased. However, if an organism fails to reproduce, genetically speaking, it makes no contribution to future generations. In species with a short life span like mice, individuals may die before environmental conditions become more favorable. This is why it was predicted that protein expression in testes would be maintained in mice fed a protein deficient diet. This study highlighted the fact that organisms possess different strategies for reproduction and determining whether reproduction will be maintained or decreased is dependent on the organism's life history.

Yet another study examined the effects of dietary protein on rats. In the study, pregnant rats were either fed a standard laboratory chow or an equal calorie low protein chow. The offspring of the rats fed the low protein chow had a lower mean body weight than rats born from mothers who were fed a normal protein chow (Snoeck, 1990). These results led to the assumption that in protein deficient rats, proteins from various parts of the body were being broke down for use and thus, contributed to the lower body weight

in the offspring of pregnant rats fed a low protein chow. These results were additionally supported as a lower mean mass of 29.8 (g) was observed in protein deficient mice, in comparison to the mean mass of 29.8 (g) in the control mice.

Moreover, the effects of protein efficiency were observed in the protein synthesis in the livers of rats. The subjects used for research were either fed a standard laboratory chow or a low protein chow and the effects of protein deficiency were quantified by measuring the concentrations of mRNA in the rat livers (Pain, 1978). A significant decrease of mRNA concentrations was observed in the protein deficient mice (Pain, 1978). The fact that liver functioning decreased in this study led to the assumption that other organs would shrink in response to protein deficiency.

In taking all the studies into consideration, protein deficiency causes lower mean body weights as proteins from organs are broken down in order to maintain vital life functioning. The prediction that regardless of dietary condition the functioning of the heart would be maintained was supported. In all the measurements used to infer organ function ability (organ wt/ body wt, total DNA and protein content) no significant differences were observed between the control and protein deficient subjects. This is more than likely attributed to the fact that functioning of an organism's heart is crucial for its survival. If the heart fails to work properly a myriad of problems exist as a result of poor circulation. Metabolic waste and byproducts of respiration must be maintained at low levels in the body, otherwise the ability of an organism to adequately function is severely jeopardized. Furthermore, cells through the body need a stead supply of nutrients (glucose, O_2, hormones, etcetera) need to be circulated throughout the body, and

thus, due to its importance in circulation it was predicted that heart function would be maintained.

The results also clearly supported the prediction that functioning in the pancreas would decrease in protein deficient mice. A statistical difference was observed between the experimental and control mice in the organ wt/ body wt values and in the total protein content. This is more than likely due to the fact that one of the key roles of the pancreas is the digestion of protein and in the mice fed a no-protein diet digestion of protein was not necessary. If an organism is protein deficient, proteins within the body will be broken down in order to synthesize other proteins critical for survival. Interestingly, in the measures of total DNA content no statistical difference between the control and protein deficient mice was observed. Since DNA is localized in the nucleus of cells, measurements of the total DNA content of an organ gives a rough estimate into the number of cells. The similar means of total DNA in the pancreas and the rather large difference in total protein suggests that while the numbers of cells appear to be the same, the overall size of the cells seems to be decreasing in protein deficient mice.

The hypothesis that reproduction would be maintained during protein deficiency was supported by the results. The argument that when faced with an environmental stress reproduction would be shut down until conditions improved, was not statistically supported. However, although not significant, a decrease in the total number of protein was observed in protein deficient mice. The standard deviation for the testes was extremely large due to the small sample size and having a larger sample size may have yielded significant differences. Despite the problems associated with sample size, the results indicated that reproduction is high on the hierarchy of biological functions.

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Figures and Results:

Table 1: Quantification *p*-values

Organ	Organ wt/ Body wt	Total DNA	Total Protein
Pancreas	0.007***	0.769	0.001***
Heart	0.459	0.404	0.336
Testes	0.572	0.637	0.135

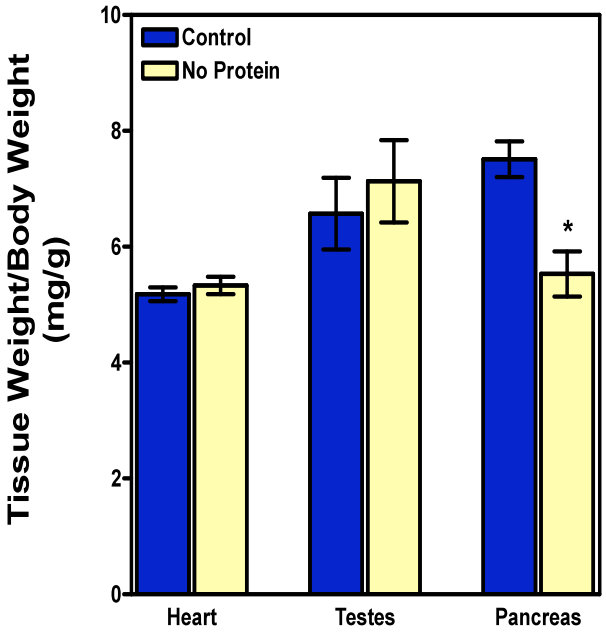

Figure 1: Tissue weight/ Body weight Graph

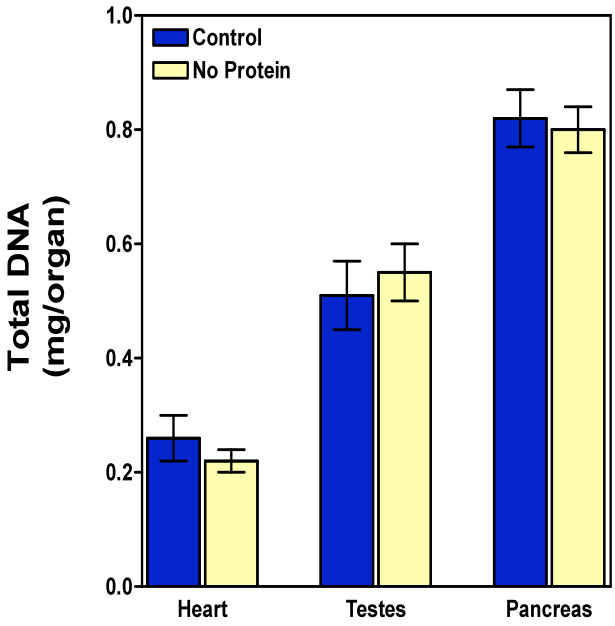

Figure 2: Total DNA Graph

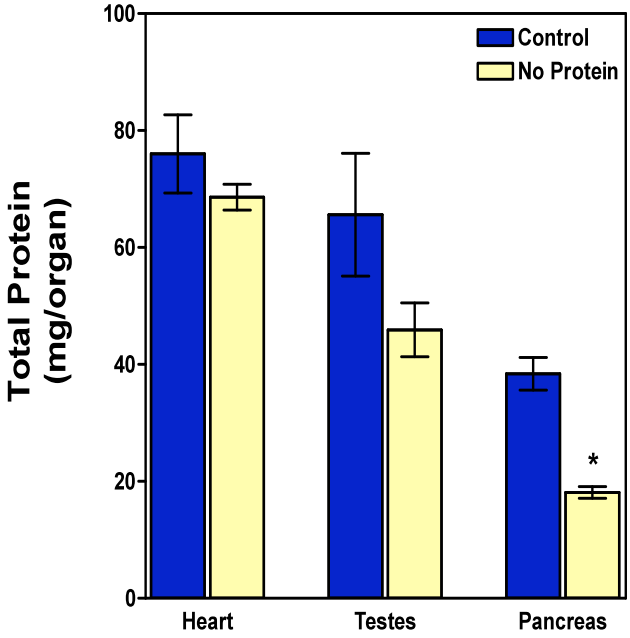

Figure 3: Total Protein Graph

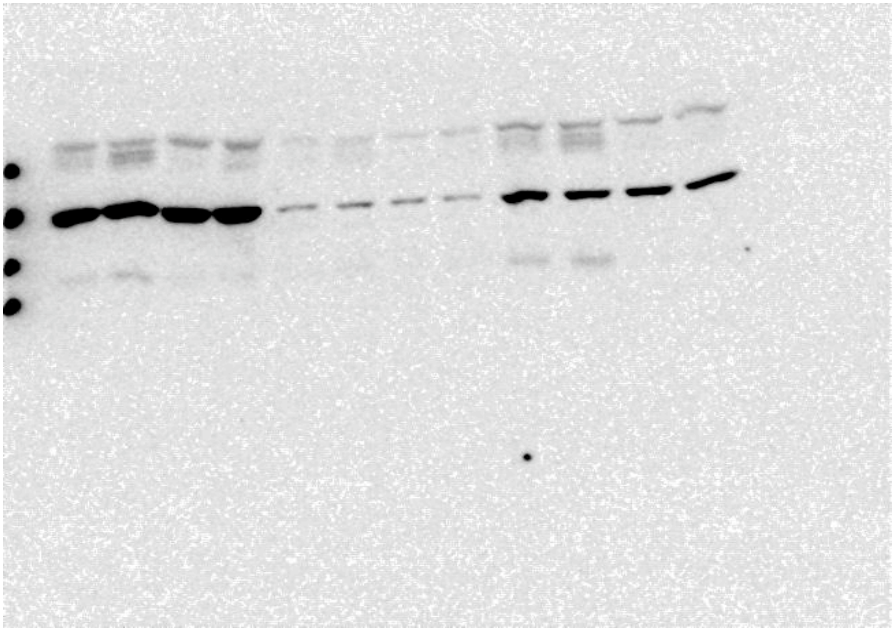

Figure 4: Immunoblot for gapdh

From left to right lanes go molecular weight markers, 2 heart control, 2 heart no protein, 2 pancreas control, 2 pancreas no protein, 2 testes control and 2 testes no protein.

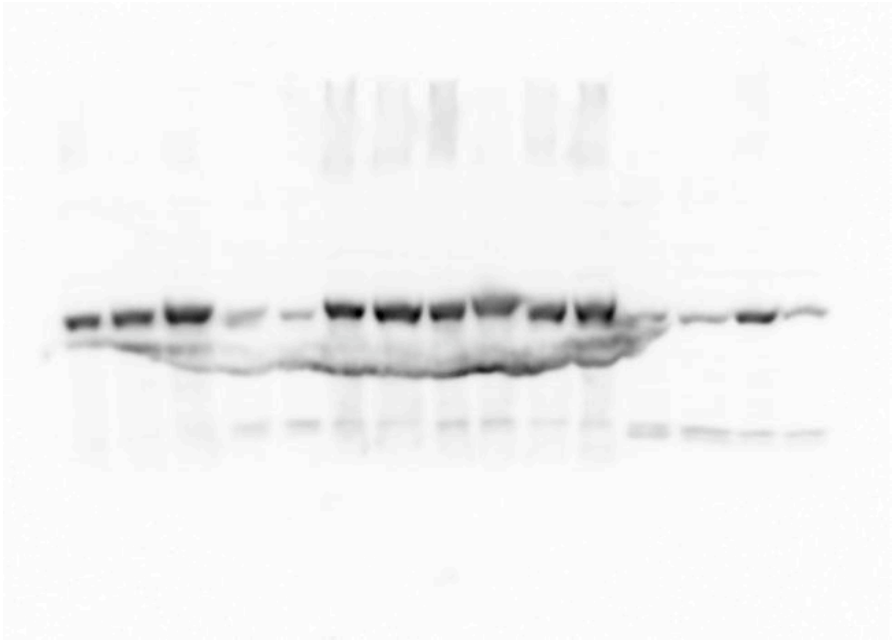

Figure 5: Immunoblot for s6p

From left to right (beginning on lane 4) lanes go 2 pancreas no protein, 2 pancreas control, 2 heart no protein, 2 heart control, 2 testes no protein, and 2 testes control. A clear change in protein expression is apparent between the control and no protein conditions in the pancreas.