Literary Review

A History of Isotopic Tissue Turnover and Metabolic Rate

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Introduction:

Stable isotope analysis has been used by researchers to study trophic interactions and assess overall ecosystem structure. The research done has been based on the idea that the isotopic signature of an organism's diet is eventually incorporated into its body. Throughout the various studies that have been done using stable isotope analysis, researchers have continually observed that there is some relationship between high metabolic activity and more rapid incorporation of the isotopic signature of one's diet. This paper is meant to construct a history of how this relationship has been observed and how it has subsequently been tested.

Review:

One of the first studies to explore the isotopic signature of carbon in animals due to diet was Michael J. DeNiro and Samuel Epstein's 1978 paper titled "Influence of diet on the distribution of carbon isotopes in animals." The objectives of this study were to establish a relationship between an animal's carbon isotopic signature and that of its diet and to find out whether or not this isotopic signature can be used to reconstruct an animal's dietary history (DeNiro 1978). This study was influenced by various other papers which showed that the carbon isotopic signature of animals in the wild, which is quantified by relative abundance of ^{13}C, was related to the carbon signature of the plants they were eating. Additionally, these studies showed that there were significant differences between the ^{13}C values of certain plants. More specifically, plants which used the C_4 photosynthetic pathway are more enriched in ^{13}C than plants which the C_3 pathway. To follow-up on this research, DeNiro and Epstein structured a study in which various insects along with mice were fed an isotopically constant diet. Their whole body, or in the case of the mice, specific tissues, was collected and the overall isotopic signature of these samples were subsequently determined.

The first major result from this study was that the animal carbon was indeed "enriched in $\delta^{13}C$ relative to the diet carbon" (DeNiro). This result was positive in eight of the thirteen animals that were analyzed. A secondary result was that there was some variation in ^{13}C values within individuals of the same species that were being fed the same diet. In the case of mice, DeNiro and Epstein analyzed the ^{13}C values in individual tissues. Their hope was that they could find tissues that could accurately reflect the ^{13}C values in the diet. In one set of experiments, DeNiro and Epstein found that brain, hair, and spleen samples were more enriched in ^{13}C relative to the diet whereas the other tissues sampled (heart, kidney, liver, lung, muscle, and pancreas) were depleted. However, another set of experiments showed that liver and kidney samples were indeed more enriched while the brain sample was slightly depleted. Due to the inconclusiveness of these findings DeNiro and Epstein proposed that specific tissues could not be used to determine the overall abundance of ^{13}C due to the diet without further investigation.

According to DeNiro and Epstein assessing ^{13}C values in animals is an efficient method for determining the animal's dietary history. However, this can only be done in cases where an animal's "potential diet sources have sufficiently different $\delta^{13}C$ values" (DeNiro). One example of this would be terrestrial versus aquatic plants because their isotopic signature often does not overlap. A second example would be C_3 and C_4 plants, as discussed above.

The measurements of ^{13}C in individual mouse tissues by DeNiro and Epestein led Larry Tieszen et al. to investigate how quickly these tissues take up the carbon isotopic signature of their diet (following a diet change) in their paper, "Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet." The process of taking up the isotopic signature of a new diet, which is termed tissue turnover, is measured by the time required for half of the tissue to turnover, or the tissue half-life. In their study, Tieszen et al.

switched a group of gerbils from a corn (C_4 plant) diet to a wheat (C_3 plant) diet. Hair, fat, liver, muscle, and brain samples were taken from each gerbil and both the ^{13}C levels and the half lives of each tissue were determined.

Of all the tissues, hair was the most enriched in $\delta^{13}C$ relative to the diet. In contrast, the fat samples taken were highly depleted in $\delta^{13}C$ relative to the diet. This is to be expected, however, because studies have shown that $\delta^{13}C$ is actively left out in the process of lipid synthesis. The liver, muscle, and brain samples mirrored the diet most closely, as they were only slightly enriched in ^{13}C. With respect to tissue turnover, the liver sample was the fastest, with a half-life of 6.4 days. The next fastest was the fat sample, which had a half-life of 15.6 days. The muscle and brain samples were slightly slower, with half-lives of 27.6 days and 28.2 days, respectively. Finally, hair showed the slowest turnover with a half-life of 47.5 days. In response to this data, Tieszen et al. proposed that more metabolically active tissues may turnover carbon more rapidly. To explore this hypothesis, the half-lives of the examined gerbil tissues were compared with the known metabolic rates of rat tissues. Assuming that these two species have similar metabolic rates, Tieszen et al. found a relationship between increased tissue metabolic rate and a faster rate of tissue turnover.

While the 1978 research done by DeNiro and Epstein propose that no individual tissue could be used to accurately assess an animal's carbon signature, Tieszen et al.'s findings show that liver, muscle, and brain samples could be of use. These tissues closely resembled their diet with respect to ^{13}C content and they all turnover relatively quickly. Despite this, Tieszen et al. contend than no single tissue would be adequate for determining the isotopic signature of a whole animal. Instead, they proposed that scientists should look at multiple tissues together when assessing ^{13}C uptake and turnover.

Tieszen et al.'s study marked the first exploration of tissue carbon turnover in mammals. Similar research did not exist for many other types of animals, however. This led Keith A. Hobson and Robert G. Clark to look into avian tissue turnover is their 1991 paper, "Assessing Avian Diets Using Stable Isotopes I: Turnover of ^{13}C in Tissues." Japanese Quail were switched from a wheat based diet to one based of corn. Liver, whole blood, muscle, and bone collagen samples were subsequently taken and ^{13}C enrichment and turnover rates were established for each tissue. After the diet switch, each tissue became enriched in ^{13}C. Further, the rates of tissue carbon turnover followed a similar pattern to those established by Tieszen et al. The liver sample had the fastest rate of turnover quantified by a half-life of 2.6 days. Whole blood and muscle samples were slower, with half-lives of 11.4 days and 12.4 days, respectively. Models tracking this turnover showed that there was complete tissue carbon turnover for all three of these tissues. On the other hand, bone collagen had the slowest half-life (173.3 days) and models showed that it did not reach a state of complete carbon turnover by the end of the 200 day experiment.

These results further support Tieszen et al.'s hypothesis that metabolically-active tissue undergo tissue carbon turnover at an increased rate as liver carbon turned over most rapidly. In light of this, Hobson and Clark contend that more studies must be done in order to find how changes in whole-body metabolic rate affect carbon turnover in individual tissues. They propose that variations in body size, age, and activity level (all of which can affect metabolic rate) must be explored with respect to rates of carbon assimilation.

Age and the related rate of growth of animals has been an isotopic turnover factor that has been widely studied. This began in 1982 with Brian Fry's and Connie Arnold's study titled "Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (Penaeus aztecus)." In this study, Fry and Arnold found that young brown shrimp underwent accelerated carbon turnover during

periods of growth and that the rate of turnover was primarily related to the rate of weight gained. In addition, Hobson and Clark explored the effect of age on carbon and nitrogen assimilation in birds in their 1992 study titled "Assessing Avian Diets Using Stable Isotopes II: Factors Influencing Diet-Tissue Fractionation." Although their data could not conclusively verify that age directly influence the rate of isotopic carbon and nitrogen assimilation, they noticed that younger or smaller crows were more affected by a diet switch.

These findings led Sharon Herzka and G. Joan Holt to explore the influence of growth on isotopic carbon and nitrogen turnover in juvenile fish in their study 2000, "Changes in isotopic composition of red drum (Sciaenops ocellatus) larvae in response to dietary shifts: potential applications to settlement studies." Herzka and Holt establish that "the rate of isotopic change is a function of both growth and metabolic turnover," where growth refers to the influence of "newly added biomass" and metabolic turnover refers to "the breakdown and replacement of existing body tissues" (Herzka). Two of the primary objectives of this study were to find the rate of isotopic turnover in the juvenile fish and to determine the relative importance of growth versus metabolic replacement on overall turnover.

Herzka and Holt found that ^{13}C and ^{15}N levels shifted towards those of the new diet. This shift was noticeable in as little as 2 days, which corresponded with a doubling of biomass in the juvenile red drum fish. Further, they determined that complete carbon and nitrogen turnover was achieved when the fish had increased their biomass six-fold. Additionally, exponential decay models indicated that metabolic turnover did not have any influence on the isotopic shift in this experiment. Instead, these models showed that the turnover was due entirely to growth. Based on this data, Herzka and Holt suggest that, for the larval red drum fish, "patterns of isotopic change can be adequately predicted based on growth rate estimates."

The findings discussed above are reasonable because one would expect growth to heavily influence the overall isotopic turnover of an animal which is rapidly adding new biomass. However, more recent studies have attempted to discover whether growth has as much influence on turnover in adult fish. MacAvoy et al.'s 2001 study, "Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey," showed that adult channel catfish had a relatively low rate of isotopic turnover. It was estimated that these fish would undergo complete ^{13}C turnover in 400-450 days. This is very long compared to the juvenile red drum species that achieved complete turnover in a matter of weeks. Further, Rania A. Tarboush et al.'s study, "Contribution of catabolic tissue replacement to the turnover of stable isotopes in Danio rerio," showed that growth accounted for very little of the overall turnover in adult zebra fish. Tarboush et al. found that metabolic tissue replacement was responsible for as much as 80% of the isotopic turnover. They postulate that the large influence of metabolic tissue replacement could be due to the fact that these fish were small and lived in warm water (both factors are known to increase metabolic rate). Nevertheless, these results support the idea that age and rate of growth are important factors affecting isotopic turnover.

Although the relative importance of growth and metabolic tissue replacement on fish has been well-studied, less research has been done exploring this relationship in mammals. This led MacAvoy et al. to publish their 2005 study, "Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis." One of the premises behind this study was that "the isotopic turnover rate of bird/mammals which experience very little growth at the adult stages, is likely to be quite different than that of invertebrates or fish, which experience indeterminant growth." The primary purpose of this study was to examine the rate of isotopic change in mice with respect to the influence of growth and metabolic tissue

replacement. A secondary objective is to explore how nutrients may or may not be selectively routed to different tissues in the process of isotopic turnover.

Blood, muscle, and liver samples were taken and the isotopic signature of these tissues essentially mirrored that of their new diet by the end of the experiment in both carbon and nitrogen turnover. In particular, they found that liver was more enriched in ^{15}N with respect to the blood and muscle samples. Overall, turnover rates were much higher than those that were estimated based solely on growth. MacAvoy et al. concluded that "growth is responsible for at most 10% of turnover rate, with the remainder owing to metabolic tissue replacement."

In addition, this study uncovered interesting data on differences in turnover rates between different tissues. With respect to ^{13}C, MacAvoy et al. found that blood underwent the quickest turnover (70 days). They propose that blood samples would be a useful in future research because they would accurately reflect short-term dietary change and, as a renewable tissue, blood can be sampled without killing the animal. MacAvoy et al. also found that the rate of tissue turnover varied greatly between the turnover of ^{13}C and ^{15}N in liver samples. Liver turned over the slowest in ^{13}C, as it had not completely equilibrated with the new diet by the end of the 112 day experimental period. However, the liver was the quickest to turnover ^{15}N. 90% of the ^{15}N shift took place within the first 28 days of the experiment and the half life was 7 days. MacAvoy et al. propose that this difference is due to the fact that nitrogen is found almost exclusively in proteins and nucleic acids, and that the rate of liver turnover is closely related to the rate of protein turnover.

The second objective in this study was to explore the idea of nutrient routing in different tissues. In order to track this MacAvoy et al. had to make sure that proteins, carbohydrates, and fats in the diet were isotopically distinct so that they could accurately track the movement of

these dietary molecules. With respect to the muscle and blood samples, they found that 75% of the new carbon associated with the isotopic turnover came from protein instead of carbohydrates or fats. This finding directly contradicts one of the basic assumptions of most isotopic turnover studies: “that all dietary carbon compounds are incorporated into tissues in proportion to dietary concentration” (MacAvoy 2005). Also, MacAvoy et al. proposed that the high ^{15}N enrichment in liver was due to differential fractionation in this tissue. In other words, they contend that the high metabolic activity in the liver results in rapid protein turnover and a subsequent accumulation of ^{15}N. These findings are important because they show that isotopic shifts are subject to specific fractionation, which varies among different elements and different tissues.

Arneson et al. further explored the differential routing of nutrients in mice in their 2006 study, “Metabolic protein replacement drives tissue turnover in mice.” The methods used in this study were similar to MacAvoy et at. ((2005). Arneson et al. found that 90-95% of newly turned over carbon originated from protein sources. They also found, as previous studies had, that tissue turnover was dominated by metabolic tissue replacement. Therefore, Arneson et al. concluded that protein was the major driver in metabolic tissue replacement, and subsequently the major driver of adult isotopic tissue turnover.

After MacAvoy et al. (2005) and Arneson et al. (2006), several claims could be made relating metabolism to isotopic tissue turnover. First, it was found in several studies that more metabolically active tissues, such as liver, turned over more rapidly than less metabolically active tissues. Second, it was found that, in adult animals, tissue turnover is controlled almost exclusively by metabolic tissue replacement. Finally, it was shown that metabolic tissue replacement, and therefore overall isotopic tissue turnover, is driven primarily by protein sources in the diet. These findings establish a useful framework for studying metabolism and turnover.

However, previous studies have not measured whole-body metabolic rate and directly studied its relationship with isotopic tissue turnover.

This led MacAvoy et al. to publish their 2006 study, "Correlation of metabolism with tissue carbon and nitrogen turnover in small mammals." The purpose of this study was to measure both the rate of tissue turnover and the basal metabolic rate of mature mice and rats and establish a statistically significant relationship between the two. In addition, MacAvoy et al. compiled data which included the known tissue turnover rates and basal metabolic rates of several avian species.

Blood samples were regularly taken from the mice and rats throughout the experiments. MacAvoy et al. found that the blood tissue half lives for ^{13}C and ^{15}N turnover in mice were 17.3 days and 15.4 days, respectively. For rats, the half lives for blood were 24.8 days for ^{13}C turnover and 27.7 days for ^{15}N turnover. Basal or resting metabolic rates were taken throughout the experiment and the averages were 3.2 ml O_2/min for mice and 8.9 ml O_2/min for rats. These values are not an accurate determinant of relative metabolic rate between the two species, however, because of the difference in weight between mice and rats. When adjusted for mass, mice had a metabolic rate of 6.84 ml O_2/h/g while rats had a metabolic rate of 1.84 ml O_2/h/g. This result is in accordance with understanding that smaller animals generally have higher mass-adjusted metabolic rates.

Overall, MacAvoy et al. found that there was a correlation between basal metabolic rate and the rate of tissue turnover. They found a statistically significant, positive relationship between increased basal metabolic rate and an increased rate of tissue turnover. Further, when the known metabolic and tissue turnover rates of the avian species were plotted, the same positive relationship was established. In fact, when regression analysis was performed, the data

fit the curves with 87% accuracy for ^{13}C turnover and 90% accuracy for ^{15}N turnover. However, the mouse and rate data could not be accurately plotted with the avian data because the birds have a faster metabolic rate per gram than the small mammals.

MacAvoy et al. contend that the relationship established in this study could be useful in future research projects. They propose that if this relationship can be tested further and made stronger, it would help researchers who want to find "a way to predict time to isotope equilibrium for species for which metabolic rate is known but the isotope turnover is not." This model which incorporates whole-body metabolic rate cannot be used to predict the rate of turnover in other tissues, however. In order to make such predictions, new, tissue-specific models must be created.

Despite the positive results from MacAvoy et al. (2006), a few studies concluded that there was no discernable relationship between increased metabolic rate and an increased rate of tissue turnover. The first of which was a 2003 study conducted by Christian C. Voigt at al. titled "Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species." Voigt et al. assessed the rate of tissue turnover in bats because they were known to have very high mass-specific metabolic rates. Surprisingly, none of the bat tissues that were sampled (blood, wing membrane, and hair) became isotopically equilibrated with their new nectar-based diet. In addition, the estimated half-life of the blood sample was approximately 116 days. This number is very large compared to the 17.3 day half-life of the mice in MacAvoy et al. (2006) despite the fact that the bats have a higher mass-adjusted metabolic rate.

To explain these results, MacAvoy et al. (2006) propose that the rate of tissue turnover in these mature bats should be slow because they are being fed a diet that is deficient in protein. As dietary protein was shown to drive metabolic tissue replacement, and therefore overall isotopic

tissue turnover (MacAvoy 205, Arneson 2006), one would expect an animal on a low-protein, sugar-based diet to not turn over tissue carbon rapidly.

Another study which contradicts the positive relationship between metabolic rate and the rate of tissue turnover is S.A. Carleton and Carlos Martinez del Rio's 2005 study, "The effect of cold-induced increased metabolic rate on the ^{13}C and ^{15}N incorporation in house sparrows (Passer domesticus)." In this study, Carleton and Rio raised one group of sparrows at 22°C and another group at 5°C. The volume of oxygen consumed, which is an indicator of metabolic rate, was 1. 9 times higher in the group of sparrows that was kept at a colder temperature. However, Carlton and Rio found that "there were no statistically significant differences in the fractional incorporation rate between birds at 5 and 22°C for either carbon or nitrogen." Carleton and Rio further contend that the temperature change did not affect the rate of tissue turnover.

While Carleton and Rio suggest that the conditions that the sparrows were placed in were within their environmental range, MacAvoy et al. (2006) disagrees. Instead, they contend that the metabolic rates determined by Carleton and Rio were field metabolic rates and not basal (or resting). MacAvoy et al. further suggest that field metabolic rate "may have only an indirect, and perhaps negligible, effect on the isotopic tissue turnover rate."

One final study that contradicts the positive relationship between increased metabolic rate and an increased rate of tissue turnover is Keith A. Hobson and Elizabeth Yohannes 2007 study, "Establishing elemental turnover in exercising birds using a wind tunnel: implication for stable isotope tracking of migrants." Most studies up to this date had been done on captive animals. In light of this, Hobson and Yohannes attempted to simulate the amount of exercise that birds would get in the wild by letting a group of Rosy Starlings fly regularly in a wind tunnel. They

expected that those birds which were exercised would turnover carbon more rapidly because exercise is known to increase metabolic activity.

For 4 weeks prior to the diet shift, all of the Rosy Starling were trained in the wind tunnel daily. Once the diet was switched, one group of birds flew in the wind tunnel for several hours daily for the entire 45-day experimental period. After processing all of the data, Hobson and Yohannes found that there was no significant difference between the two groups with respect to the rate or carbon turnover. They also found that both the exercise and non-exercise groups gained mass over the course of the experiment. The former gained an average of 15.24% of their initial body mass while the latter gained an average of 5.57% of their initial body mass.

These findings indirectly contradict the positive relationship between metabolic rate and the rate of turnover because the metabolic rates of the Rosy Starlings were not calculated in this study. Instead, they were exercised, which was, in theory, supposed to translate into higher basal metabolic rates. Additionally, the fact that all of the birds received exercise during the first four weeks of the study could have affected the results by making the two experimental groups more similar. One other caveat is that all of the birds gained mass throughout the course of the study. Therefore, growth must have driven part of the carbon turnover experienced by the birds. This could have altered the results because the relationship between increased metabolic rate and an increased rate of isotopic tissue turnover is dependant on metabolic tissue replacement, not growth.

The Next Step:

Several studies have challenged the positive relationship between metabolic rate and the rate of isotopic tissue turnover proposed by MacAvoy et al. (2006). Therefore, more research needs to be done to either validate or disqualify this correlation. Several specific steps must be

taken to do this. First, more studies must be conducted which consistently measure the metabolic rates of study subjects. This type of data was lacking from Hobson and Yohannes (2007). Second, researchers must conduct studies comparing basal and field metabolic rate with respect to their effect on the rate of isotopic tissue turnover. Finally, research must be done on the various factors which effect metabolic rate. In particular, the effects of sustained physical activity, exercise, must be understood as they relate to tissue turnover. This is particularly important because most studies are conducted on latent, laboratory animals instead of the active animals that are present in the wild.

A study like this was conducted by Hobson and Yohannes in 2007. However, there were several problems with the methods, which were explained in the body of this paper. Currently, a study is being conducted at the American University which is exploring the effects of exercise-induced increased metabolic rate on isotopic tissue turnover in adult mice. The mice were receiving exercise in the form of swimming. Unfortunately, the study has been suspended because the mice have reacted poorly to an increased level of exercise.

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Honors Capstone in Environmental Studies

Research Log 2007-2008

Over the course of the year, I have conducted research examining tissue turnover in mice (Mus musculus). I began the year working on a study whose purpose was to assess the effect of exercise on basal metabolic rate and the overall rate of blood tissue turnover. Unfortunately, problems associated with the experimental design of the study led to the abandonment of this specific project. However, I was able to use the same mice to begin a new study which alters the abundance of protein in a diet and examines its effect on the rate of carbon turnover in blood tissue. This paper summarizes the research that I have competed this year and discusses the expected findings for the research that has yet to be completed.

The isotopic signature of an organism's diet eventually becomes incorporated into its body tissue. Therefore, if a study animal is switched to an isotopically distinct diet, one can effectively track how fast the new diet is being incorporated and to which tissues they are being incorporated in to. Studies analyzing this process have been conducted since the 1970s and various findings have been established.

One pattern that has been observed is that metabolically active tissues, such as liver and blood, tend to turnover more rapidly than less metabolically active tissues, such as bone and fat. This trend led MacAvoy et al. to conduct a study in 2006 which aimed to determine whether animals with a higher overall metabolic rate experience faster carbon and nitrogen tissue turnover compared to animals with slower metabolic rates. To accomplish this, MacAvoy et al. (2006) performed a diet switch on mouse and rat subjects and took both blood samples and basal metabolic rate readings throughout the course of the study. In the end the mouse subjects had higher mass-adjusted basal metabolic rates than the rat subjects. Additionally, the mice

experienced faster blood turnover. Therefore, MacAvoy et al. (2006) concluded that there was a direct correlation between higher basal metabolic rate and an increased rate of blood turnover.

At the end of their study, MacAvoy et al. propose that several factors, such as external temperature, body temperature, age, and activity all can affect metabolic rate. In the study that I conducted, I specifically examined the effect of exercise on metabolic rate and carbon and nitrogen tissue turnover. The primary hypothesis of this study was that increased exercise (activity level) will increase the metabolic rate of mice, resulting in faster turnover rate of blood tissue as measured by stable carbon and nitrogen isotopes.

Eighteen mice were split into three groups of six for this study. All the mice were equilibrated on a control diet for several weeks at the onset. One group was going to remain on the control diet whereas the other two groups were going to be switched to two different and isotopically distinct experimental diets. In addition, all three of these groups were split into exercise and non-exercise groups. The non-exercise groups were to remain in their cages for the duration of the study while the exercise group was originally going to be exercised using a small treadmill for thirty minutes per day, five days per week for a total of two months. Additionally, the study protocol called for two types of data collection. First, basal metabolic rates were to be calculated by measuring the mice's rate of oxygen production. Second, the rate and extent of tissue turnover was to be determined by taking weekly blood samples for the final six weeks of the study. Finally, these samples were to be dried and sent to the University of California-Davis to undergo stable isotope analysis.

Once all of the mice had become equilibrated with the control diet, we attempted let them adjust to exercising on the treadmill. Unfortunately, the mice were not receptive to this mode of exercise. The treadmill, which was built by American University students over the summer, was

not properly contained in its frame. This led many of the mice to slip underneath the track and get stuck. Additionally, some of the mice simply would not run while the track was moving and would instead be thrown into the back of the treadmill frame. After assessing the effectiveness of the treadmill-based exercise and considering the potential threats to the health of the mice, we decided to abandon this method.

The next step was to find a new way to exercise the mice. After consulting with the American University biology staff, we decided to "swim" the mice in a large water bath. By putting them is this bath, we assumed that, in order to survive, they would have to swim, and therefore expend energy. While this may seem cruel, this method has been used in previous studies and the mice turned out to be surprisingly good swimmers. However, when we started this regimen, it became clear that thirty minutes of exercise five days per week would be too strenuous for the mice, as we observed them struggling to swim after about twenty minutes. In response to this we cut down the exercise to fifteen minutes per day, three times per week.

We continued on this path for several weeks. Unfortunately, we were unable to move past this step of the experiment for two reasons. First, after measuring the mice's oxygen output for several weeks, we were unable to discern any significant change in metabolic rate when comparing the exercise and non-exercise groups. This was problematic because the entire study was based on achieving an increase in basal metabolic rate that was significant enough to test whether or not it had an impact of the rate of blood tissue turnover. The second problem that we encountered was that some of the mice did not react well to the swimming. In fact, 4 mice drowned upon touching water in their third week of exercise. It is thought that shock was the cause of death. In addition, two mice (from exercise groups) died in their cages at around the

same time that the other four mice passed away. Given these problems, we were forced to abandon this specific study.

Overall, we found it exceedingly difficult to choose and implement an exercise regimen which was safe for the mice and rigorous enough to influence an appreciable increase in basal metabolic rate. In order to move forward, a new study was undertaken that did not require exercise. Instead, two new strains of mice were purchased which had genetically different metabolic rates. The goal for this study is to perform a simple diet switch and then, using stable isotope analysis, determine whether the mouse strain with the naturally higher metabolic rate turns over blood tissue more rapidly than does the strain with the slower metabolic rate.

While this experiment was being undertaken by another student, I focused on conducting a new study with the mice left over from the original experiment. Research done by Dr. Stephen MacAvoy and Dr. Lynne Arneson (both American University faculty) has indicated that the vast majority newly-synthesized tissue carbon (75-95%) comes from dietary protein sources. These authors argue that protein carbon is most influential in the synthesis of new tissue (tissue turnover), whereas carbon from carbohydrates is used primarily as an energy source. In order to build on this research, I examined influence of protein and carbohydrates on isotopic blood tissue turnover by placing mice on a protein-sufficient diet and a protein-deficient diet.

The twelve mice remaining from the previous study were split into three groups of four. The first group was kept on a control diet (the same diet that all twelve mice had been equilibrated on). Groups two and three were switched to a 0% protein diet and a 10% protein diet, respectively. Immediately prior to the diet switch, preliminary blood samples (Week 0) were taken. In this study, blood was drawn from the tail using a small razor blade. Blood samples were deposited into labeled vials and these were subsequently placed in a 95 degree Fahrenheit

oven overnight. Following the diet switch, blood samples were taken weekly for the next 5 weeks.

Unfortunately, group two, which was placed on the 0% protein diet, reacted poorly to its diet. Following the diet switch, body weights dropped significantly (losses ranged from 30-35% of body weight) by Week 2. Additionally, all of the mice in this group experienced hair loss and one mouse developed cancer. Due to their deteriorating health and recommendations from the resident veterinarian, these mice were taken off the 0% protein diet following Week 2 and they were put down the following week. This result was somewhat surprising because I was under the impression that the diet, which was formulated by Harlan (our supplier for custom mouse diets and the mice themselves), would have been safe to use. However, after talking to one of Harlan's lab experts, I learned that this diet was supposed to be used with the addition of a separate protein source. In hindsight, it may have been more beneficial to put this group on a low-protein diet instead of the zero protein diet.

Between Week 2 and Week 5, blood samples were taken from groups one and three. Once dried, I performed lipid extraction on these samples. This procedure involves treating the sample with a solvent, dichloromethane, and heat. This procedure allows the lipids from the blood to be suspended in the solvent, which can be easily discarded. The remaining dried blood was then removed from the vials and placed in small tin cups, which were weighed. Packaged cups were then sent to the University of California-Davis to undergo stable isotope analysis using equipment that American University does not possess. Unfortunately, these samples have yet to be processed and hence we have not, to date, secured data for this study.

While data is still lacking, I expect to see certain results. MacAvoy et al (2005) and Arneson et al. (2006) determined that the vast majority of new tissue is synthesized using carbon

derived from dietary protein sources rather than carbon from carbohydrate sources. Given this, I expect carbon to turnover more rapidly in the mice fed the 10% protein diet compared with the mice fed on the 0% protein diet. Further, I expect increased protein retention in group 2, as they have had no new source of protein in their diet. Finally, this study will enable us to draw a comparison between protein from animals and plant sources, as the 10% protein diet contains casein (animal protein) and the control diet is comprised entirely of plant protein.

Studies Cited:

1. Arneson LS, MacAvoy S, Bassett E (2006) Metabolic protein replacement drives tissue turnover in adult mice. Can. J. Zool. 84: 992-1002.
2. MacAvoy SE, Macko SA, Arneson LS (2005) Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. Can. J. Zool. 83: 631-641.
3. MacAvoy SE, Arneson LS, Bassett E (2006) Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. Oecologia 150: 190-201.