THE RELATIONSHIP BETWEEN TISSUE TURNOVER AND METABOLIC RATE IN TWO STRAINS OF RATS (*RATTUS NORVEGICUS*)

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ABSTRACT

Stable isotope signatures show the rate of tissue turnover in two strains of rats (*Rattus norvegicus*) with different metabolic rates (MR). MR is hypothesized to be positively correlated with tissue turnover rate. If MR, a relatively simple measurement, can be correlated with tissue turnover, then this relationship could be used to study important ecological questions, such as those involving migration and seasonally available nutrient sources. Here, oxygen consumption was used to measure MR. After a diet switch, the changing signatures of carbon and nitrogen from whole blood were mathematically modeled. The mass and MRs were significantly different between strains, but half-life and the metabolic component of turnover (m) were not. No significant correlation was found between MR and m between the strains. Results suggest that within a species with a range of MRs, tissue turnover occurs at relatively the same rate.

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CHAPTER 1

INTRODUCTION

The use of stable isotopes for ecological and environmental purposes has grown considerably over the past decade (Michener & Lajtha ed 2007). Stable isotopes can be used to measure tissue turnover, which can then be applied to the study of food webs, ecosystems, dietary choices, and animal migrations (reviewed in Hobson 1999; Dalerum & Angerbjorn 2005; Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL 2007). They can act as tracers, moving through trophic levels in predictable ways so that trophic relationships can be determined (Minagawa & Wada 1984; Lajtha & Michener 1994). It is also possible to use stable isotopes for long term and short term diet studies, by comparing samples over time in the same tissue, comparing tissues with different metabolic rates in the same individual, or by comparing sections of tissue which have progressive growth, such as hair or feathers (Dalerum & Angerbjorn, 2005). By analyzing the turnover rate of dietary components like proteins and carbohydrates, researchers can better understand the impact of shifting diet among organisms (Lajtha & Michener 1994; MacAvoy SE, Macko SA, and Arneson LS 2005).

This study aims to see if there is a correlation between the rate of isotopic incorporation into whole blood tissue after a diet switch (rate of tissue turnover), and metabolic rate (MR) for two different strains of rat. It is hypothesized that rats with a higher MR will show a faster rate of tissue turnover, as demonstrated by a faster

incorporation of a new diet's carbon (C) and nitrogen (N) stable isotopic signature into their blood. It is predicted that MR will be correlated with m, which is the metabolic component of tissue turnover. If a predictive relationship can be made between metabolic tissue replacement and isotopic tissue turnover, then it may be possible to infer the tissue turnover rate of animals in the field, just by knowing the MR.

Stable Isotopes

Stable isotopic signatures of an organism are a measure of the amount of heavy versus light isotopes present in a sample (Peterson & Fry 1987). They are measured as del values (δ), which represent parts per thousand difference from a standard. If a sample has a positive δ value, then it is enriched in the heavy isotope relative to a standard. If a sample has a negative δ value, it is depleted in the heavy isotope relative to a standard. Isotopic signatures are calculated based on the following equation:

$$\delta^{H}X = [({}^{H}X / {}^{L}X)_{sample} / ({}^{H}X / {}^{L}X)_{standard}] - 1) * 1000 \qquad (1)$$

Here, X represents any element [C and N in this study, but also hydrogen (H), oxygen (O), and sulfur (S)]. The superscripts H and L represent the heavy and light isotope mass numbers (^{13}C and ^{12}C; ^{15}N and ^{14}N). The standard that is used for C comparison is carbonate from the fossil *Belemnitella americana* from the PeeDee formation in South Carolina, and is referred to as the PDB standard (Craig 1953, 1957). The degree of C fractionation is usually very small, ranging from 0.5-1‰ for consumers relative to their food source, but this can vary based on tissue type, food quality, and feeding preferences

(McCutchan JH, Lewis WM, Kendall C, and McGrath CC 2003). It is commonly assumed that C has little trophic enrichment (DeNiro & Epstein 1978; Fry & Sherr 1984; Peterson & Fry 1987). On the other hand, individuals are on average enriched in N by approximately 3-5‰ over their diet (Minagawa & Wada 1984; Peterson & Howarth, 1987; Koch PL, Fogel ML, and Ostrum NE 1994; Michener & Schell, 1994). This diet-tissue discrimination is due to the fact that animals excrete the isotopically light ^{14}N in urine, leaving the body enriched in ^{15}N (Shoeninger & DeNiro, 1984; Peterson & Fry, 1987).

It has been shown that different species on the same diet, as well as individuals within a species on different diets, will have similarly enriched whole body values of $\delta^{13}C$ compared to their diet (DeNiro & Epstein 1978). $\delta^{13}C$ values are lower for herbivores than they are for carnivores, due to the difference in C signature of lipids, proteins, and carbohydrates in their food sources (DeNiro & Epstein 1978; Focken & Becker 1998). As plants do not contain many lipids, which are depleted in ^{13}C (DeNiro & Epstein 1978), herbivores may obtain more lipids from carbohydrates and therefore have a lower $\delta^{13}C$ value.

Fractionation and Diet-Tissue Discrimination

The astounding variety of ways that stable isotopes can be used for ecological studies stems from the way they behave once ingested by an organism. Heavy and light isotopes of a particular element will move at different rates through the metabolic processes of an organism. The lighter isotopes (^{12}C and ^{14}N in this study) will tend to

move faster through assimilation and metabolic reactions, as enzymes can more easily break down these lighter isotopes. Therefore, the reactant pool ends up being enriched in the heavier isotopes (^{13}C or ^{15}N), which react more slowly because they form stronger bonds within molecules, and have a higher activation energy so it takes more energy from the organism to utilize them (Peterson & Fry 1987; Griffiths 1991; Hogberg 1997; McCutchan et al. 2003). The term 'fractionation' refers to the fact that these isotopes move at different rates through an organism. It can be thought of as the difference in isotopic ratio between an animal and it's diet (Beltran et al. 2009).

When an organism consumes a diet of the same stable isotopic signature over time, its tissues will begin to isotopically resemble the diet in predictable ways (Fry & Sherr 1984; Minagawa & Wada 1984; Peterson & Howarth 1987; DeNiro & Epstein 1978, 1981). Therefore, the isotopic signature of an animal's tissues will be a reflection of the local food web, as long as the animal has resided there for a length of time (Peterson & Fry 1987; Tieszen & Bouton 1988; Michener & Schell 1994). In this way, the stable isotopic signature of the tissues is a measure of assimilated food, not just food that was ingested by the organism (Tieszen LL, Boutton TW, Tesdahl KG and Slade NA 1983). Due to fractionation however, the organism will not isotopically resemble its food source exactly, but will vary; this variation is referred to as diet-tissue discrimination.

Though both C and N exhibit predictable diet-tissue discrimination, many factors can influence this rate of turnover. Enrichment of ^{15}N can occur due to stress from fasting or lack of water (Ambrose & DeNiro 1986; Sealy JC, van der Merwe NJ, Lee Thorp JA, Lanhman JL 1987; Hobson & Clark 1992; Ambrose 1993; Hobson KA, Alisaukas RT,

and Clark RG 1993; Hobson KA, Schell DM, Renouf D, Noseworthy E 1996), low protein diets (Gaye-Siessegger J, Focken U, Abel H, and Becker K 2004), nursing offspring, hibernation (Nelson et al. 1998; Liden & Angerbjorn 1999), and changes in trophic level (Fry 1988; Hobson & Welch 1992). Stable isotope ratios are also affected by the nutritional content of their diet (Hobson & Clark, 1992) and possibly age (Ponsard & Averbuch 1999; Keeling & Nelson 2001; Witt & Ayliffe 2001). The metabolic rate of the tissue in question also influences stable isotopic enrichment, where tissues with a lower MR (such as blood or muscle) have lower diet-tissue discrimination than do tissues with a higher MR (such as liver, brain, and heart) in some species such as seals and mice (Grande 1980; Arneson & MacAvoy 2005). The relationship between MR and tissue turnover will be discussed in detail in later sections.

Photosynthetic pathways

It is possible to use stable isotopes as tracers because different sources of food have different ratios of $^{13}C/^{12}C$ so that each food source has it's own stable isotopic signature. This is because different types of plants have different photosynthetic pathways, which in turn gives them different ratios of heavy to light C isotopes (Bender 1971; Sternberg & DeNiro 1983; Griffiths 1991, 1992). A plant's $\delta^{15}N$ signature can also give information about the source of N for the plant; whether it stems from the soil, bacteria, or rainwater (Gallon 1992; Handley & Raven 1992; Högberg 1997). The characteristics of the different methods of photosynthesis (C3, C4, and Crassulacean Acid Metabolism [CAM]) lead to $\delta^{13}C$ values that are unique to each type of plant. C3 plants,

which follow the Calvin Cycle of photosynthesis, exhibit $\delta^{13}C$ values in the range of -22 to -33‰, whereas C4 plants have higher values ranging from -10 to -20‰ (Bender, 1971). CAM plants only respire at night, so they exhibit less evapotranspiration than the other two types of plants, which respire during the day when it is warmer and dryer (Kluge & Ting 1978). Because CAM and C4 plants do not have their stomata open as much as C3 plants due to habitat differences, they must use stored CO_2 instead of atmospheric CO_2 to photosynthesize (Sternberg & DeNiro 1983). This use of stored CO_2 leads to their enrichment of ^{13}C compared to Calvin Cycle photosynthesizing plants (Bender 1971; Sternberg & DeNiro 1983; Griffiths 1991). Because plants such as corn and wheat have different methods of photosynthesis (C4 and C3, respectively), food with a corn base will be more enriched in ^{13}C compared to wheat based foods (Hobson & Clark 1992a). Therefore, not only is it possible to trace these stable isotopes in organisms, but it is possible to use the ratio of ^{13}C to ^{12}C to determine an individual's reliance on different types of primary producers (DeNiro & Epstein 1978; Wolf & Martinez del Rio 2003), as well as to characterize a niche space (Newsome et al. 2007).

Tissue Turnover Rates

Turnover can be defined as the change in isotopic composition, due to growth and metabolic tissue replacement, that is associated with a change in diet (MacAvoy SE, Macko SA, and Garman GC 2001). Different tissues within an individual will have different rates of turnover, depending on the MR of the specific tissue. MR of individual tissues can be measured by isolating fresh samples of the tissue in a buffered medium,

such as Krebs-Ringer phosphate, in a closed chamber so that oxygen levels can be measured to determine rate of oxidation (Altman & Dittmer 1968, and references therein). Because tissues turn over at different rates, an individual organism can provide dietary information over a span of time, as different tissues will provide a "time window" of information about previous diets (Tieszen et al. 1983; Hobson & Clark 1992a; Newsome et al. 2007). This has been referred to as an "isotopic clock" to see when an animal shifts diets (Phillips & Gregg 2003). For example, whole blood can be used to show two different periods of integration, because the cellular and plasma fractions of blood have different rates of protein turnover (Waterlow JC, Garlick PJ, and Millward DJ 1978; Hobson & Clark, 1992a, 1992b). Tissues with drastically different turnover times, such as bone and hair, can be used to track an individual's diet over the course of many years (Yeakel et al. 2009), and tissues which are not metabolically active once they are grown (such as feathers) can be a record of a past diet (Hobson and Clark 1992a).

Animal Age

Another factor that can influence the rate of tissue turnover is the age of the organism. Individuals that are still growing will show increased turnover because of tissue replacement due to growth, so that a juvenile and an adult of the same species may have different rates of isotopic change (Tieszen et al. 1983). Fry & Arnold (1982) found that, for post larval brown shrimp, tissue turnover was accelerated during growth so that it was a function of weight gain, whereas for adults, turnover occurred during maintenance metabolism as a function of time. MacAvoy et al. (2005) showed that

younger rats have a shorter isotopic half-life (and thus faster tissue turnover), so that the age of animals at the start of the experiment may impact rates of tissue replacement, and turnover will differ between adult and juvenile mammals. Growth rate is very important for determining tissue turnover, especially for animals that do not stop growing over the course of their lifetime, such as ectotherms (Martinez del Rio C, Wolf N, Carleton SA, and Gannes LZ 2009). Because of this, it is possible that there may be a difference in the contributions of growth and metabolic (catabolic) processes to isotopic incorporation between ectotherms and endotherms (MacAvoy et al. 2005).

Previous Studies on turnover

The length of time it takes to "become what you eat" depends on many factors, including age of the organism (Tieszen et al. 1983), animal and tissue in question (Tieszen et al. 1983; Phillips & Gregg 2003; Carleton & Martinez del Rio 2005; MacAvoy et al. 2005), body size, growth rate, protein turnover (Sakano H, Fujiwara E, Nohara S, and Ueda H 2005; Martinez del Rio et al. 2009), dietary components (DeNiro & Epstein 1978), and possibly the metabolic rate of the organism as a whole. It has been demonstrated that the metabolic rate of the tissue in question influences the rate of that tissue's isotopic turnover (Tieszen et al. 1983), so that C turnover will differ between metabolically active tissues in a variety of organisms. Studies have demonstrated this in yellow-rumped warblers (Podlesak et al. 2005), American crows (Hobson & Clark 1993), mice (MacAvoy et al. 2005; Arneson LS, MacAvoy S, and Basset E 2006), alpacas (Sponheimer et al. 2006), gerbils (Tieszen et al. 1983), quails (Hobson & Clark 1992),

and zebra finches (Bauchinger & McWilliams 2009). Many different studies have been performed on a variety of taxa (including birds, fishes, and mammals) to examine diet-tissue discrimination (mice - DeNiro & Epstein 1978, 1981a; 1981b; Tieszen et al. 1983; red fox - Roth & Hobson 2000; Bearhop S, Waldron S, Votier SC, and Furness RW 2002; garden warblers - Hobson & Bairlein 2003; McCutchan et al. 2003; Poldesak DW, McWilliams SR, and Hatch KA 2005; brown shrimp - Fry & Arnold 1982; Hobson & Clark 1992; horses - Ayliffe et al. 2004). This is usually accomplished by means of a diet switch study, where the organism is equilibrated on a diet with a known stable isotope signature, before being switched to another diet with a different isotopic composition (Martinez del Rio & Anderson-Spercher 2008). It is then possible measure the rates of tissue turnover over time through systematic sampling and analysis, which is the method utilized in this study.

Ecological Applications

Stable isotopes are incredibly useful for a variety of ecological purposes. With known fractionation values of C and N, it is possible to estimate omnivory, trophic position, food chain length, energy flow, and energy sources within a habitat (Cabana & Rasmussen 1996; Vander Zanden MJ, Shuter BJ, Lester NP and Rasmussen JB 1999b). Models can be constructed of food webs, trophic structures (Peterson & Fry 1987), bioaccumulation contamination (Cabana & Rasmussen 1994), impacts of invasive species on food webs (Vander Zanden MJ, Casselman JM, and Rasmussen JB 1999a), and even incorporation of sewage derived N into the food web (Van Dover CL, Grassle JF, Fry B,

Garritt RH, and Starczak VR 1992). Stable isotopes measure assimilated diet over long and short terms, so they can be used to study past food webs, as well as diets of animals that are hard to observe, or for reconstructing diets when direct dietary information is unknown (Peterson & Fry 1987; Hobson & Clark 1992a; Angerbjorn A, Hersteinsson P, Linden K, and Nelson E 1994; Koch PL, Heisinger J, Moss C, Carlson RW, Fogel ML, and Behrensmeyer AK 1995; Cerling et al. 2006). Stable isotopes have been called nature's recorders of ecological processes (West JB, Bowen GJ, Cerling TE, and Ehreringer JR 2006), and as such, are an important tool in conservation biology because they can track changes in ecological characteristics of organisms (Newsome et al. 2007). When planning conservation measures it is very important to know the breeding, wintering, and stopover grounds of the species in question, and stable isotopes can give information about these (Hobson 1999) because the different ratios in an organism's tissues can identify diet changes and trace the migratory routes and habitats that animals have previously used (Fry 1981; Schell DM, Saupe SM, and Haubenstock N 1989; Fleming TH, Nunez RA, and Lobo-Sternberg LS 1993; Koch et al. 1995). Along the same line, it is possible to use the ratios to see the extent that a migrating population of organisms is preyed upon by local predators (MacAvoy et al. 2001). It is possible to track animal movements using isotopes because when animals move between food sources, they bring information about their previous food web in the form of stable isotopic ratios, so that it is possible to track movements between habitats that are inshore vs. offshore, marine vs. freshwater, and that have C3 vs. C4 vs. CAM photosynthesizing plants (Hobson 1999). By analyzing the stable isotope ratios of various tissues, it is possible to

see if an organism is in equilibrium with it's environment, or to see how long it has been in contact with the nutrients in it's habitat (Hobson et al. 1996; Maruyama A, Yamada Y, Rusuwa B, and Yuma M 2001; Harvey CJ, Hanson PC, Essinton TE, Brown PB, and Kitchell JF 2002).

Background on Metabolic Rate

Metabolism is defined as the sum total of all the chemical reactions taking place in an organism (Randall D, Burggren W, and French K 2002). Animals metabolize food by consuming O_2 to break down the macromolecules present (proteins, lipids, and carbohydrates), and in the process CO_2 is produced. The ratio of CO_2 produced to O_2 consumed (called the respiratory quotient, or RQ) can in some cases be used to determine the proportion of carbohydrates, proteins, and fats being metabolized, as each food molecule gives off a characteristic energy yield per liter of O_2 consumed (Schauf C, Moffett D, and Moffett S 1990). Because food substances consistently release the same amount of heat and require the same amount of O_2 when being oxidized to water and CO_2, all three measures – heat production, CO_2 production, and O_2 consumption - can be used to measure MR (Randall et al. 2002). As is commonly done, O_2 is used in this study to measure MR. Three assumptions must be met in order to use O_2 to measure MR:

1. the chemical reactions are aerobic (which is the case because the energy obtained from anaerobic reactions is minor except during intense aerobic activity)

2. the amount of heat produced when O_2 is consumed is consistent regardless of metabolic substrate (this is not the case because more heat is released when O_2 breaks down carbohydrates, rather than fat or protein, but this cannot be corrected for)
3. O_2 stores in the body are small compared to stores of CO_2 in animals' tissues, so that changes in O_2 consumption reflect MR more so than changes in CO_2 (this is true for most animals) (Randall et al. 2002).

Metabolic rate (MR) is related to body size (Schmidt-Nielsen 1984), so as the size of the organism increases, MR per tissue gram decreases according to $m^{0.75}$ where m is the mass of the organism in question (Kleiber 1932, 1947). Put another way, larger animals have lower MRs per gram of tissue than smaller animals (Kleiber 1961). However, whole body MR differs from mass-specific MR. For example, the overall MR (measured as ml O_2 consumed per unit time) of a very large animal will be higher than that of a very small animal, because larger animals need more oxygen and have a greater amount of metabolizing tissues. However, when mass of the individual is accounted for, larger animals have a lower MR/g (measured as ml O_2 consumed per unit time *per gram*). Therefore, whole body MR has a positive relationship with mass, while mass-specific MR has a negative relationship with mass. The tissues of a small animal will use O_2 faster than the same mass of tissues in a large animal. It is important to keep in mind that while size influences the MR of different species, the tissues in an individual organism

also have very different MRs which can lead to different turnover times (Tieszen et al. 1983). Unless otherwise noted, MR in this study refers to mass-specific MR (ml O_2/hr/g).

This being said, MR is a function not only of mass, but also of temperature (Gillooly FJ, Brown JH, West GB, Savage VM, and Charnov EL 2001), age, activity level, and stress (Hulbert & Lewis 2000). Animals that are similar sizes can have very different MRs (Muller & Diamond 2001; Speakman 2005). Increases in activity level have also been shown to increase the MR of both endotherms and ecotherms (Speakman 2005).

Basal MR (BMR) is measured under strict conditions, as it aims to obtain a uniform measure of MR that is comparable across studies. To obtain the BMR, the individual must be an adult, resting during the normal time of rest, it must not be temperature stressed or digesting food, and it must be inactive (McNabb 1988). BMR can also vary in terrestrial vertebrates with diet and other parameters (McNabb 1988). Because Field MR (FMR) and BMR scale differently with size (Koteja 1991) they cannot be used interchangeably. It has been shown that a correlation does exist between FMR and BMR for rodents (Koteja 1991; Ricklefs RE, Konarzweski, Marek, and Serge Daan 1996), but since FMR can vary by many parameters, BMR is widely used as a standardized parameter to compare MRs. The type of MR obtained in this study will be discussed in detail in later sections.

Relationship between MR and Isotope Turnover

It is believed that animals with a higher MR have higher incorporation rates for C and N isotopes. In 1983, Tieszen et al. found that the turnover of isotopes in tissues was related to the metabolic activity of that tissue, and that organisms and tissues with high MR (measured as rate of oxygen consumption) will have faster rates of tissue isotope incorporation. This has been interpreted to mean that organisms and tissues with a higher MR should have faster tissue turnover (Hobson & Clark 1992a; Voight CC, Matt F, Michener R, and Kunz T 2003; Klassen M, Thums M, and Hume ID 2004). Tissue turnover rate is correlated with both growth and metabolic tissue replacement (Fry & Arnold 1982; Hesslein RH, Hallard KA, and Ramlal P 1993; MacAvoy et al. 2001, 2005), and in organisms where growth does not account for all of the observed isotopic turnover, metabolism becomes important (Tieszen et al. 1983; Hobson & Clark 1992). Previous experiments have shown that it is possible to experimentally identify growth and metabolic tissue replacement as separate components of isotope incorporation (MacAvoy et al. 2005; Arneson et al. 2006).

Several studies have shown that tissue turnover depends more on metabolic tissue replacement than on growth, for a variety of organisms. Tarboush RA, MacAvoy SE, Macko SA, and Connaughton V (2006) showed that metabolic tissue replacement accounts for 68-80% of the observed changes in isotopic signature in the muscles of the zebrafish (*Danio rerio*) after a diet switch. Hobson and Clark (1993) found that tissue turnover in American crows is dominated by metabolism; similarly, it was found that metabolic tissue replacement accounted for approximately 90% of tissue turnover in mice

(MacAvoy et al. 2005). MacAvoy et al. (2005) also found that tissue turnover was an order of magnitude higher than would be expected solely from growth of mice. For adult brown shrimp (*Penaeus aztecus*) it was found that turnover is related more to maintenance metabolism than growth (Fry & Arnold 1982). Hobson and Clark (1992) hypothesized that C will turn over more slowly in larger animals because O_2 consumption is negatively correlated with body size. Finally, it has been shown that a faster turnover is associated with a higher MR per gram body mass, and that rats have a slower tissue turnover rate than do mice, which are smaller (and so have a higher MR) (MacAvoy SE, Arneson LS, and Bassett E 2006).

Many studies have shown that metabolically active tissues have a faster isotopic turnover than do tissues that are not metabolically active. Tissues like liver, fat, and the pancreas, which are more active (Schoenheimer 1949; Waterlow et al. 1978), tend to have higher rates of isotope turnover than non-metabolically active tissues like bone collagen, connective tissue, and muscle (Thompson & Ballou 1956; Libby WF, Berger R, Mead J, Alexander G, and Ross J 1964; Stenhouse & Baxter 1979; Tieszen et al. 1983; Hobson & Clark 1992; Logan J, Haas H, Degan L, and Gaines E 2006). It has been shown for gerbils that the turnover of C correlates linearly with the MR of the tissue in question, with liver turning over much faster than hair (Tieszen et al. 1983). A similar result has been found in Japanese quail when comparing turnover rates of liver and bone collagen (Hobson & Clark 1992a). Other organisms for which it has been found that tissues with a higher MR turn over faster include Pacific herring (Miller 2006), the salt

marsh mummichog (Logan et al. 2006), and the ocellate river stingray (MacNeil MA, Drouillard KG, and Fisk AT 2006).

For birds, the time it takes for an individual to isotopically resemble its diet is well known and predictable based on MR. Because many turnover studies have been performed on a variety of birds of different sizes, it is possible to construct a logarithmic relationship between isotopic turnover and MR (Roberts & Baudinette 1986; Hobson & Clark 1993; Lindstrom 1997; Woodin & Stephenson 1998; Haramis GM, Jorde DG, Macko SA, and Walker JL 2001; Bearhop et al. 2002; Hobson & Bairlein 2003; Person et al. 2003; Evans-Ogden LJ, Hobson KA, and Lank DB 2004; Mckechnie & Wolf 2004). However, this logarithmic relationship does not help to describe the relationship between MR and turnover for mammals, because mice and rats have a longer blood turnover time per gram of body mass than do birds (MacAvoy et al. 2006). More data is necessary to determine the type of relationship between MR and tissue turnover for mammals.

Importance of this Study

In 2005, Carleton and Martinez del Rio made an urgent call to gather more data in order to construct allometric relationships between body size and rate of incorporation for the most commonly used tissues in a diversity of taxa. Obtaining diet-tissue discrimination factors for many different tissues with different turnover rates may help to determine when dietary changes occur owing to migration, seasonal variation in food availability, or organism maturation (Arneson & MacAvoy 2005). There is currently insufficient data to show how mammal MR scales with mass and tissue turnover, as many

studies are on birds, with a few notable exceptions (MacAvoy et al. 2006; Martinez del Rio et al. 2009), so more data is necessary to see if mammals will scale logarithmically like birds. It has been acknowledged that MR may significantly impact isotope turnover rates, even if they vary within individuals of the same species, and that more studies are needed in order to find the relationship between isotope turnover and MR (Evans-Ogden, et al. 2004).

MacAvoy et al.'s (2006) study on the relationship between metabolism and isotope turnover in mice and rats was the first study to attempt to elucidate a predictive relationship between MR and tissue turnover, and they found that there was indeed a "strong and predictive relationship between metabolism and isotope turnover in adult homeotherms" (pg. 199). However, because they used two different species (mice vs. rats), they were not able to determine the type of correlation. Hobson and Clark (1992) stressed that more studies were needed to see how variations in MR influence isotopic turnover in tissues, whether the variations are due to body size, developmental stage, or activity level - all of which could also vary within a species. If a predictive relationship between MR and tissue turnover can be developed, then it will be possible to use known MRs to predict tissue turnover rates for organisms. To our knowledge, this is the first study to attempt to find a relationship between MR and tissue turnover between individuals of the same species.

CHAPTER 2

MATERIALS AND METHODS

Organisms

Twenty-four female rats of two different strains were obtained from Harlan Laboratories. Twelve rats were Sprague-Dawley (SD), and 12 rats were Wistar (W). It is important to note that, to our knowledge, this is the first study to explore the relationship between MR and turnover within a given species. Previous studies have shown the relationship between species (MacAvoy et al. 2006). Female rats were chosen because studies have indicated that male rats tend to show more signs of stress when group housed, whereas female rats show more signs of stress when they are housed alone (Brown & Grunberg 1995). As all rats were group housed (3 rats per bin), female rats were chosen so as to encourage minimum stress levels. Both of these strains are outbred, and were chosen as such to avoid long term health issues which many inbred strains face. Both strains are albino, have a docile disposition, and are noted to be good research models for both nutritional and general purpose studies (Harlan Laboratories). SD rats were 20-21 days old at arrival, and W rats were 21-26 days old. As the purpose of this experiment is to determine if strains with different MRs exhibit different tissue turnover rates, it was important to select strains that have a different MR. Harlan Laboratories provides growth chart data for their organisms, and the SD and W strains exhibit markedly different growth rates. According to these charts, female SD rats are

expected to reach approximately 220 grams by 12 weeks of age, whereas female W rats are projected to grow to approximately 340 grams by 12 weeks. It is worth noting that these weights are estimates, as the cage size and density of animals differs between the data provided by Harlan and the experimental set up employed here. The rats used in this experiment reached an average weight of 250 grams (SD), and 390 grams (W) after 12 weeks (these represent averages of all rats, regardless of experimental grouping).

Experimental Design

Upon arrival, the rats were randomly separated into bins by strain, with 3 rats per bin as previously noted. All rats were initially fed diet control diet 2018 *ad libitum* (see below for a description of diets), and all were given constant access to water throughout the experiment. All rats were allowed to equilibrate on the control diet while they were growing, so that the experimental period did not begin until rats were grown and tissue turnover due to growth would be minimized compared to turnover due to metabolic activity (West GB, Brown JH, and Enquist BJ 2001; MacAvoy et al. 2005). After 120 days on diet 2018, two bins (6 rats) of each strain were randomly selected using a random number generator to become the experimental rats. I chose to use the bins as the unit of randomization instead of individual rats so that the same rats would stay together throughout the course of the experiment, again to minimize the additional stress associated with forming a dominance hierarchy (Tamashiro KLK, Nguyen MMN, and Sakai RR 2005). It has been demonstrated that female rodents display increased levels of stress in situations of social instability or disruption, and one of the major stress responses is weight loss, which may be linked to an increase in MR (Tamashiro et al.

2005). Day 120 on the control diet was day 0 for the experiment. On day 6, one of the experimental SD rats died of unknown causes. Each of the rats was given a unique identity by making tick marks on their tails using a Sharpie. In the data, for example, the rat referred to as SD1-2 is in bin number 1, and has 2 tick marks on her tail. Because each has a unique identity, it was possible to group house the rats, while keeping track of each individual's tissue turnover and MR.

Care of Organisms

IACUC approved the use of animals for this experiment under protocol numbers 2010-SM021910 and 2010-SM080910. The bins in which the rats were housed were cleaned, according to protocol, by replacing the bedding at least once every week. Every two weeks, the bins, bin lids, water bottles, and water bottle stoppers were cleaned according to protocol with an acid wash in an appropriate chamber. During bin cleaning, and once per week apart from cleaning, the rats were allowed outside of the bins for approximately 10 minutes at a time. This was done to minimize stress levels, as well as to encourage natural behavior (such as sniffing, climbing, and hiding in provided boxes). It is known that Field MR (FMR) is higher than Basal MR (BMR) in some species (Nagy 1987; Hobson & Clark 1992), and in a lab setting where animals are confined and not regularly exercised, it is possible that tissue turnover will be slower than would be expected in the wild due to the differences in FMR and BMR (Hobson & Clark 1992).

Experimental and Control Diets

Harlan's Teklad Global Rodent Diet 2018 was the control diet used for this study. This diet contains ground wheat, ground corn, wheat midds, soybean meal, corn gluten

meal, and brewers yeast, for a total of 18% crude protein, 6% fat from soybean oil, and no less than 5% crude fiber (Harlan Laboratory). The experimental beet sugar diet was also produced by Harlan Teklad, but was a custom-made research diet for our study. This diet contains protein from casein, beet sugar, soybean oil, and a mix of vitamins and minerals. Though the two diets were chosen because they have different stable isotopic signatures, both diets provide essential nutrients so that the rats were not nutritionally stressed at any point throughout the study. It has been shown that nutritional stress can lead to changes in the stable isotopic value of tissues (Hobson & Clark 1992). The beet sugar diet is 18.3% protein, 60% carbohydrate and 7.2% fat by weight. The amino acid content was very similar between the two diets, as different concentrations of essential amino acids could conceivably affect the rate of fractionation from diet to body tissues. The percent of essential amino acids in each diet is seen below, in Table 1.

Table 1
Comparison of Essential Amino Acid Content of Control and Experimental Diets

Amino Acid	2018	Experimental
Lysine	0.92	1.45
Tryptophan	0.20	0.21
Histidine	0.47	0.52
Phenylalanine	0.99	0.91
Leucine	1.94	1.66
Isoleucine	0.85	1.04
Threonine	0.67	0.79
Methionine	0.35	0.48
Valine	0.95	1.24
Arginine	1.06	0.68

Note. All amino acid values are percentages.

Metabolic Rate Sampling

A Qubit system High Range Respiration package was used, in conjunction with Vernier® LoggerPro®, to measure MR as a function of decreasing oxygen (O_2) and increasing carbon dioxide (CO_2). Oxygen was used to measure MR as it is the final electron acceptor during oxidative phosphorylation, which creates a lot of energy in the form of ATP, and so is a good measure of energy output. Stores of O_2 in the bodies of most animals are also very small, so minute to minute O_2 consumption is assumed to reflect MR, whereas animals have a large store of CO_2 in their tissues, so CO_2 by itself would be a less reliable means of measuring MR (Randall et al. 2002). The respiration equipment (which includes an air pump, a CO_2 bag filled with a span gas [used to calibrate the CO_2 sensor, the span gas consisted of 5.125% CO_2 and ~95% N gas], flow meter, syringe with soda lime to remove CO_2, an animal chamber with a temperature probe and fan, syringe with Drierite to remove moisture, an oxygen sensor, and an infrared CO_2 analyzer) has been used for previous studies, and needed to be calibrated. Before obtaining reliable data, it was necessary to ensure that all parts were working properly, and that the battery-like O_2 sensor would remain calibrated over the course of several days. Multiple conversations with Qubit technicians enabled proper set up, calibration, and trouble shooting of the equipment.

Two different sized animal chambers were available for use: a 700 mL and 6.6 L chamber. Initially, measurements were taken using the 700 mL chamber, as the Qubit technicians advised that gas exchange would be harder to observe in the large 6.6 L chamber. The following calculation was used to determine the appropriate sampling

length in order to ensure that all measured gasses were those emitted from the rat, and not lingering room air inside the chamber:

$$Fx=C \qquad (2)$$

where F is the flow rate, x is a time constant, and C is the size of the animal chamber. Generally, it takes 5 time constants to completely flush the gas from the chamber so that all measured gasses are from the animal (personal communication with Quibit technician). Eight rats were chosen randomly for the first round of metabolic rate testing. The flow rate was 0.4 (400 mL/min) and the 700 mL chamber was used, as has been done in previous studies (MacAvoy et al. 2006). There were many problems associated with using this set-up, mainly that the rats were stressed in the small chamber, and a steady reading of gasses was not reached (see *Results*), so for the second round of MR testing, the large chamber was used with a flow rate of 0.6 L/min. This new flow rate was chosen for multiple reasons. It is essential to choose a flow rate high enough so that the rat is not stressed, and room air needs to come through the chamber at the same rate as the rat's respiration, so that CO_2 does not build up inside the chamber and cause asphyxiation. The flow rate was also increased so that the length of time needed to flush the 6.6 L animal chamber was still a reasonable sampling time per rat (55 minutes according to the above equation; rounded up to an hour per rat). This set-up gave better results (the animals seemed less stressed, and the measurements for CO_2 production and O_2 consumption reached a much more stable reading – see *Results*). However, as each rat needed to be sampled for one hour to obtain a reliable MR, sampling was spread over the course of three days (8 rats sampled per day), and the CO_2 sensor was recalibrated to the span gas

and room air each day. Unfortunately, small calibration differences each day led to variations in the measurements obtained (see *Results*). Therefore, for the third and fourth rounds of MR testing, all rats were sampled in one calibration of the equipment, over a course of approximately 27 hours.

There were several inherent issues with the MR testing, including the fact that the equipment being used was not the most current model, and had been in storage for approximately 2 years. Also, in order to calculate MR, masses of the animals must be known (see *Results*). As the animals were not sedated for any portion of the sampling, reliable and consistent weights were difficult to obtain. This problem was circumvented for the third and fourth rounds of MR sampling (when all samples were collected consecutively) by taking the average of five weights for each rat, being sure to obtain a steady reading each time, prior to MR sampling. Ideally, a final steady reading of O_2 and CO_2 should be used to calculate MR. However, even after one hour of sampling, a steady gas reading was not obtained, as the levels of O_2 and CO_2 followed a slight wave pattern, fluctuating up and down. Therefore, final values of CO_2 and O_2 are presented as averages of the last 10 minutes of sampling. It is possible that error is introduced if an average of the final values is not an accurate reflection of MR. As animals were not restrained or sedated during sampling, any movement may have an influence on CO_2 and O_2 levels, as activity level influences MR (Hulbert & Lewis 2000).

The strict definition for BMR is that of an animal at rest during it's normal time of rest, not under thermogenic stress, and not digesting food (McNabb 1988; Speakman 2005). Animals in this study were not necessarily at rest, as they were free to move

around the animal chamber, though by the end of the hour sampling period, most animals usually settled down and were not moving about. Because rats were not denied access to food or water prior to MR sampling, each individual rat's weight may shift slightly depending on their last fecal and the presence of undigested food. Rats were consistently weighed before MR sampling, and usually over the course of an hour in the MR chamber the rats urinated and defecated, which would change their weight slightly. As sampling spanned approximately 27 hours, some rats were sampled during rest while others were sampled during their normal time of activity, but this did not affect the MR readings (see *Results*). The relationship between BMR and MR obtained in this study will be discussed in later sections (see Discussion on "Metabolic Rates").

Blood Collection

After the diet switch (day 0), blood samples were regularly obtained for isotopic analysis. Blood samples of approximately 25-50 μl were collected from the experimental rats once a week for the first 16 weeks, while the control rats were sampled biweekly, as it was not anticipated that their tissue isotopic signature would change. After the first 16 weeks of sampling, blood was collected biweekly from the experimental animals and once per month from the controls. This design was appropriate because previous studies had shown that isotopic equilibrium was likely to have occurred at this point (MacAvoy et al. 2006).

Blood samples were taken via the lateral tail vein. All rats were weighed each week prior to blood collection, including the biweekly sampled controls. Before collecting blood, the rat's tail was wiped with a warm paper towel to increase blood flow

to the tip, then cleaned with an alcohol swab and dried. The end of the tail was then nicked with a scalpel, moving anterior with each consecutive week. Heparinized plastic 75mm hematocrit capillary tubes were used to collect the blood samples, which were placed in labeled glass vials and allowed to dry in a drying oven at 60°C for at least 3 days to remove moisture. As heparin is a protein used to prevent blood clotting, it is possible that C and N isotopes from the heparin coating were introduced into the blood sample. In order to test for this variable, blood was taken from 5 control SD rats using the above-described method, as well as without using the heparin tubes, and then these samples were analyzed for statistical differences.

Lipids were removed before analysis of all samples (DeNiro & Epstein 1977) by reflux with methylene chloride for 35 minutes. Samples were air-dried, ground, weighed (all samples weighed at least 0.6 mg), and packaged in tin capsules. Samples were then shipped for analysis of $\delta^{13}C$ and $\delta^{15}N$ at the UC Davis Stable Isotope Facility.

Calculating Growth, Turnover and Half-life

Because both growth and metabolic tissue replacement contribute to tissue turnover, it is important to separate those two components. Growth was calculated using the following equation

$$k = ln\ (Ms/M_0)/t \qquad (3)$$

where k is the growth rate, M_0 is the "initial" mass, and M_s is the mass in grams on day t. In order to monitor growth throughout the experiment, k was calculated in two-week intervals. M_0 therefore does not represent day 0 of the experiment, as this would give skewed growth rates as day t increases. In order to account for this, k was calculated for

every 2 week period throughout the experiment, so that M_0 was always 2 weeks prior to M_s for any given day t.

In order to determine the rate of turnover for both C and N isotopes, the following equation was used to model the rate of isotope incorporation into the blood of the rats:

$$C(t) = C_E + (C_0 - C_E)e^{-(k+m)t} \qquad (4)$$

Here, C represents the isotope signature (of either C or N) at day t of the experiment, C_E represents the isotopic signature when the animal is in equilibrium with it's diet (calculated using the signature of the diet and the calculated fractionation value), C_O is the initial isotopic signature before the diet change, k is the growth constant as described previously, and m is the metabolic constant, or the portion of turnover due to metabolic tissue replacement. To estimate C(t), k+m is determined using the least sum of squares method. The value of k+m was adjusted to give the smallest possible outcome of equation 5, where all weeks are summed. The estimated value of k+m can be adjusted to give the smallest sum of differences, which shows the best fit.

$$\sum [observed\ C(t) - estimated\ C(t)]^2 \qquad (5)$$

In order to determine m for each rat, equation 4 can be rearranged:

$$m = -[(\ln [(C - C_E)/(C_0 - C_E)])/t + k] \qquad (6)$$

Once both m and k are calculated, it is possible to determine the half-life of the isotope. Half-lives represent how long it takes for half of the existing tissue to reflect the isotopic signature of the new diet, and are calculated as follows:

$$t\ (1/2) = \ln (2) / (m + k) \qquad (7)$$

Statistical Tests

For the first full round of MR sampling, which was spread over the course of three days, an ANOVA analysis was used to see if there was a significant difference between the MRs of each strain (SD and W) over the three days of calibrating the equipment. As an average of the final 10 minutes of CO_2 and O_2 values were used instead of a final steady reading, it was necessary to see if the standard deviations of the averages were significantly different between strains, and for this an independent t-test was used. Finally, a two-sample t-test was used to see if there was a significant difference in MR between the two rat strains.

The data for the last two rounds of sampling were analyzed slightly differently, as they were run over a continuous time period, in the same calibration of the MR equipment. Independent t-tests were used for the following statistical analyses: to see if there was a significant difference in the standard deviation of the final CO_2 and O_2 values between strains (as before); to see if there was a significant difference between the MR (measured as ml O_2/g/hr) between the strains (as before); and to see if there was a significant difference between the masses of the two strains.

Independent samples t-tests were also performed to see if there was a significant difference between strains for all variables (mass, MR, metabolic tissue replacement rate [m], and half-lives). In order to determine if heparin influenced isotope readings, an independent samples t-test was performed on the heparin vs. no heparin sampling methods.

Pearson's Correlations were used to test for significant correlations between MR, mass, m, and half-lives for each isotope.

CHAPTER 3

RESULTS

Metabolic Rate

Metabolic rate (MR) was measured on four separate occasions. Unless noted otherwise, MRs in figures refer to mass-adjusted MR (ml O_2/hr/g) of both the experimental and control animals of each strain. The first measurement was taken on a random sample of 8 rats immediately prior to day 0 of the diet switch, using the 700 mL animal chamber and a flow rate of 0.4 L/min. It was determined that this set-up was not ideal, as the rats appeared to be stressed in the smaller animal chamber, temperature inside the chamber rose throughout the sampling time, and even after 30 minutes of sampling, the CO_2 and O_2 levels fluctuated significantly. CO_2 concentration inside the chamber reached very high levels, so the decision was made to use the larger (6.6 L) animal chamber instead.

The second round of metabolic rate testing was spread over the course of three consecutive days, as discussed, so the MR equipment needed to be calibrated each time it was turned on, and the small differences in calibration between each day resulted in variation of measured MR between days that was higher than the variation between the strains. A one-way ANOVA was performed to see if there was a difference between the MR within a strain on the three different days of sampling, in order to determine if different calibrations of the MR equipment could significantly affect the results. The MR of both strains was significantly different across the three days of sampling ($p= 0.002$ and

0.001 for SD and W, respectively – see Figure 1 and Figure 2). This showed that the separate calibrations of the MR equipment could potentially lead to unreliable results. In all box-and-whisker type figures that follow, the black line represents the 50th percentile of the data, the top and bottom of the "box" are the 75th and 25th percentile, respectively, and the "whiskers" extend to the highest and lowest values. Any outliers (values which are more than 1.5 times the range represented in the interquartile box) are represented as circles beyond the whiskers.

Because the MRs could not reliably be compared across days, the next step was to determine if the difference between SD and W MRs remained constant over the course of the three days of different calibrations. If the difference in MR remained constant between days of sampling, then this could be used to measure MR even with calibration differences in the equipment. An independent samples t-test was run to compare the

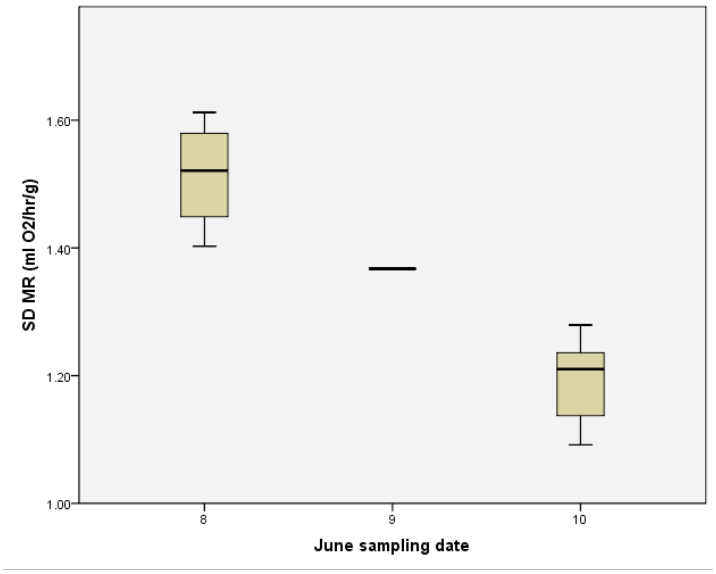

Figure 1: Comparison of MR for SD Rats Over Three Days of Different Calibrations of the MR Equipment. This led to significantly different MRs (p=0.002, ANOVA analysis) on different days. Only one SD rat was sampled on June 9th.

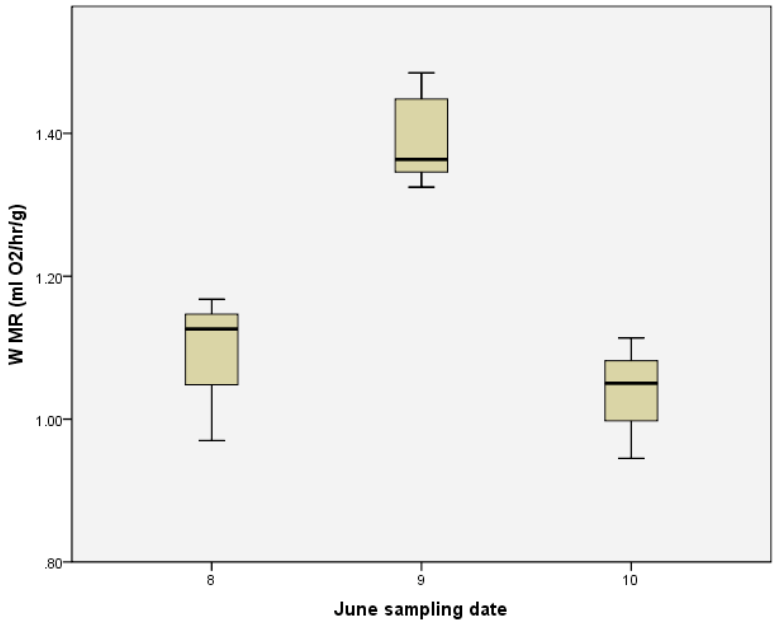

Figure 2: Comparison of MR for W Rats Over Three Days of Different Calibrations of the MR Equipment. The MRs were significantly different over the different days (p=0.001, ANOVA).

difference in MR between strains (MR of SD minus MR of W) between the three days of sampling. A t-test was used instead of an ANOVA because on the second day, only one SD rat was sampled, so differences could not be obtained, and so the first day was compared to the third day of testing. The difference between strains on different testing days was significant (p=0.001). Therefore, it was determined that the error introduced by calibration on the three consecutive days was too great to reliably compare all the rats of each strain to one another, and that in order to obtain comparable MRs, all rats needed to be sampled during one calibration of the MR equipment. For the subsequent MR sampling events, all MRs were obtained in one calibration. These sampling events are referred to as “July” and September” throughout the course of the analysis, and are explained in more detail later.

Even when using the larger animal chamber and a full hour of sampling per rat, the final readings for CO_2 and O_2 did not reach steady values. They were, however, significantly steadier with the large chamber and higher flow rate than they were with the small chamber and lower flow rate. Because a final steady reading was not achieved, an average of the final CO_2 and O_2 values was used to calculate MR. The following box plots (Figure 3 and Figure 4) compare the standard deviations in the averaged final 10 minutes for both CO_2 and O_2 gas, for all times MR data was collected. It is clear that during the sampling in May, when the small animal chamber was used with the low flow rate, the deviations in final gas values were much higher than with the alternative experimental set up. An ANOVA showed that there was a significant difference between these runs ($p= 1.09E^{-13}$ for CO_2, and $p= 3.36E^{-18}$ for O_2), showing that the larger chamber and higher flow rate give significantly steadier final readings on which to take an average final CO_2 or O_2 value. This could be because the rats were much less stressed in the larger chamber with a steady temperature, less CO_2 accumulated inside the chamber, and the rate of O_2 flowing through was higher, resulting in much less fluctuation of gasses.

The fluctuation of CO_2 and O_2 during the last 10-20 minutes of sampling formed a very gentle "wave" pattern, so an attempt was made to include one full cycle (from "crest" to "crest") of the "wave" to take the average. The CO_2 and O_2 values for the SD rats took approximately 10 minutes to cycle, whereas the W rats cycled in approximately 20 minutes. Independent samples t-tests were used to determine the length of time to include in the averaged final values for SD and W, by comparing how much the gas values fluctuated in the last 10 vs. 20 minutes. The standard deviations between an average of the last 10 minutes for SD and last 20 minutes for W were significantly

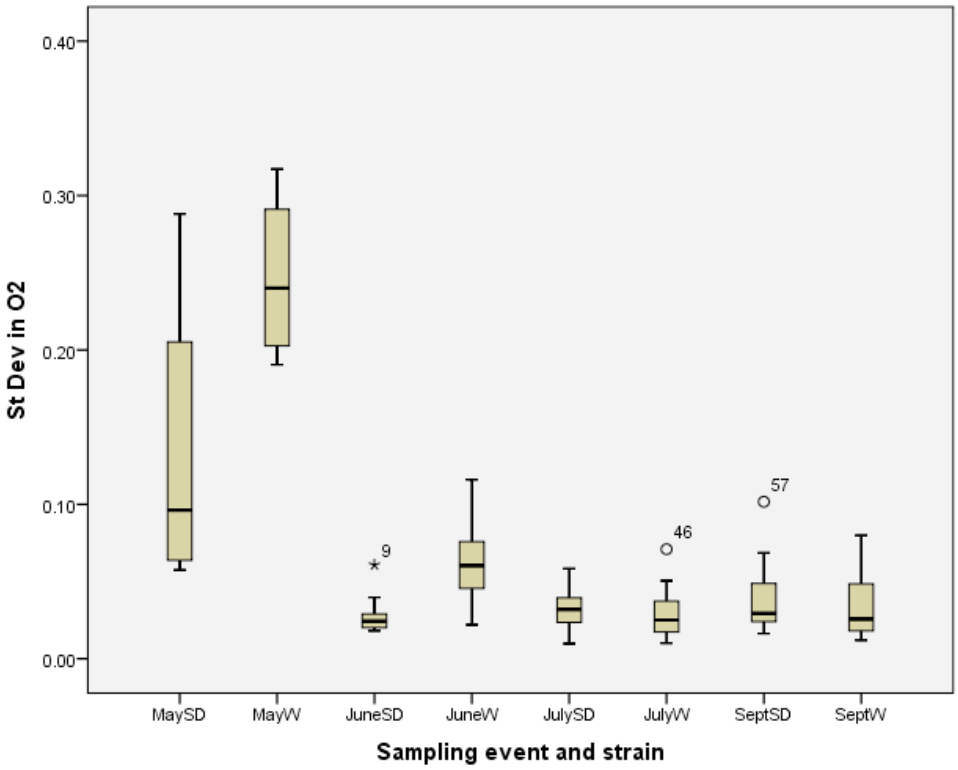

Figure 3: Comparison of Standard Deviations on Final Averaged O_2 Values Across all MR Sampling Runs. Those samples taken with the larger chamber and higher flow rate (June, July and September sampling events) show significantly less variation in O_2 levels during the final 10 minutes of sampling, represented by the significantly smaller standard deviations in the average final gas reading.

different (p= 0.004 for CO_2 and 0.001 for O_2). The standard deviation of the last 10 minutes of final CO_2 and O_2 values for W was then compared to the same for SD rats, and it was found that they were not significantly different (p= 0.473 for CO_2 and 0.187 for O_2), so an average of the final 10 minutes of CO_2 and O_2 values for both strains was used as the final CO_2 or O_2 value for all statistical analysis of MR.

For the third and fourth rounds of MR sampling, all rats were sampled during one calibration of the equipment, over a period of approximately 27 hours. The first overnight sampling occurred in the middle of the diet change (July 29-30), and the final MR

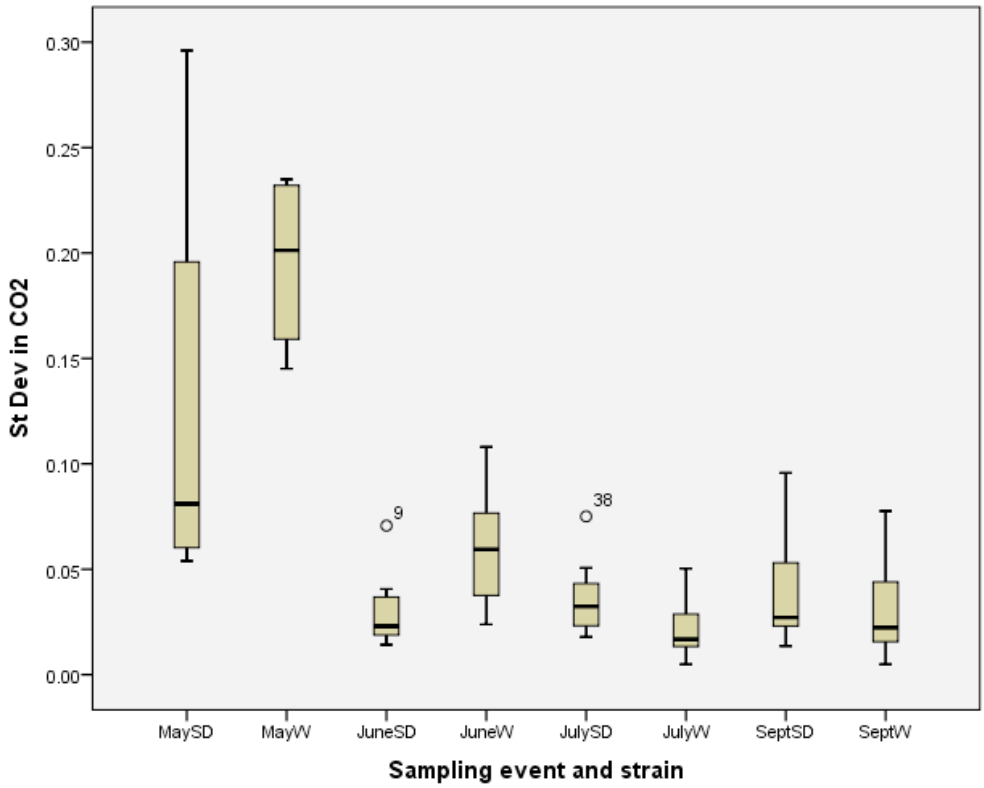

Figure 4: Comparison of Standard Deviations on Final Averaged CO_2 Values Across all MR Sampling Runs. Those samples taken with the larger chamber and higher flow rate (June, July and September sampling events) show significantly less variation in CO_2 levels during the final 10 minutes of sampling, represented by the significantly smaller standard deviations in the average final gas reading.

overnight sampling occurred at the end of the first 16 weeks (September 20-21), and so they are referred to as "July" and "September" sampling events in all comparisons. As before, the standard deviations around the averaged final CO_2 and O_2 values (averaged over the last 10 minutes of sampling for both strains) were compared using an independent samples t-test. There was a significant difference between the two strain's standard deviation of final CO_2 values ($p=0.029$), but no difference for the standard deviations of averaged final O_2 values ($p=0.625$) for the first overnight round of testing in July. For the second round in September, there was no significant difference between the two strains' standard deviations for averaged final CO_2 ($p=0.38$) or O_2 ($p=0.411$) values.

This allows us to be reasonably confident that the gas values for both strains reached an equally steady reading, and that we can take an average of the values of CO_2 and O_2 for the last 10 minutes of sampling to use as the final gas values for calculating MR.

Because all rats were sampled under the same calibration of the MR equipment, it is possible to compare the calculated MR between strains. Unless otherwise noted, MR in this study refers to the MR per gram of tissue, and is calculated as mL O_2 per hour per gram, as follows:

1. The change in O_2 is found by subtracting the final (averaged) value from the initial recorded O_2 value
2. ml O_2 per min is found by dividing the change in O_2 by 100, then multiplying this by (-600) because the flow rate was 600mL per minute and the difference between the final and initial O_2 values is negative
3. the value for ml O_2/min (which represents the MR which is not mass-adjusted) is divided by the individual rat's mass to get ml O_2/min/g
4. this value is multiplied by 60 to get the final MR value, in ml O_2/hr/g

MR was calculated for each rat, and an independent samples t-test showed that the MR was significantly different between strains, both during the July ($p=0.010$) and September ($p=4.17E^{-4}$) sampling runs. The following box plots (Figure 5 and Figure 6) compare the MR of each strain for both rounds of sampling. As predicted, the smaller SD rats have a significantly higher MR than the larger W rats, when calculating MR per gram of tissue. The average MR for SD rats in July was 1.17 (±0.24) ml O_2/hr/g, and in September the average SD MR was 1.23 (± 0.17) ml O_2/hr/g. For W rats, the average MR in July was 0.93 (± 0.15) ml O_2/hr/g and in September it was 0.95 (± 0.16) ml O_2/hr/g.

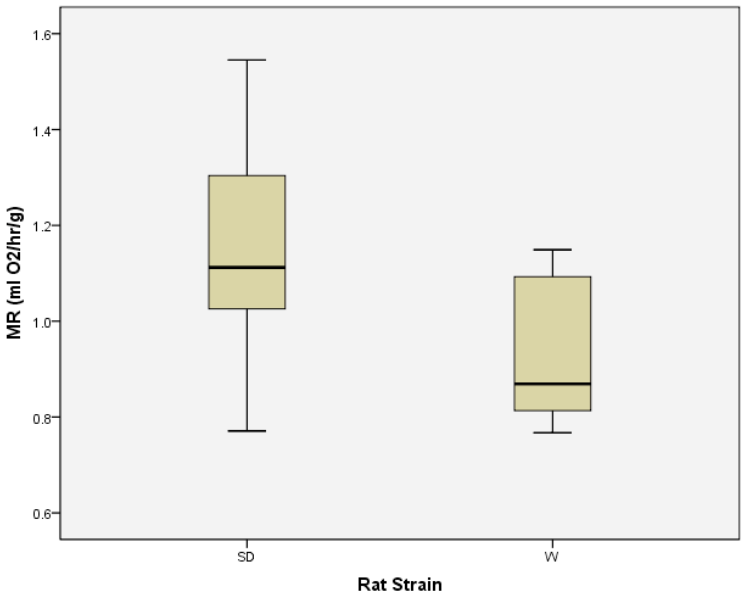

Figure 5: Comparison of MRs of the Two Rat Strains During July Overnight Sampling. MRs are significantly different (p=0.01).

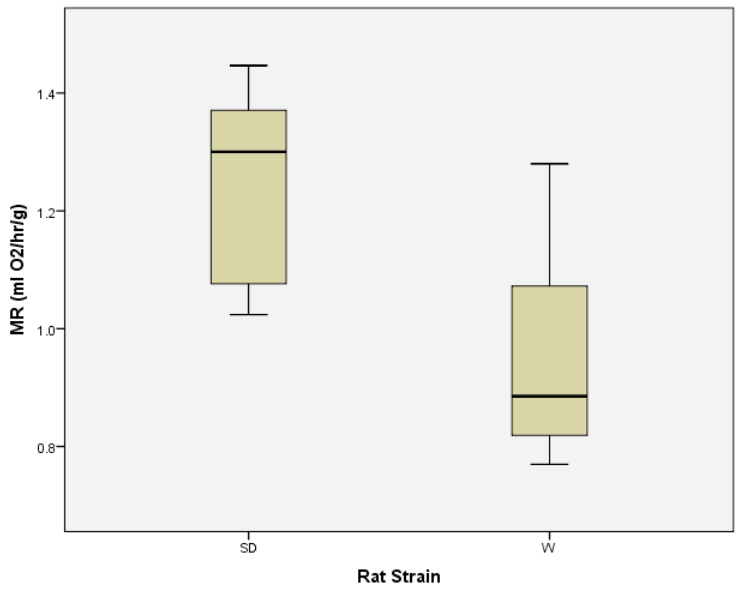

Figure 6: Comparison of MRs between Two Rat Strains During September Overnight Sampling. MRs are significantly different ($p=4.17E^{-4}$).

In order to compare the two overnight runs to one another, as well as to the initial MR sampling in June (which was spread over three days), the difference in MR between strains was compared (as before, the difference was calculated as SD MR minus W MR).

The difference was used because direct comparison was not possible, as the calibration of the MR equipment introduced too much error to directly compare between days. An independent samples t-test showed that the difference in MR did not significantly differ between overnight samplings (p=0.41). This shows that the MR of both strains did not change significantly from the middle of the study to the end. An ANOVA was performed to compare the two overnight runs to the initial 3-day run, again using the difference in MR as the basis for comparison. As seen below in Figure 7, there was no significant difference between the four sampling runs – two days in June, one in July, and one in September (p=0.413). On one of the June dates, only one SD rat was sampled (the rest were W) so it was not possible to take a difference, therefore only two of the three dates in June are represented in the below comparison. The ANOVA shows that the MR of both strains did not change significantly throughout the course of the study.

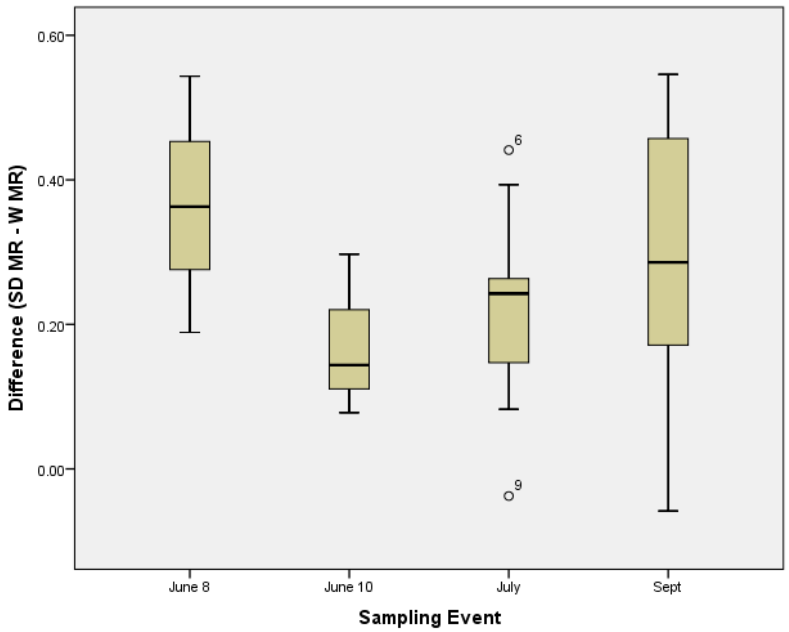

Figure 7: Box Plot to Compare all MR Sampling Runs to One Another, Using the Difference between Strains (SD MR Minus W MR). June 9 is excluded in this analysis because only one SD rat was sampled, so a difference could not be obtained.

In order to determine if diet had an effect on MR in either strain, t-tests were performed comparing the control and experimental rats of each strain to one another for both the July and September MR sampling events. The SD strain showed no significant difference between the MRs of the control group and experimental group for either the July or September sampling events (p=0.462 and 0.870, respectively). Figure 8 below compares the MR of the SD control and experimental groups for both sampling events. Because there was not a significant difference in experimental groupings, it can be assumed that the difference in diet did not affect the MR of SD rats.

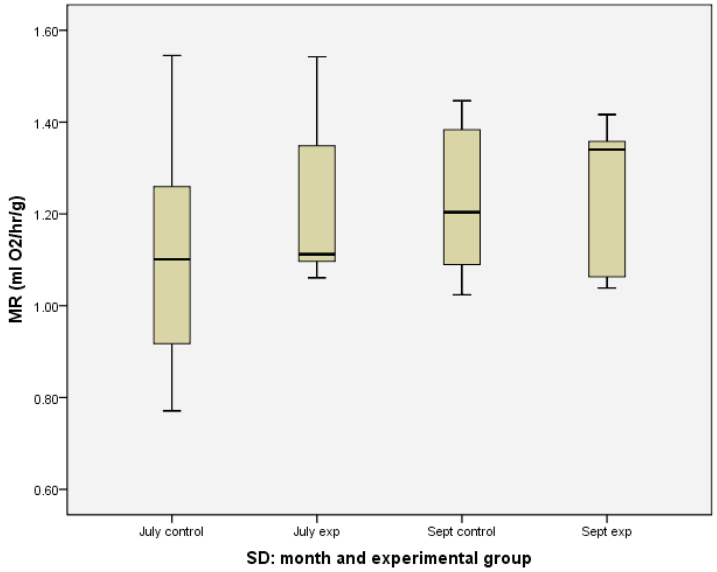

Figure 8: MR of Control and Experimental SD Rats During both the July and September MR Sampling Events. There was no significant difference in MR between experimental groupings (p=0.462 and 0.870 for July and September).

Although the diet did not affect the MR of SD rats, it is possible that W MR could have been affected by diet. There was no significant difference in MR between experimental and control W individuals for the July sampling event (p=0.282), but the

experimental group had a significantly lower MR than the control group during the September sampling event (p=0.033) (see Figure 9 below).

To determine if this difference in MR was due to differences in mass between the control and experimental group of W rats, a t-test was performed to compare the mass of control and experimental groups for both MR sampling events. However, there was no difference in mass of W rats for the July (p=0.197) or September sampling events (p=0.084). Figure 10 below shows the masses of W rats in both control and experimental groups, at the time of each MR sampling. Though the differences are not significant, the experimental W rats weighed more on average than the controls during both sampling events, and the difference is more pronounced in September (see Figure 10).

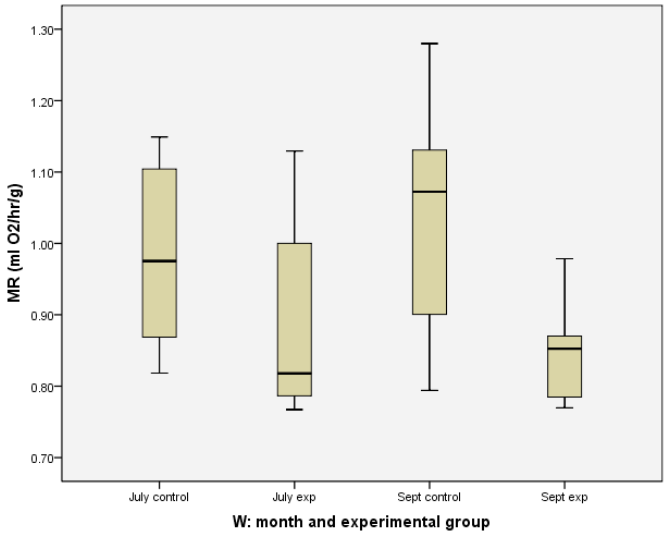

Figure 9: MR of Control and Experimental W Rats During both the July and September MR Sampling Events. The control rats had a significantly higher MR than the experimental rats during the September sampling event (p=0.033).

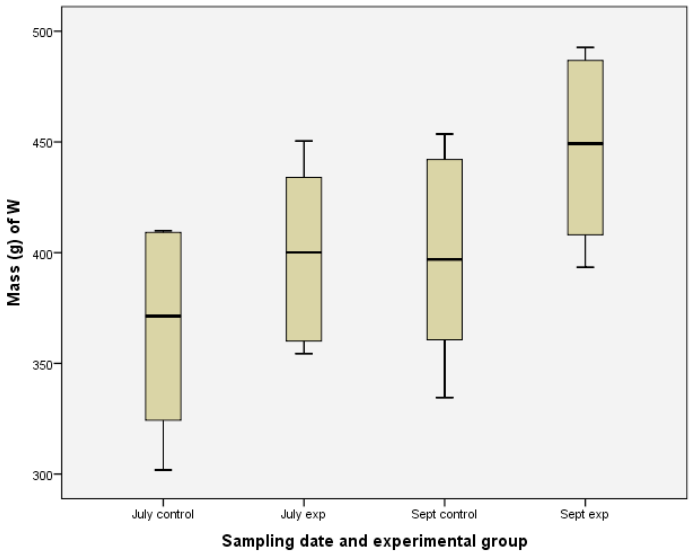

Figure 10: Comparisons of W Masses between Experimental Groups for both July and September Sampling Events. There were no significant differences between control and experimental groups for either sampling.

The average growth rate, k, was an order of magnitude larger for W than for SD rats (see Table 2). The experimental rats of both strains had a slightly higher growth rate than the control rats within each strain (see Table 2). When compared between strains, the assumption of equal variances was violated, so a Wilcoxon Signed Rank test was used as the non-parametric statistical analysis to compare the growth rate between strains, which was significantly different ($p=0.003$); this is unsurprising as the W rats grew more than the SD rats. When k is compared within a strain to see if experimental grouping affected the growth rate, there is no significant difference between experimental and control SD rats ($p=0.685$), but the experimental W rats grew at a significantly higher rate than the control W rats ($p=0.005$) (see Table 2 for average values of k for experimental grouping and strain). A comparison of the growth rates can be seen below in Figure 11. Where experimental and control SD rats show a similar growth rate, experimental W rats grew faster than control W rats.

Table 2
Comparison of Average Growth Rates Between Strains and Experimental Groups

	Average k	St. Dev
Control SD	2.60E-04	1.12E-04
Experimental SD	2.82E-04	3.89E-05
Control W	1.58E-03	2.86E-04
Experimental W	2.03E-03	1.24E-04

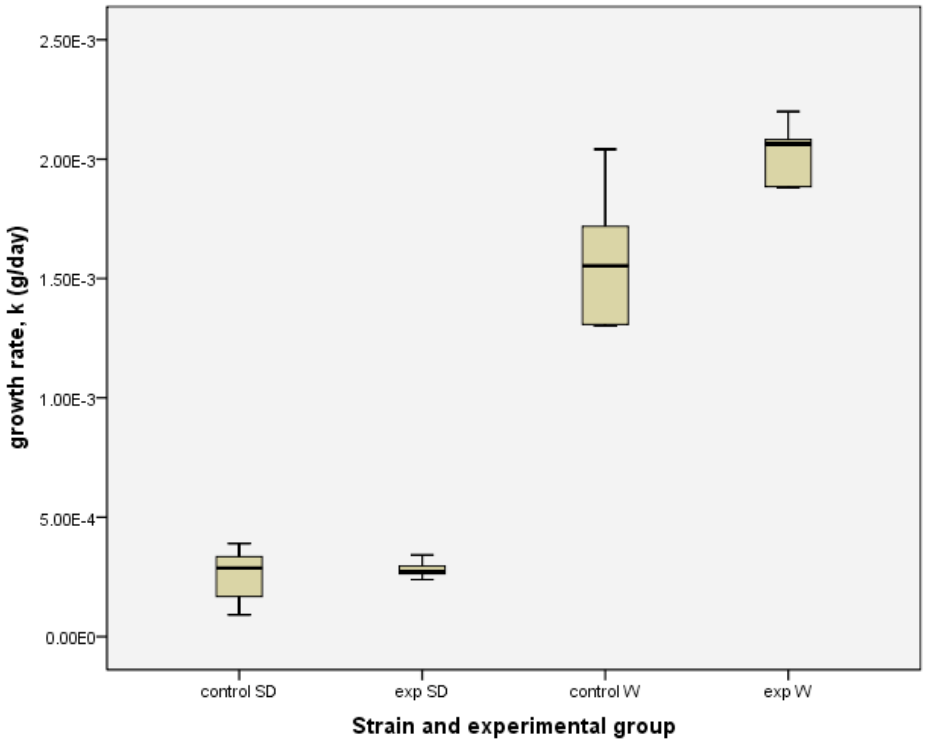

Figure 11: Growth Rates Across Strains and Experimental Grouping.

The metabolic tissue replacement rate, m, for C was not affected by this difference in k, though W rats have a higher m for N than do SD rats (see Table 4 in "Components of Turnover: growth (k) and metabolic tissue replacement (m)"). It is interesting to note that when all rats (control and experimental groups of each strain) were included in the analysis for significant differences in MR between strains, the statistical variances were equal. On the other hand, the assumption of equal variances for

independent samples t-tests was violated when only the experimental rats of each strain are compared to one another. An independent samples t-test of the MRs sampled in September of *only* the experimental groups of each strain showed unequal variances, as the range of MRs obtained for W rats was much smaller than for SD rats (see Figure 12 below). A Wilcoxan Signed Rank test was used to compare the experimental rats' MRs in September. This analysis showed that the experimental groups of each strain have significantly different MRs (p= 0.043). It is interesting to note that experimental grouping did not affect the MRs sampled in July, and the assumption of equal variances was not violated. July MRs were significantly different between strains when all rats were compared (p=0.01) as well as when control rats were excluded from comparison (p=0.01).

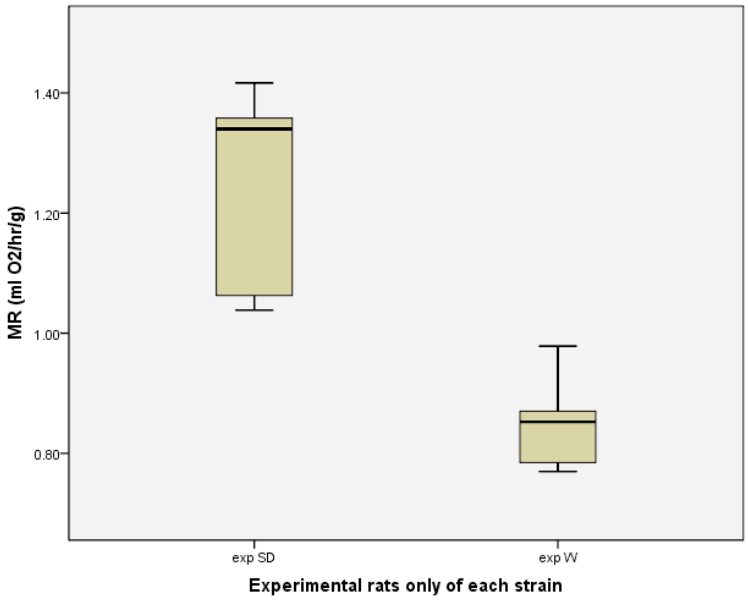

Figure 12: Comparison of MRs Sampled in September of Only the Experimental Rats of Each Strain. Because of unequal variances, a t-test could not be used to analyze the difference and a Wilcoxan Signed Rank test is employed instead.

The following tables summarize the MRs (in ml O_2/hr/g) of each individual rat, over all three sampling periods (Table 3), as well as the average MR of SD rats in this study and in previous studies (Table 4). The very first sampling run (in May) is excluded from this table because the smaller chamber and lower flow rate were used, and only 8 random individuals were sampled.

Table 3
Final Calculated Metabolic Rates (ml O_2/hr/g) for all Rats Across all Sampling Events, as well as Averages Within Strain per Sampling Event.

Rat	June 8-10	July 29-30	September 20-21
SD1-1	1.37	1.06	1.34
SD1-2	1.40	1.35	1.36
SD1-3	1.50	1.10	1.04
SD2-1	1.28	1.54	1.42
SD2-2	1.14	1.11	1.06
SD3-1	1.61	1.55	1.38
SD3-2	1.55	1.26	1.30
SD3-3	1.17	1.21	1.45
SD4-1	1.24	0.77	1.09
SD4-2	1.09	0.92	1.02
SD4-3	1.21	0.99	1.11
SD Avg:	**1.32**	**1.17**	**1.23**
W1-1	1.21	0.82	0.79
W1-2	1.13	1.08	0.90
W1-3	1.07	0.87	1.10
W2-1	1.55	1.15	1.13
W2-2	1.30	0.87	1.05
W2-3	1.37	1.10	1.28
W3-1	1.38	1.00	0.84
W3-2	1.34	1.13	0.98
W3-3	0.92	0.81	0.77
W4-1	0.98	0.79	0.78
W4-2	1.06	0.83	0.86
W4-3	1.09	0.77	0.87
W Avg:	**1.20**	**0.93**	**0.95**

Note. All MRs presented in ml O_2/hr/g

In this study, the average MR (of the July and September sampling events) of SD rats was 1.2 ml O_2/hr/g and the average for W rats was 0.94 ml O_2/hr/g, respectively. These values are comparable to similar data in the literature for rodent MR, as seen in Table 4 below (Hart 1971; MacAvoy et al. 2006). To our knowledge this is the first study to obtain MRs for W rats, so it is not possible to compare MRs obtained in this study to the literature for this strain.

Table 4
Average MRs (ml/O_2/hr/g) of SD Rats from Three Different Studies

Study	**SD MR (ml/O_2/hr/g)**
MacAvoy et al. 2006	1.84
Hart, 1971	1.23
This study	1.20

Mass

All rats were weighed weekly beginning at Day 0, as mass is used when calculating mass-adjusted MR. It is also necessary to keep track of weights so that the growth rate may be determined, as this is part of the equation for determining tissue turnover rate as well as determining the rate of metabolic tissue replacement, as discussed previously (Hesslein et al. 1993). The following graphs (Figure 13 – Figure 16) show the growth of all rats of each strain, starting at the first week following the diet change, so that week 1 immediately follows the 120 day equilibration period.

It is interesting to note that, though neither the control nor experimental SD rats grew notably over the course of the experiment, there was a larger range of weights for

the experimental SD rats than for the control SD rats. The W rats exhibited slow but continuous growth throughout the course of the experiment, though this growth will be accounted for (k) when calculating tissue turnover so that the growth does not impact the metabolic component of turnover (m).

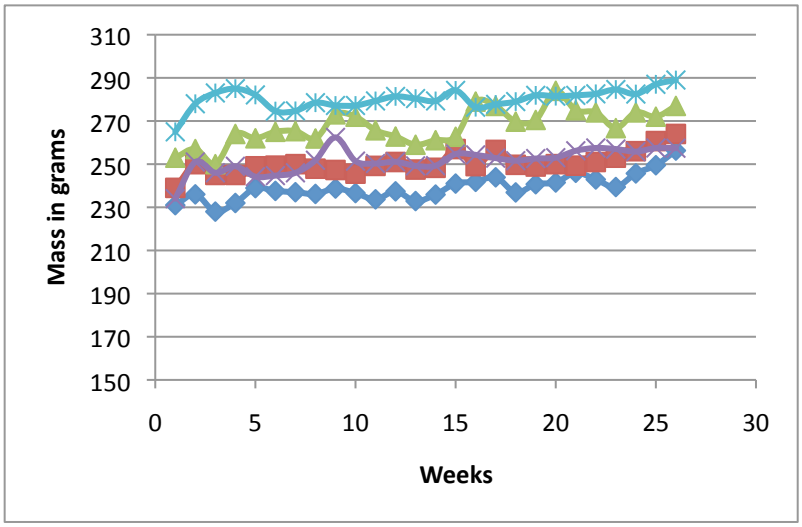

Figure 13: Mass of Experimental SD Rats for the First 26 Weeks After Diet Switch. Each line tracks the growth of an individual rat.

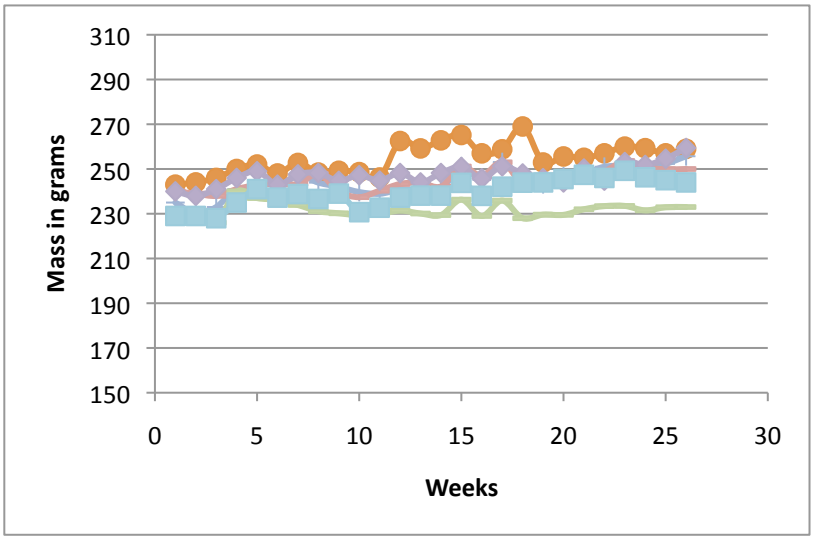

Figure 14: Mass of Control SD Rats for First 26 Weeks of Experiment. Each line tracks the growth of an individual rat.

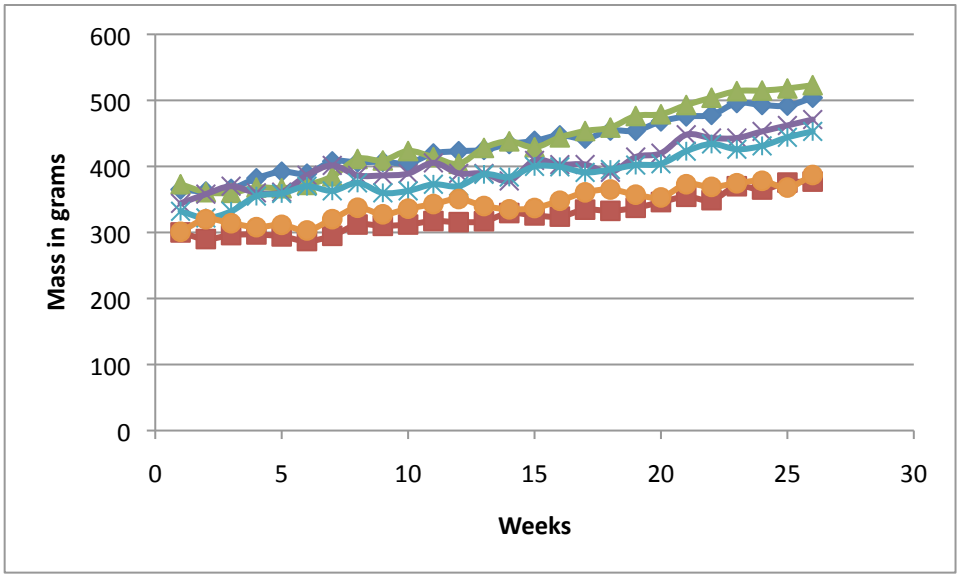

Figure 15: Mass of Control W rats for First 26 Weeks of Experiment. Each line tracks the growth of an individual rat.

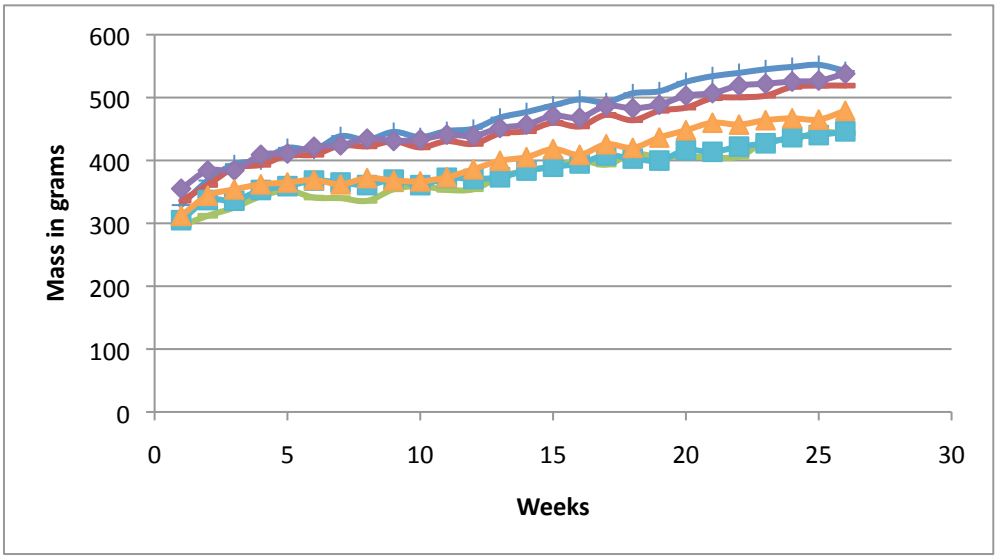

Figure 16: Mass of Experimental W rats for First 26 Weeks of Experiment. Each line tracks the growth of an individual rat.

Components of turnover: growth (k) and metabolic tissue replacement (m)

As the two strains of rats have a significantly different MR, it is possible to compare their rates of isotopic incorporation by calculating the percentage of turnover owing to growth (k) and metabolic tissue replacement (m), and determine if the different MRs of each strain influence m. In order to determine this, it is necessary to break down tissue turnover into its two components, k and m. Growth is defined as the addition of new tissue, and metabolic tissue replacement refers to the replacement of existing tissues via catabolic processes. In order to see the contributions of k and m to tissue turnover, expected turnover (calculated two ways: while setting m to zero, and then using calculated vales for m) was graphed against the observed isotopic signatures for each isotope and both strains over time. Figure 17 and Figure 18 show observed and expected average C turnover for the experimental rats of each strain, respectively, when m is set to 0 so that expected tissue turnover is based solely on k.

Figure 19 and Figure 20 show the same comparison when k+m is used to calculate the expected tissue turnover for both strains for C (see description of least sum of squares calculation above).

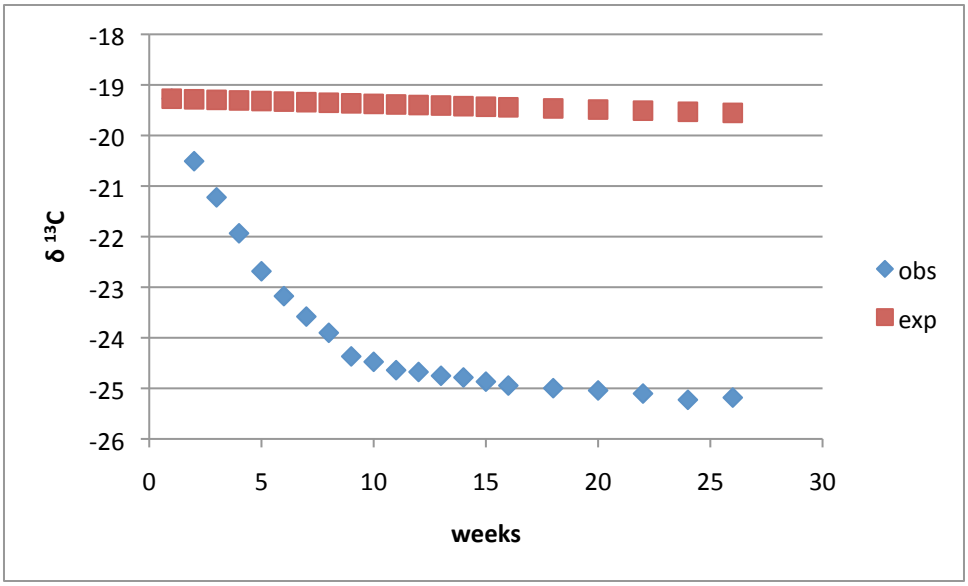

Figure 17: Expected vs. Observed C Turnover in SD Rats when Metabolic Tissue Replacement is not Taken into Account.

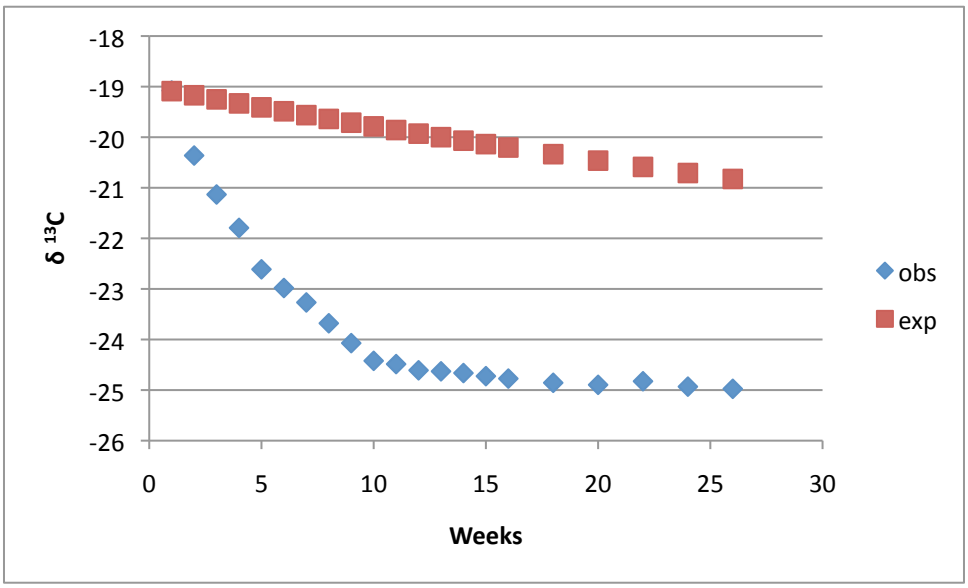

Figure 18: Expected vs. Observed C Turnover in W Rats when Metabolic Tissue Replacement is not Taken into Account.

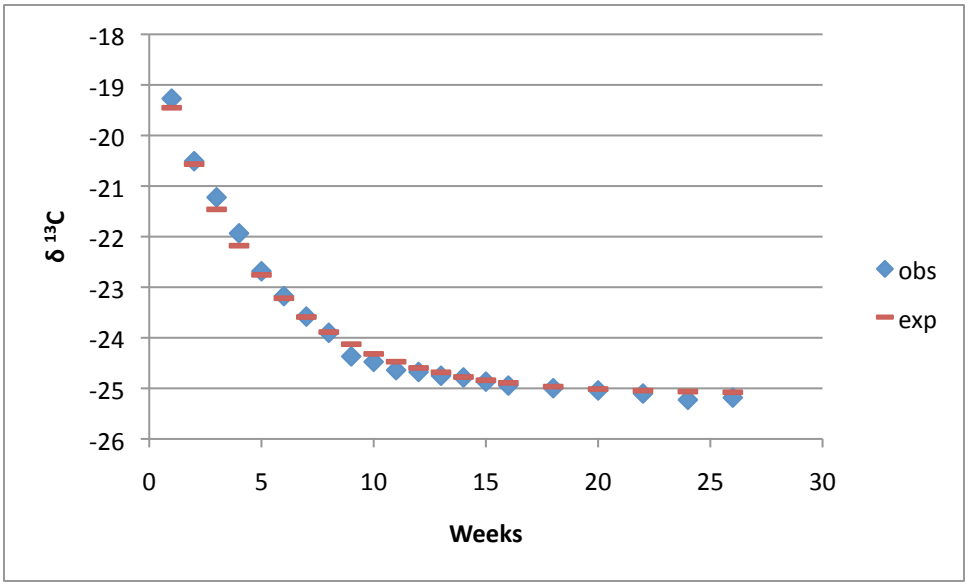

Figure 19: Observed and Expected C Turnover for SD Rats when Expected Turnover Accounts for Metabolic Tissue Replacement.

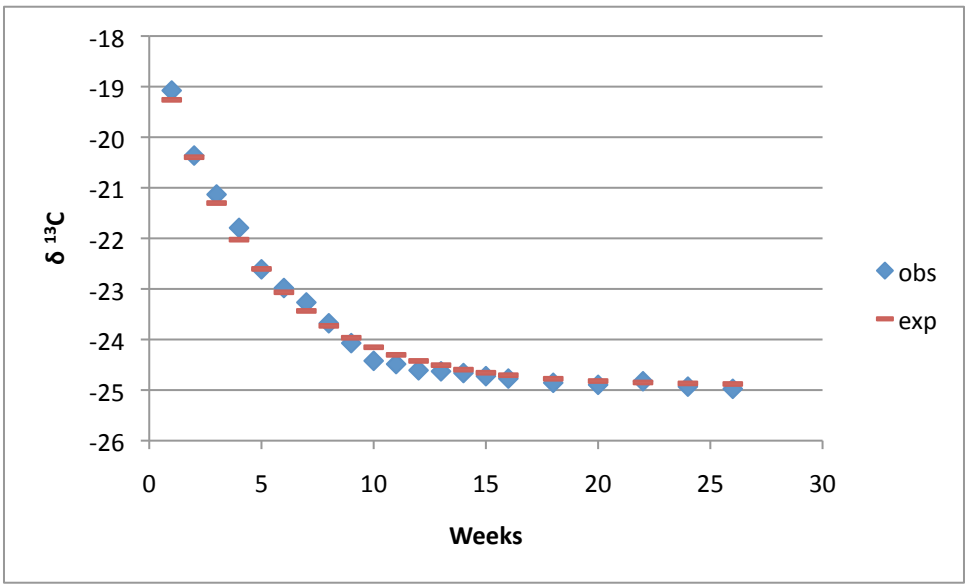

Figure 20: Observed and Expected C Turnover for W Rats when Expected Turnover Accounts for Metabolic Tissue Replacement.

When m is used to calculate expected turnover rates for C, it is a much better approximation of observed C turnover rates. The same is true for N, as shown in Figure 21 – Figure 24. Figure 21 and Figure 22 represent expected N turnover for each strain,

when only growth is used to calculate turnover. Figure 23 and Figure 24 show the much closer relationship between observed turnover and expected turnover when m is accounted for.

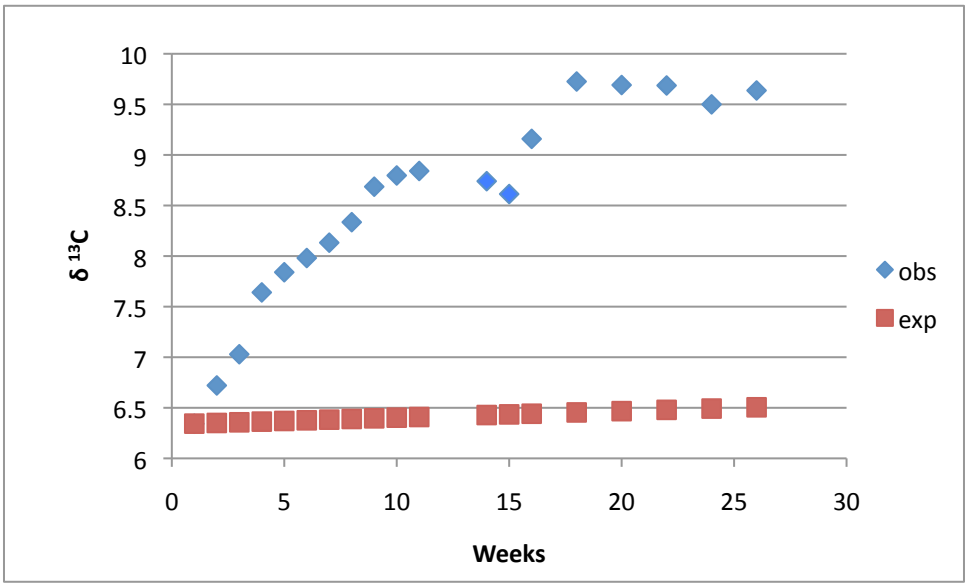

Figure 21: Expected vs. Observed N Turnover in SD Rats when Metabolic Tissue Replacement is not Taken into Account. Weeks 12 and 13 are excluded from the analysis due to incomplete combustion.

Using the least sum of squares method and equation 5 above, k+m for C was calculated to be 0.0385 for SD rats and 0.0333 for W rats. For N, k+m was calculated as 0.0179 for SD rats and 0.0209 for W rats. Tables with observed k values for each individual rat, as well as m values for the experimental rats can be found in the appendix (Table S35), and the averages for each strain are presented below in Table 5. It is worth noting that both control and experimental rats were included in the average for k, but as the isotopic signature of the control rats did not change, m+k represents only the experimental rats. Further discussion of the variation in k between control and

experimental rats is in the section entitled "*Growth rate, k, and metabolic tissue replacement rate, m"* of the discussion. As mentioned earlier, the W rats had a growth rate that was an order of magnitude faster than the SD rats.

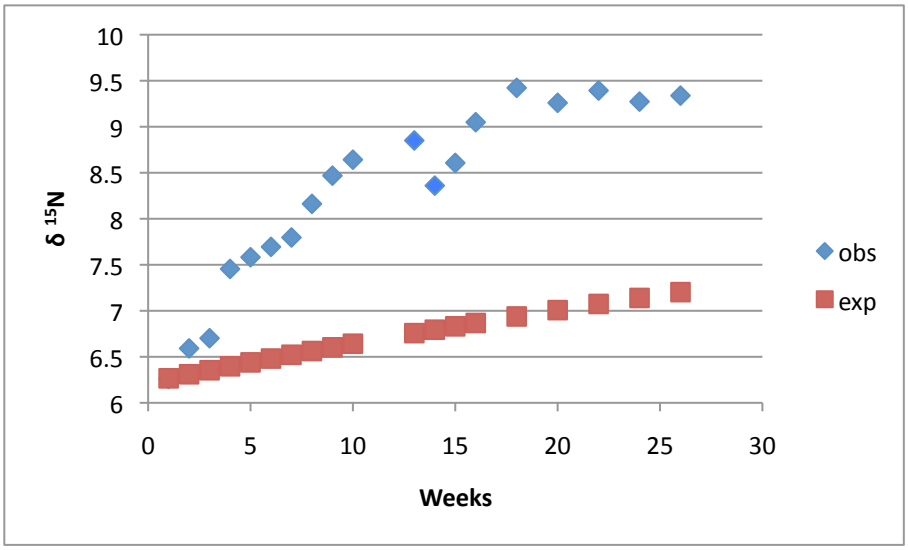

Figure 22: Expected vs. observed N turnover in W rats when metabolic tissue replacement is not taken into account. Weeks 11 and 12 are excluded from analysis due to incomplete combustion.

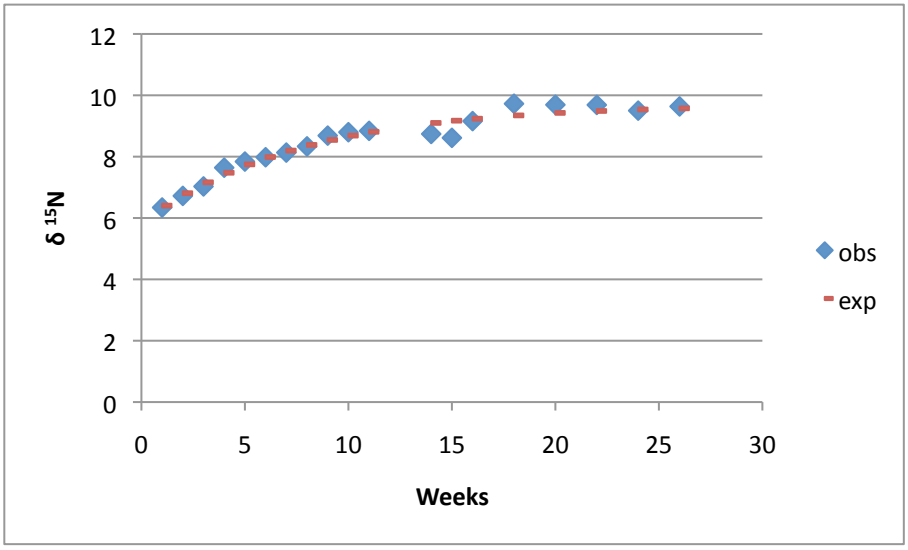

Figure 23: Observed and Expected N Turnover in SD Rats when m is Included in the Calculation for Turnover. Weeks 12 and 13 are excluded from the analysis due to incomplete combustion.

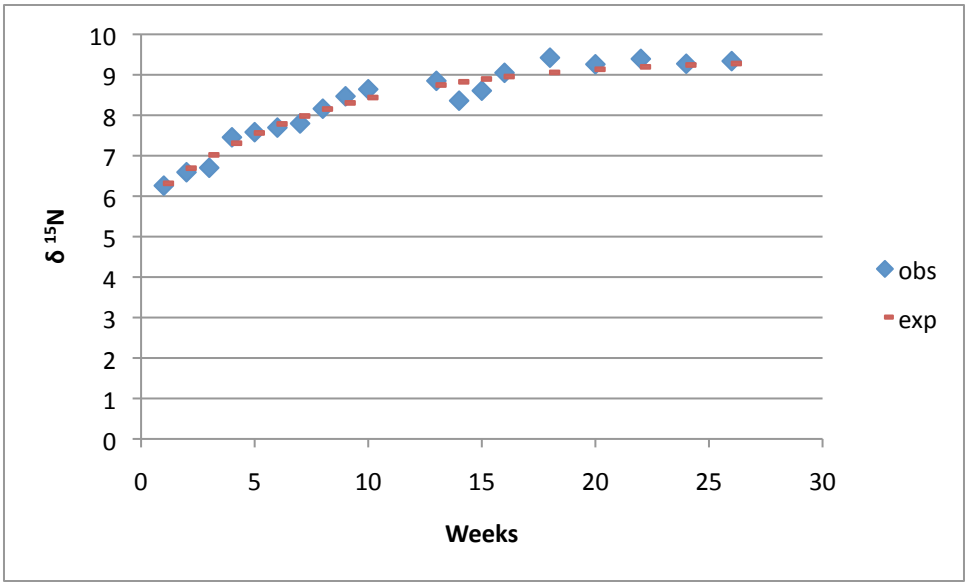

Figure 24: Observed and Expected N Turnover in W Rats when m is Included in the Calculation for Turnover. Weeks 11 and 12 are excluded from the analysis due to incomplete combustion.

Table 5
Average Growth Rate and Metabolic Tissue Replacement Rate for both Isotopes and Each Strain.

	Average k	Average m for C	Average m for N
SD	0.000282	0.0382	0.0176
W	0.002029	0.0313	0.0189

Note. Average m+k values are for experimental rats only, as control rats did not exhibit tissue turnover

Isotopic Analysis

Heparin

Although the capillary tubes used to collect blood for analysis were heparinized to prevent clotting, it can be assumed that the coating did not interfere with the isotope readings. A Paired Samples Test between blood samples taken from 5 control SD rats with and without a heparinized capillary tube showed that there was no significant

difference between the sampling methods. C and N isotope values were not significantly influenced by the presence of a heparin coating ($p=0.681$ for C and $p=0.58$ for N).

Fractionation, or Diet-Tissue Discrimination

As expected, both SD and W blood tissues eventually came into equilibrium with the experimental diet. In order to determine the isotopic signature of the tissues at equilibrium (C_E), it was necessary to determine the fractionation value for each strain on both diets. As discussed previously, animals will not mirror the isotopic signature of their diet exactly because of fractionation, so the degree of fractionation must be known in order to determine the value of C_E in the equations for turnover (see equations 4 and 6). The fractionation value is equal to the organism's isotopic signature at equilibrium minus the signature of the food. Both control and experimental diets were analyzed for C and N signature (see Table 6 and Table 7 below). Rats were considered to be in equilibrium with their diet when the isotopic signatures fluctuated less than 0.1‰ between blood sampling events. Equilibrium as defined occurred at week 20 for both strains, so the signature at equilibrium was determined by taking an average of all experimental SD and W isotopic signatures from week 20 to the end of the study (week 26). It has been shown that slight miscalculations in equilibrium values (±0.1‰) do not lead to miscalculations in tissue turnover analysis, as turnover rates are relatively insensitive to this type of error (MacAvoy et al. 2005). Calculated equilibrium values (± standard deviation in parentheses), the signatures of the experimental and control food, and the calculated fractionation values are presented in Table 6 (C signature) and Table 7 (N signature) below.

Table 6
Carbon Signature of Diets: SD and W Experimental and Control Groups at Equilibrium with Diet, and the Calculated Fractionation Value

	Initial $\delta^{13}C$ (+/- St Dev)	Equilibrium $\delta^{13}C$ (+/- St Dev)	Diet Signature	FRACTIONATION
SD control	-19.3 (0.11)	-19.3 (0.03)	-20.3	1.0
SD exp	-19.3 (0.16)	-25.1 (0.11)	-26.3	1.2
W control	-19.1 (0.17)	-19.1 (0.07)	-20.3	1.2
W exp	-19.1 (0.05)	-24.9 (0.11)	-26.3	1.4

Table 7
Nitrogen Signature of Diets, SD and W Experimental and Control Groups at Equilibrium with Diet, and the Calculated Fractionation Value

	Initial $\delta^{15}N$ (+/- St Dev)	Equilibrium $\delta^{15}N$ (+/- St Dev)	Diet Signature	FRACTIONATION
SD control	6.4 (0.21)	6.1 (0.22)	2.6	3.6
SD exp	6.3 (0.56)	9.7 (0.21)	6	3.7
W control	6.1 (0.12)	5.9 (0.3)	2.6	3.3
W exp	6.3 (0.16)	9.3 (0.21)	6	3.4

Tissue Turnover

Rats on the experimental beet sugar diet came into equilibrium with their diet around week 20, as discussed above. The following figures show C (Figure 25 and Figure 26) and N (Figure 28 and Figure 29) turnover for control and experimental animals of both strains. Only the first 16 weeks of data were analyzed weekly for the control animals, and after 16 weeks, samples were analyzed only from the experimental animals on a biweekly basis. Average isotope turnover for both strains are graphed together (Figure 27 for C and Figure 30 for N) so that turnover can be more readily compared between strains. Standard deviations for both strains were too narrow to appear on the

graphs, but can be found in Table S33 and Table S34 of the appendix (C and N, respectively). Figure S51-Figure S54 in the appendix also show each individual experimental rat's C and N turnover, graphed by isotope and strain.

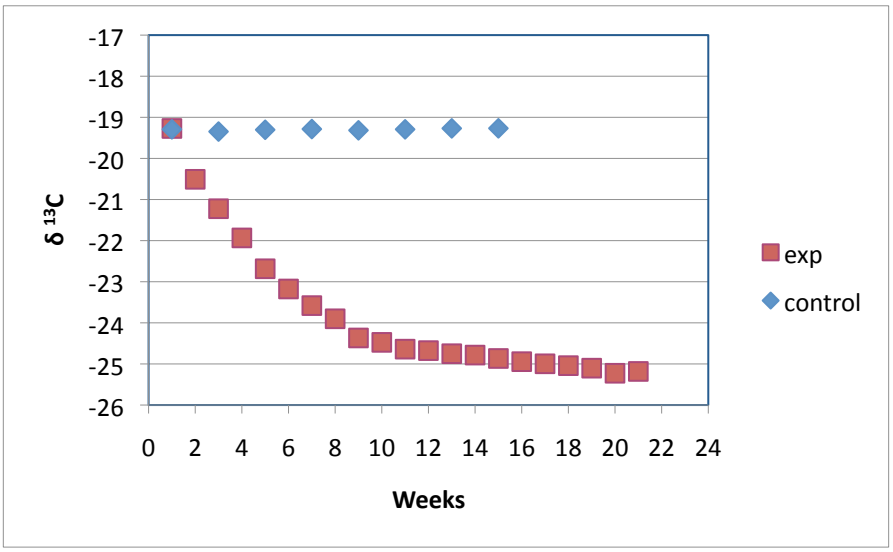

Figure 25: Carbon Turnover for SD Rats on Control and Experimental Diet.

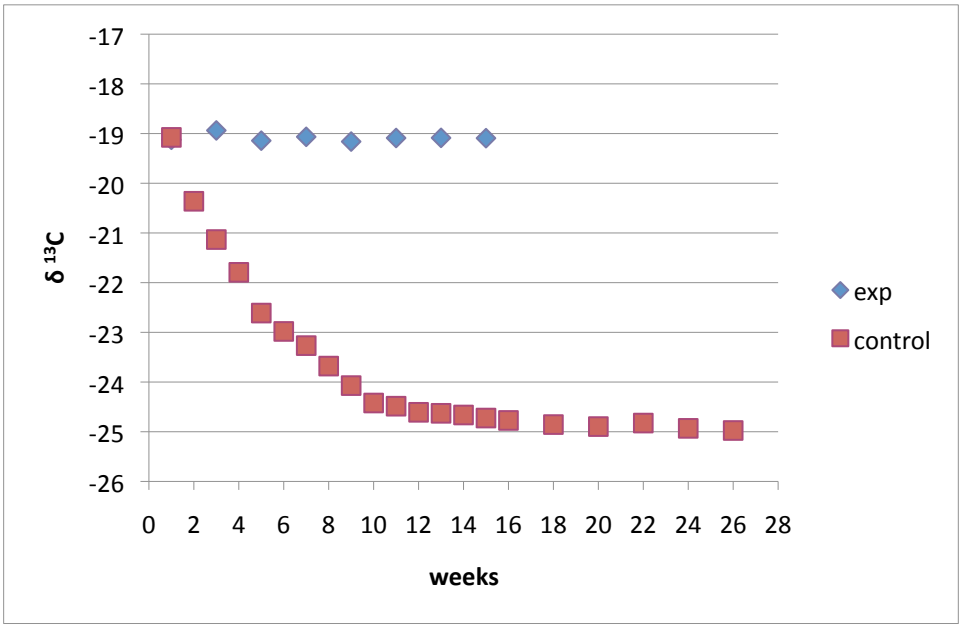

Figure 26: Carbon Turnover for W Rats on Control and Experimental Diet.

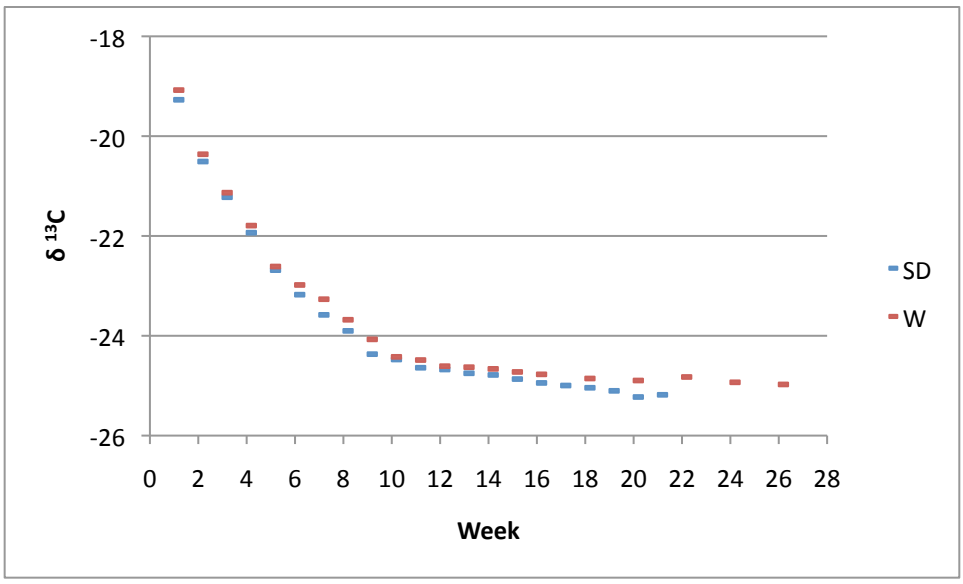

Figure 27: Average C Turnover of both SD and W Experimental Groups.

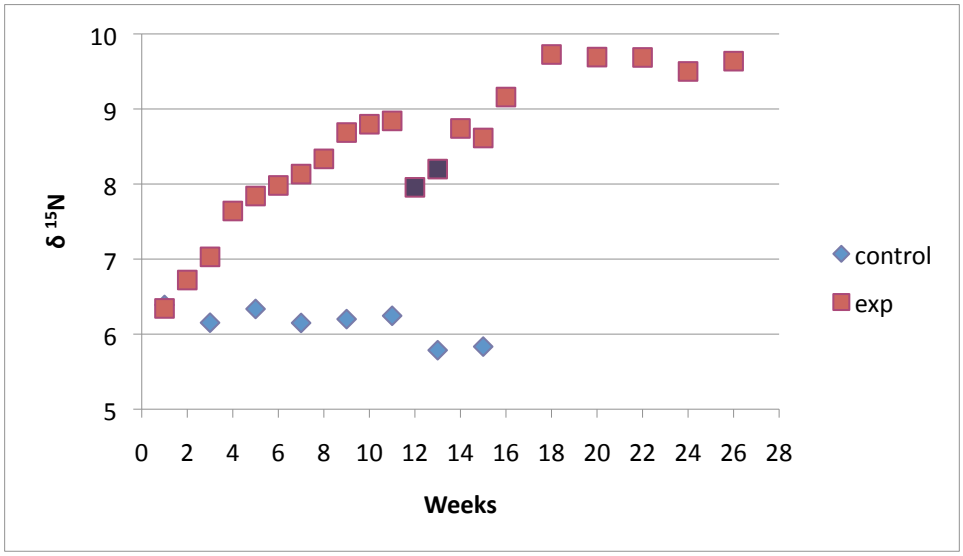

Figure 28: Nitrogen Turnover for SD Rats on Control and Experimental Diets. Purple dots represent those weeks not included in the analysis due to incomplete combustion.

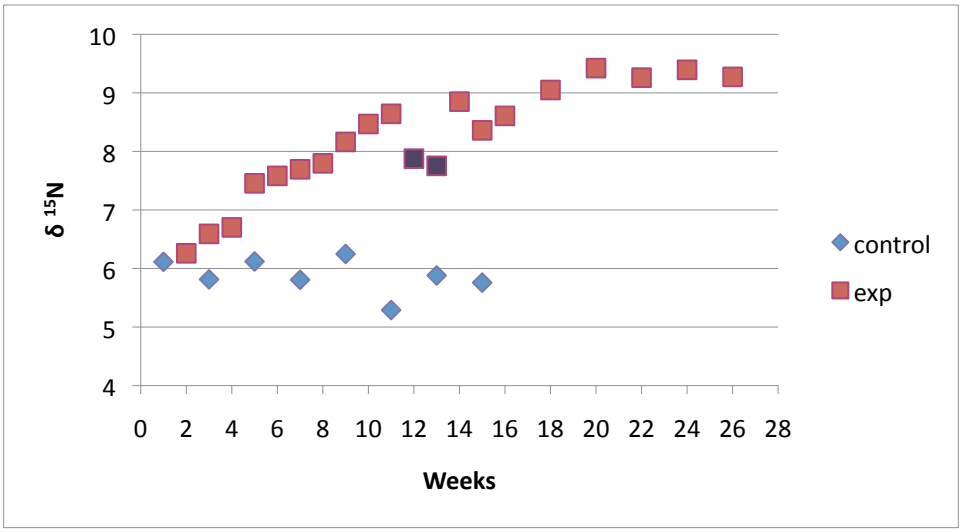

Figure 29: Nitrogen Turnover for W Rats on Control and Experimental Diets. Purple dots represent those weeks not included in the analysis due to incomplete combustion.

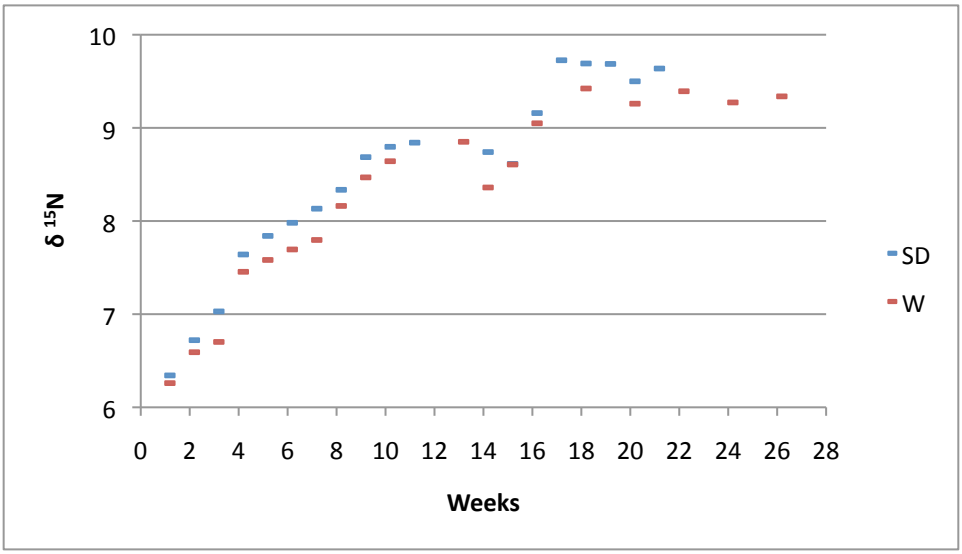

Figure 30: Average N Turnover for SD and W Experimental Groups, not Including Two Weeks Excluded from Analysis.

Because all tissue samples were analyzed using combustion, it is possible that incomplete combustion led to the dip seen in N values during weeks 11-13 (represented as purple dots in Figure 28 and Figure 29). As this dip is seen for only N samples, it is possible that the machine confused incompletely oxidized blood sample C for N. When C

is not completely oxidized to CO_2, but becomes CO, it has the same molecular weight as N_2 (28 or 29 g/mole depending on the C isotope), leading to misinterpretation by the mass spectrometer. If the dip is not due to incomplete combustion, it is likely that it is due to some other error in analysis, as this dip is present for both SD and W N samples, but not for C. These samples were all packaged in close proximity when shipped for analysis. For the purposes of performing subsequent calculations, those weeks represented as purple dots (weeks 12 and 13 for SD and weeks 11 and 12 for W) were excluded from the analysis.

Correlations

Metabolic rate and mass

Overall, the SD rats had a much narrower range of masses than the W rats. During week 1 of the study, the SD rats ranged in mass from 228-265g, while the W rats ranged from 296-373g. By week 26, the SD rats ranged from 233-289g and the W ranged from 377-542g. As discussed in the introduction, it is expected that MR that is not adjusted for mass (measured as ml O_2/unit time) will have a positive relationship with mass, while MR that is corrected for mass (measured as ml O_2/unit time/g) will have a negative relationship with mass. These relationships were demonstrated with the SD and W rats, as seen in the figures below, which show the line of best fit for a linear as well as a logarithmic relationship between the two variables. Both linear and logarithmic regressions gave significant R^2 values for the regression of MR on mass (see Table 8-Table 11). Both methods of fitting the data (linear and logarithmic) show that MR/g is significantly negatively related to mass and that unadjusted MR is significantly positively

related to mass, though because both fits are significant, it is difficult to determine if linear or logarithmic regression is most appropriate for the data set. The plotted regressions of MR vs. mass can be seen in Figure 31-Figure 34 below, where MR is first plotted as mass-adjusted MR (Figure 31 and Figure 32) and then as non-adjusted MR measured as ml O_2/min (Figure 33 and Figure 34) against mass for both sampling events. The respective values for R^2, their significance, and the equations for the lines of both the linear and logarithmic relationships are seen in the tables below their corresponding figures.

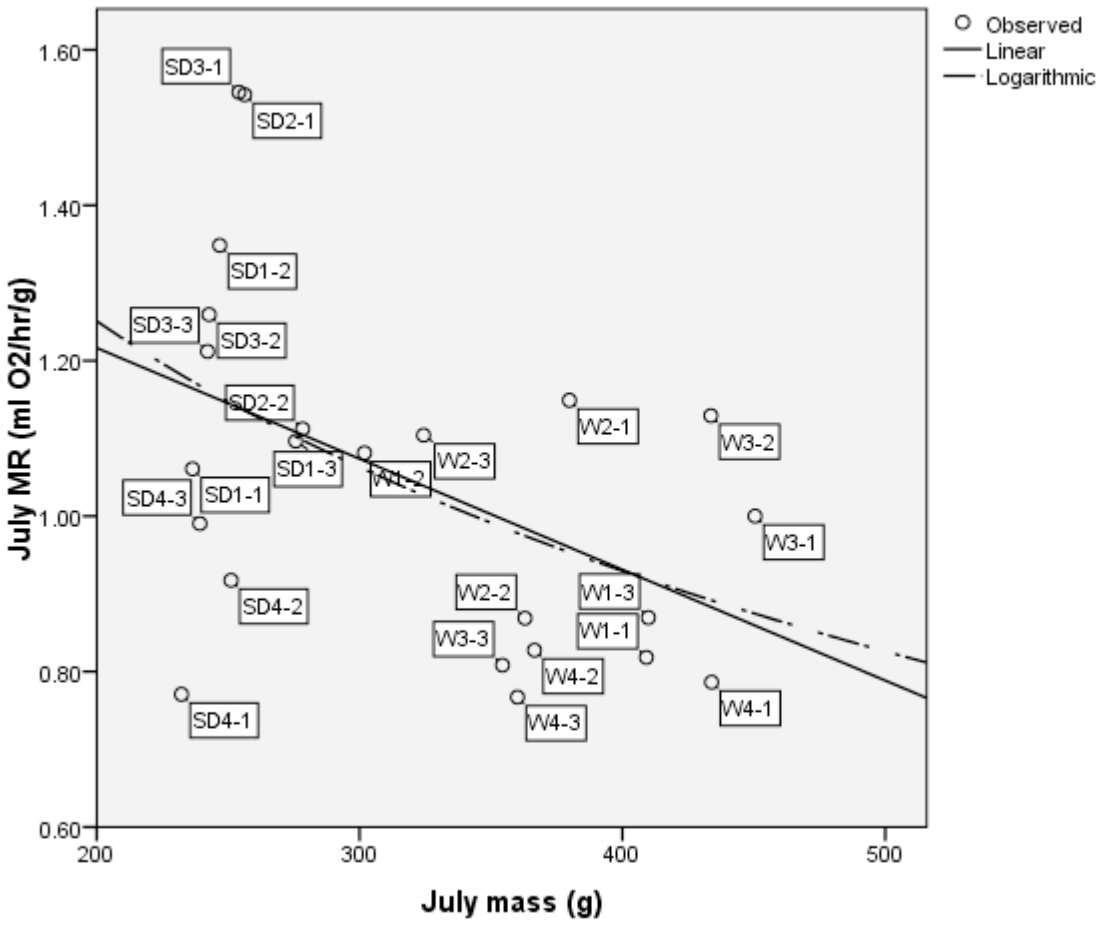

Figure 31: The Negative Relationship between Mass-specific MR (Measured as ml O_2/hr/g) Sampled in July, and Mass for all Rats.

Table 8
R^2 Value, the p-value Corresponding to the R^2, and the Equation for the Line to Compare Linear and Logarithmic Regressions of July MR on July Mass

	R^2	Sig. of R^2	Equation of line
Linear	0.221	0.024	y = -0.001x + 1.5
Logarithmic	0.224	0.022	y = -0.464*ln(x) + 3.71

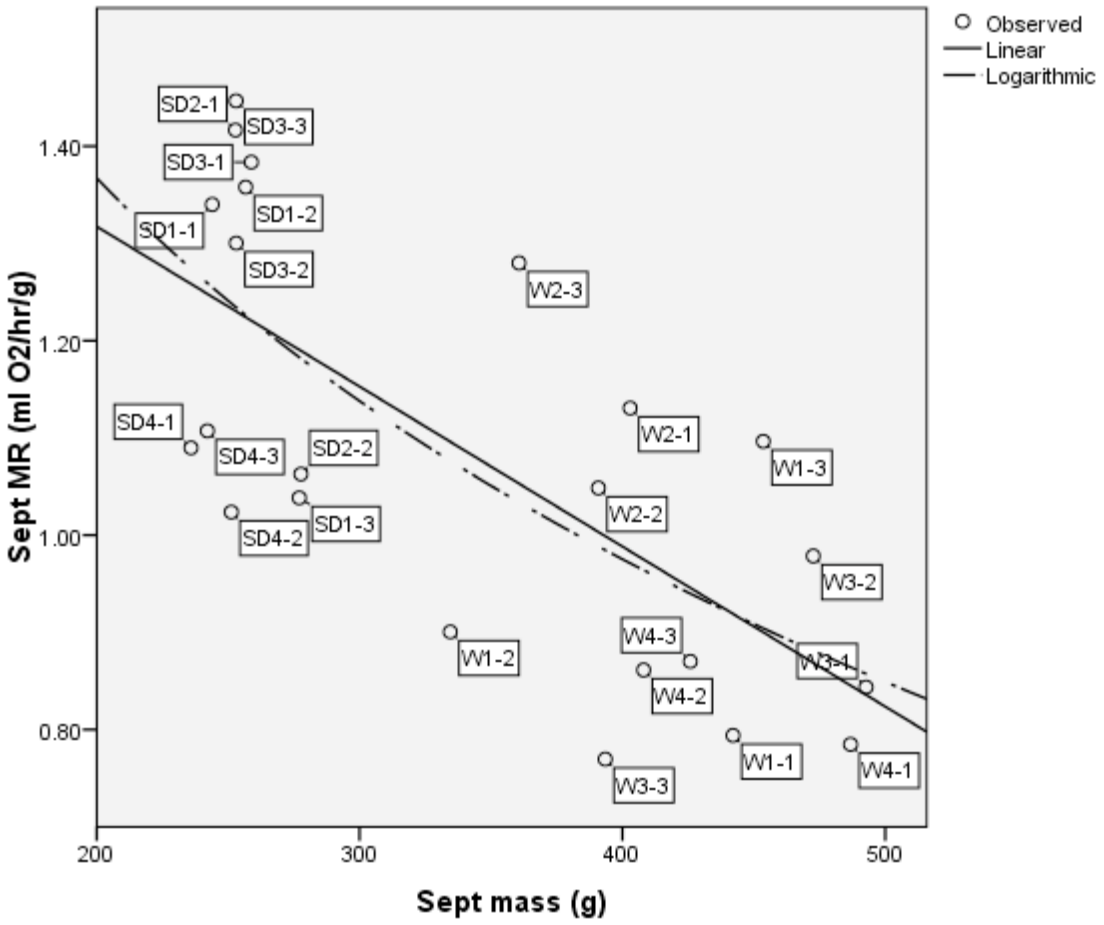

Figure 32: The Negative Relationship between Mass-specific MR (Measured as ml O_2/hr/g) Sampled in September, and Mass for all Rats.

Table 9
R^2 value, the p-value Corresponding to the R^2, and the Equation for the Line to Compare Linear and Logarithmic Regressions of September MR on September Mass.

	R^2	Sig. of R^2	Equation of line
Linear	0.493	1.86E-4	y = -0.002x + 1.65
Logarithmic	0.497	1.72E-4	y = -0.565*ln(x) + 4.36

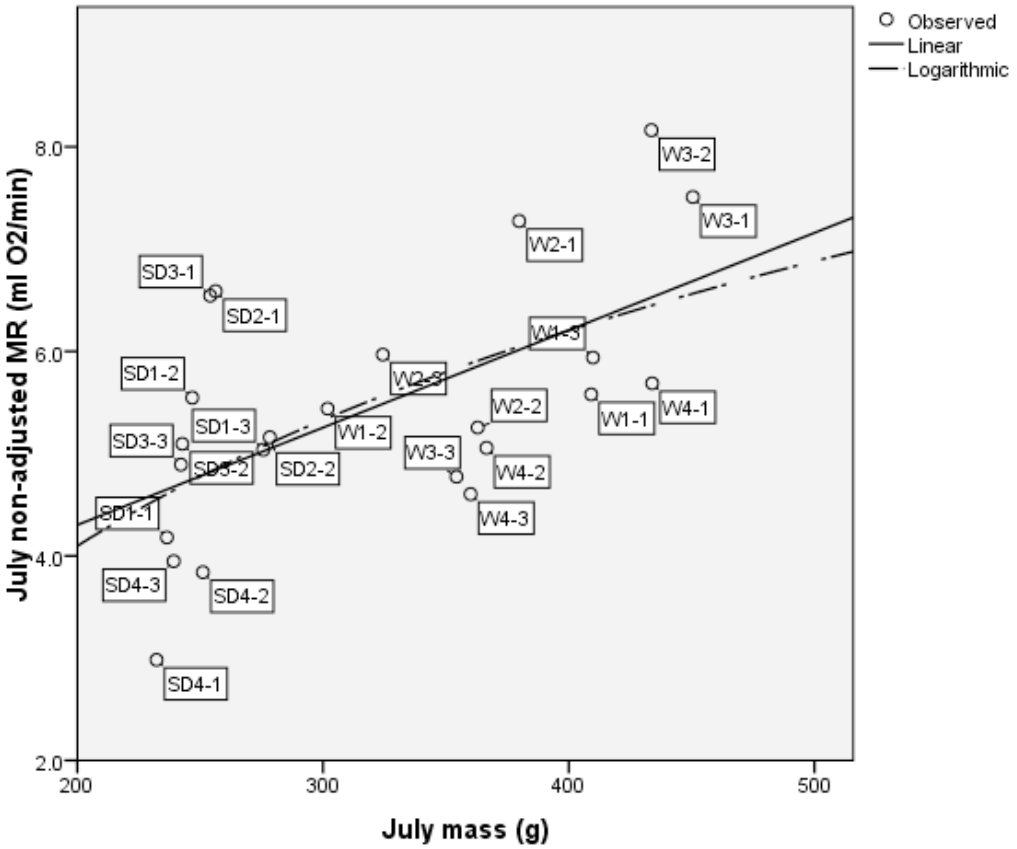

Figure 33: The Positive Relationship between Non-adjusted MR (Measured as ml O_2/min) Sampled in July, and Mass for all Rats.

Table 10
R^2 value, the p-value Corresponding to the R^2, and the Equation for the Line to Compare Linear and Logarithmic Regressions of July MR not *Adjusted for Mass on July Mass.*

	R^2	Sig. of R^2	Equation of line
Linear	0.348	0.003	y = 0.01x + 2.398
Logarithmic	0.34	0.004	y = 3.04*ln(x) – 12.01

Note. Unadjusted MR is measured as ml O_2/min

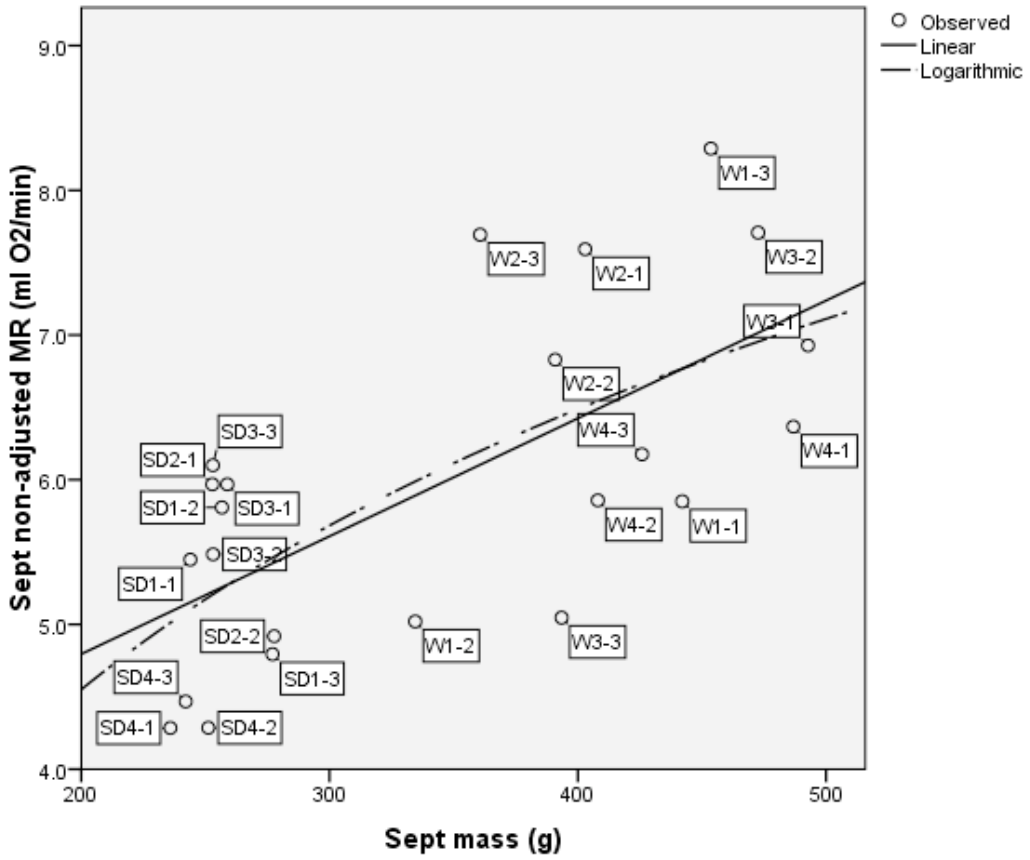

Figure 34: The Positive Relationship between Non-adjusted MR (Measured as ml O_2/min) Sampled in September, and Mass for all Rats.

Table 11
R^2 value, the p-value Corresponding to the R^2, and the Equation for the Line to Compare Linear and Logarithmic Regressions of September MR not *Adjusted for Mass on September Mass.*

	R^2	Sig. of R^2	Equation of line
Linear	0.439	0.001	y = 0.008x + 3.17
Logarithmic	0.441	0.001	y = 2.796*ln(x) – 10.26

Note. Unadjusted MR is measured as ml O_2/min

Pearson's correlations were performed to see if there was a significant correlation between mass and MR, both adjusted and unadjusted for mass. There was a significant correlation between mass and MR in all cases (for both sampling runs, and for mass-

adjusted as well as unadjusted MR). The correlation coefficients and p-values for the correlations can be found in Table 12 below. The above graphs show that there is a negative relationship between mass and MR/g, and a positive relationship between mass and whole body MR, as expected. Based on the correlation data below, mass is significantly correlated with MR, and based on the regression data presented above, mass significantly influences MR.

Table 12
Pearson's Correlation p-values for Comparing MR vs. Mass

	Correlation Coefficient for unadjusted MR (ml O_2/min) vs. mass	Sig.	Correlation Coefficient for adjusted MR (ml O_2/hr/g) vs. mass	Sig.
July MR	0.59	0.003	-0.47	0.024
September MR	0.662	0.001	-0.702	$1.86E^{-4}$

Tissue Turnover and mass

It has been demonstrated previously that *Rattus norvegicus* may not show a significant relationship between C half-life and mass (Bassett 2005). Neither strain showed any significant relationship when C or N turnover (represented as half-lives in days) was regressed against mass. The regression plots can be found below in Figure 35 and Figure 36, where both a linear and logarithmic regression gave non-significant relationships between mass and half-lives for both isotopes. Here, mass is an average of weeks 15-20 of the study, as most experimental animals came into equilibrium around week 20. Corresponding values for R^2 and the equation for the line are found below their respective figures, in Linear and Logarithmic Regressions of C Half-life on Mass, Averaged from Weeks 15-20.

Table 13 and Table 15. Unsurprisingly, a Pearson's Correlation showed that there was no significant correlation between mass and C half-life (p=0.525) or between mass and N half life (p=0.342) (see

Table 16 below).

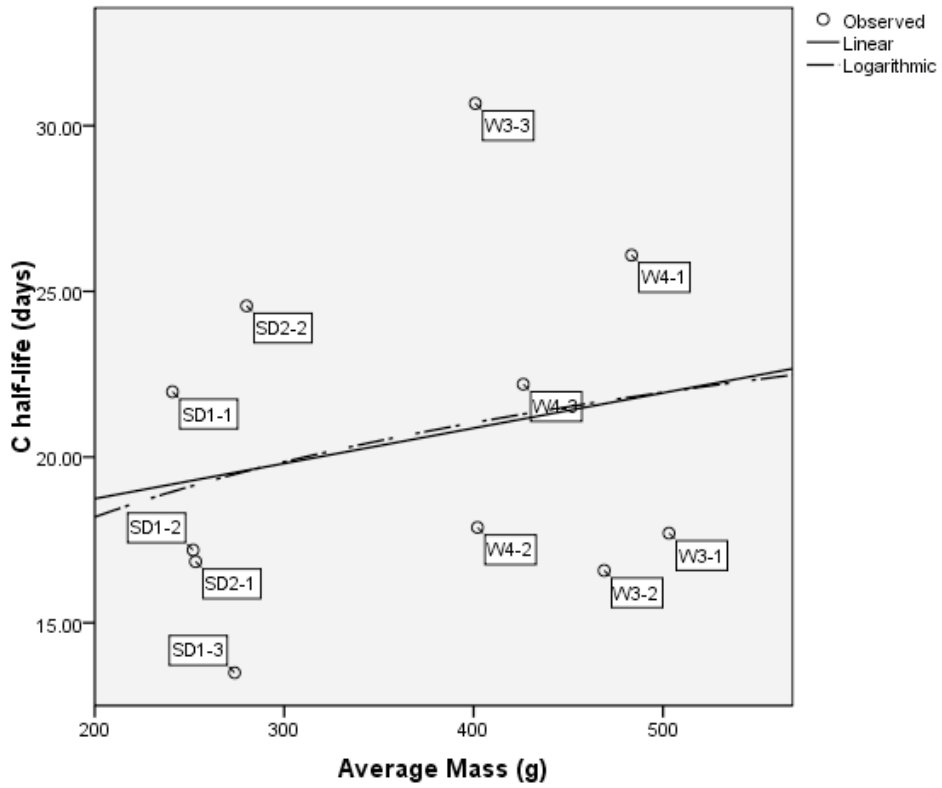

Figure 35: Linear and Logarithmic Regressions of C Half-life on Mass, Averaged from Weeks 15-20.

Table 13
Non-significant R^2 Values and the Equations for the Line of both Regressions of C Half-life on Mass.

	R^2	Sig. of R^2	Equation of line
Linear	0.046	0.525	y = 0.011x + 16.617
Logarithmic	0.056	0.485	y = 4.103*ln(x) – 3.546

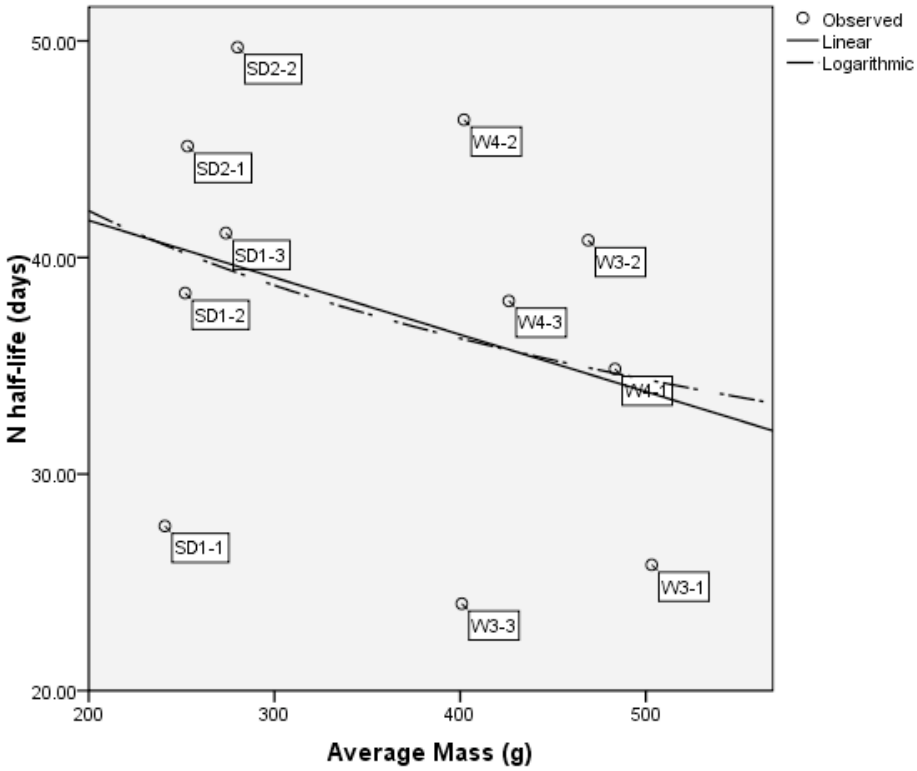

Figure 36: Linear and Logarithmic Regressions of N Half-life on Mass, Averaged from Weeks 15-20. When SD1-1 is removed, the N half-lives are significantly different (R^2 becomes 0.47).

Table 15

Non-significant R2 Values and the Equations for the Line of both Regressions of N Half-life on Mass.

	R^2	Sig. of R^2	Equation of line
Linear	0.101	0.342	y = -0.026x + 46.98
Logarithmic	0.085	0.385	y = -8.50*ln(x) + 87.20

Table 16

Pearson's Correlation Values for the Correlation Between Mass and Half-life of both Isotopes. There were no significant correlations.

	Correlation Coefficient for C half life	Sig.	Correlation Coefficient for N half life	Sig.
Avg. mass	0.215	0.525	-0.317	0.342

An independent samples t test was used to see if the strains differed in half-lives, and there was no significant difference between SD and W C or N half-lives (p=0.351 and 0.322, respectively). Therefore, these strains did not exhibit significantly different tissue turnover, regardless of a significantly different MR. It is interesting to note that one outlier is present (SD1-1), and when this individual is not included in the analysis for N, there is a significant difference in N turnover times between the two strains when compared to mass ($R^2 = 0.47$, p=0.028), yet it is in the opposite direction that would be expected if higher MR were to lead to a faster tissue turnover. It is expected that the larger W rats exhibit slower isotope turnover, though in this study W had a faster N half-life when compared to SD (see Figure 36). As each individual was tracked separately, half-lives were recorded for each rat and are presented in the appendix, in Table S38. Table 16 below shows only the average half-lives of each strain for both isotopes.

Table 16
Average C and N Half-lives for Each Isotope and Strain. Half-lives are Presented in days.

	C half-life	N half-life
SD	18.81	40.39
W	21.85	34.97

Correlating MR with m and turnover time

As this study hypothesizes a correlation between MR and tissue turnover, it is necessary to analyze the relationship between MR and m, the metabolic component of turnover, as well as between MR and half-life (which measures turnover rate), of each isotope. Regression analysis was performed to see if MR influences m, and correlation analysis was performed to see if the two variables were correlated. Independent samples t-tests were first performed to see if there was a significant difference in m between strains, for either C or N, but there was no significant difference (p=0.204 for C and p=0.671 for N). To determine if the relationship between MR and m is linear or logarithmic, regression analysis was performed, fitting a line of best fit for both a linear

relationship as well as a logarithmic one. The only significant relationship was observed in the regression between m for C and MR obtained in July, where the R^2 value for a logarithmic relationship was 0.365, with a significance of 0.049 (see Table 17). No other significant relationships were found using either fit, including the logarithmic relationship between m for C and MR sampled in September (p=0.15). R^2 values and their associated p-values can be found in Table 17-Table 20 below the corresponding figures (Figure 38-Figure 41).

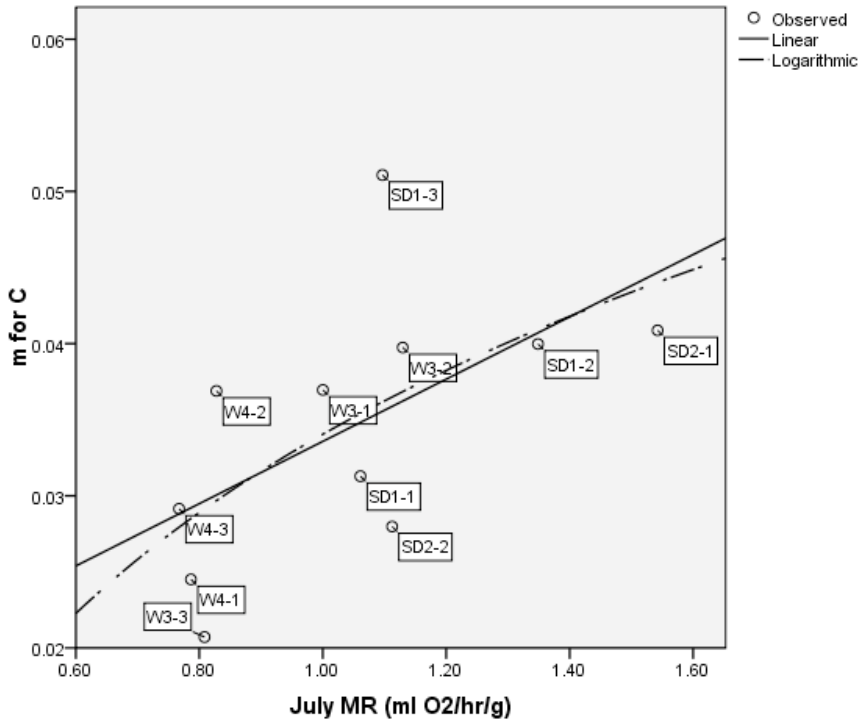

Figure 38: Linear and Logarithmic Regression of the Relationship between m for C and MR Sampled in July.

Table 17
R^2 Values and the Equations for the Line of both Regressions of m for C on July MR. The Logarithmic Regression for July is Slightly Significant.

	R^2	Sig. of R^2	Equation of line
Linear	0.334	0.063	y = 0.020x + 0.013

Logarithmic	0.365	0.049	y = 0.023*ln(x) + 0.034

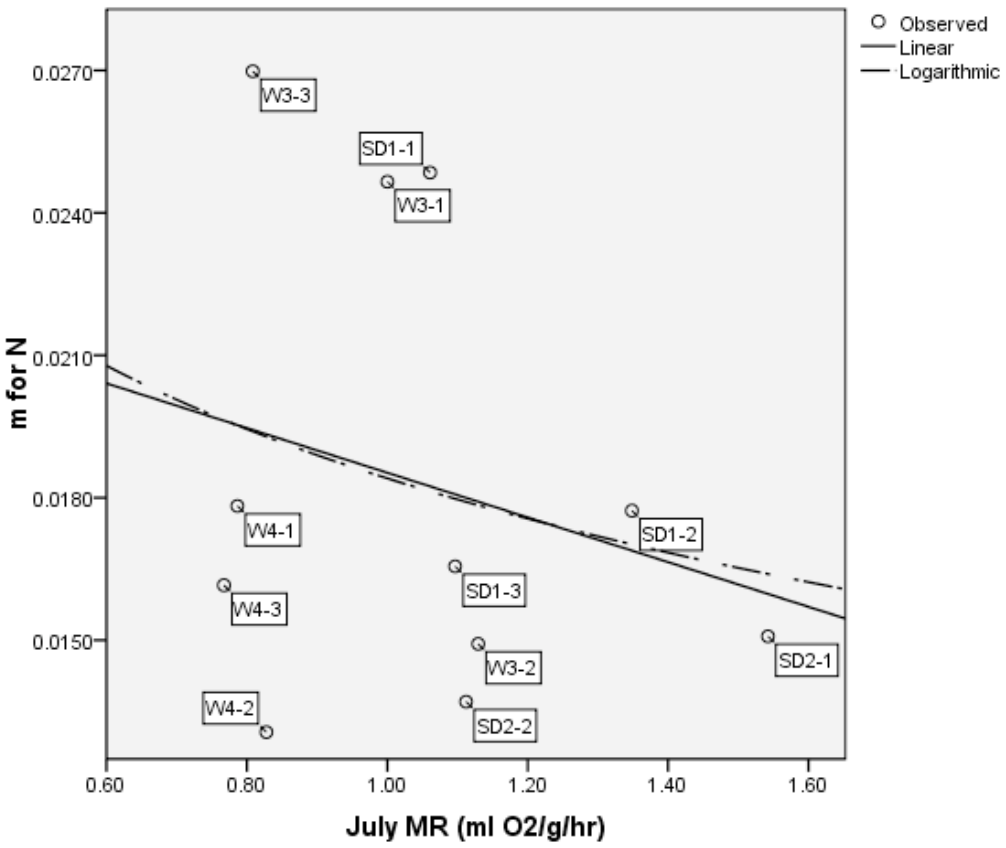

Figure 38: Linear and Logarithmic Regression of the Relationship between m for N and MR Sampled in July.

Table 18

Non-significant R^2 Values and the Equations for the Line of both Regressions of m for N on July MR.

	R^2	Sig. of R^2	Equation of line
Linear	0.056	0.482	y = -0.005x + 0.023
Logarithmic	0.047	0.520	y = -0.005*ln(x) + 0.018

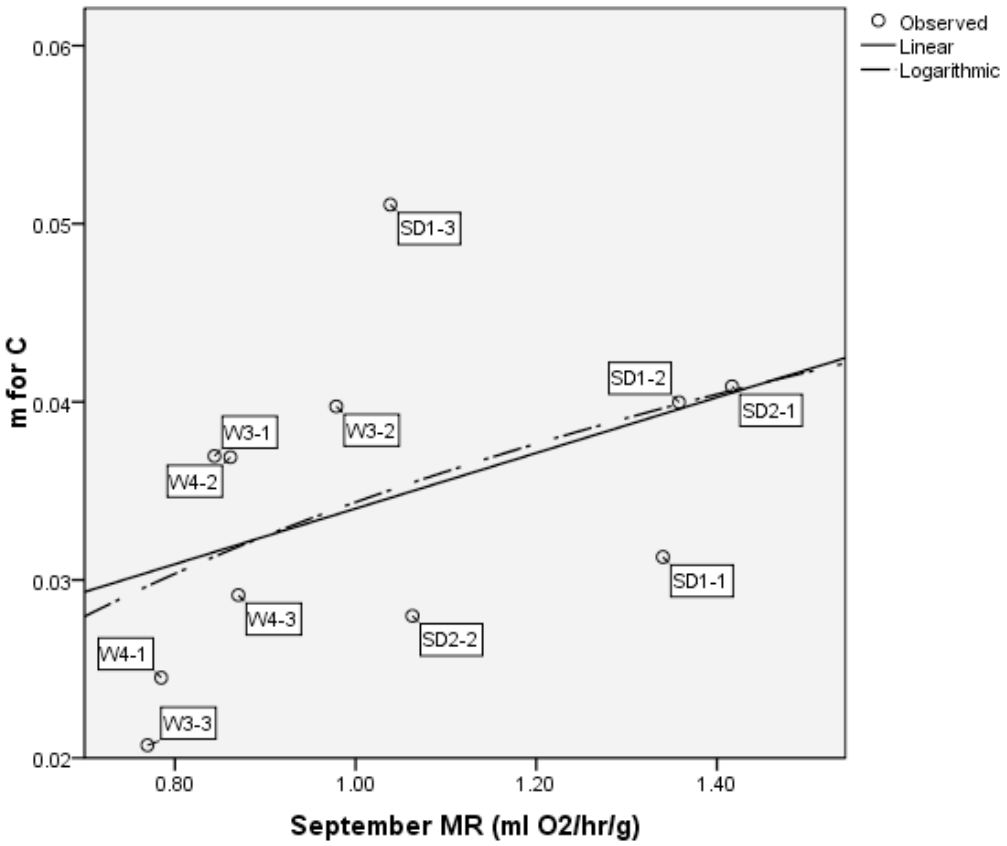

Figure 39: Linear and Logarithmic Regression of the Relationship between m for C and MR Sampled in September.

Table 19
Non-significant R^2 Values and the Equations for the Line of both Regressions of m for C on September MR.

	R^2	Sig. of R^2	Equation of line
Linear	0.185	0.187	y = 0.016x + 0.018
Logarithmic	0.216	0.150	y = 0.018*ln(x) + 0.034

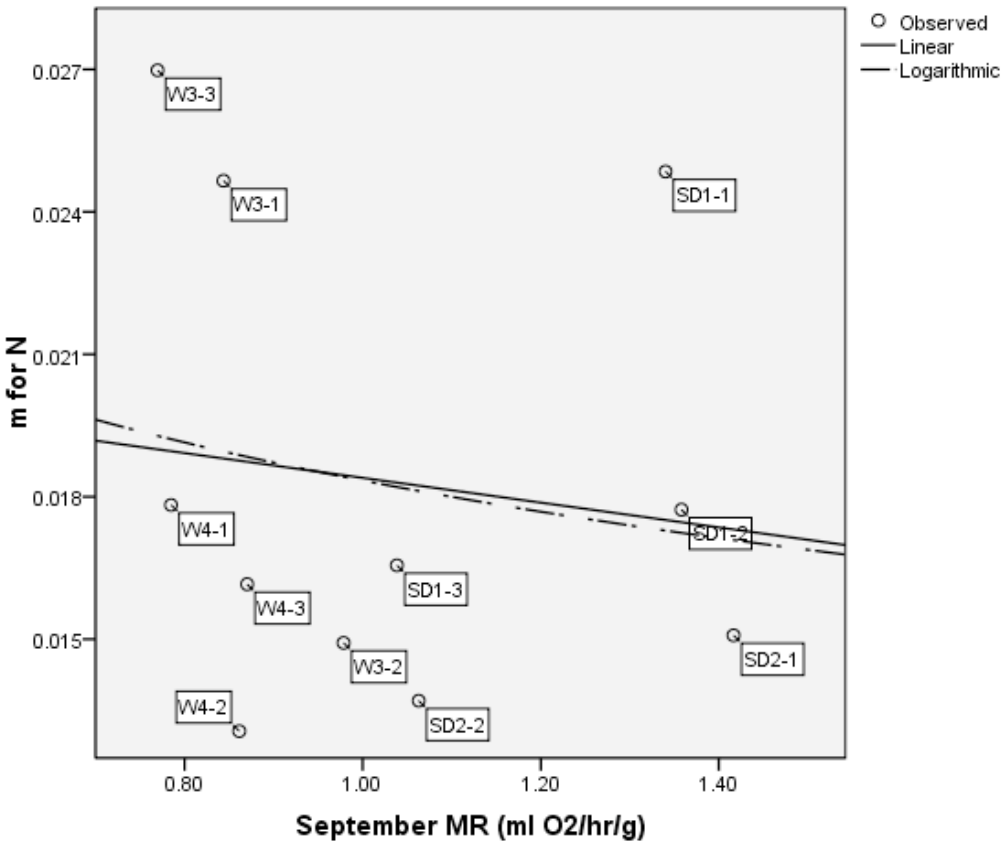

Figure 41: Linear and Logarithmic Regression of the Relationship between m for N and MR Sampled in September.

Table 20
Non-significant R^2 Values and the Equations for the Line of both Regressions of m for N on September MR.

	R^2	Sig. of R^2	Equation of line
Linear	0.016	0.707	y = -0.003x + 0.021
Logarithmic	0.028	0.626	y = -0.004*ln(x) + 0.018

As metabolic tissue replacement rate was not significantly different between strains, it is not surprising that there was not a significant correlation between m for either isotope and MR. The non-significant p-values from Pearson's Correlations (between both MR samplings and m for both isotopes) are presented in Table 21 below. Because mass

was significantly correlated with MR, Pearson's Correlation analysis was performed to see if mass was correlated with m for either isotope, but no significant correlations were found (p=0.33 for C and p=0.72 for N).

Table 21
Pearson's Correlation Coefficients and Non-significant p-values for the Correlation Between MR and m for C and N.

	Correlation Coefficient for m for C	Sig.	Correlation Coefficient for m for N	Sig.
July MR	0.578	0.063	-0.237	0.482
September MR	0.430	0.187	-0.128	0.707

Similarly, to test for a significant relationship between MR and tissue turnover, regression analysis was performed on MR vs. half-life, fitting both a linear and a logarithmic line to the relationship to see if one would give a better fit. The regressions can be seen below, in Figure 41-Figure 44, along with the non-significant R^2 values, their p-values, and the equations for the line, which are located in Table 22-Table 25, located below their corresponding figures.

Similar to the relationship between MR and m, there were no significant relationships in the regressions between MR and half-life for either isotope. Pearson's Correlations were performed to show that there were no significant correlations between MR of experimental rats and isotope turnover. The p-values for all correlations between MR and turnover time are presented in

Table 26 below.

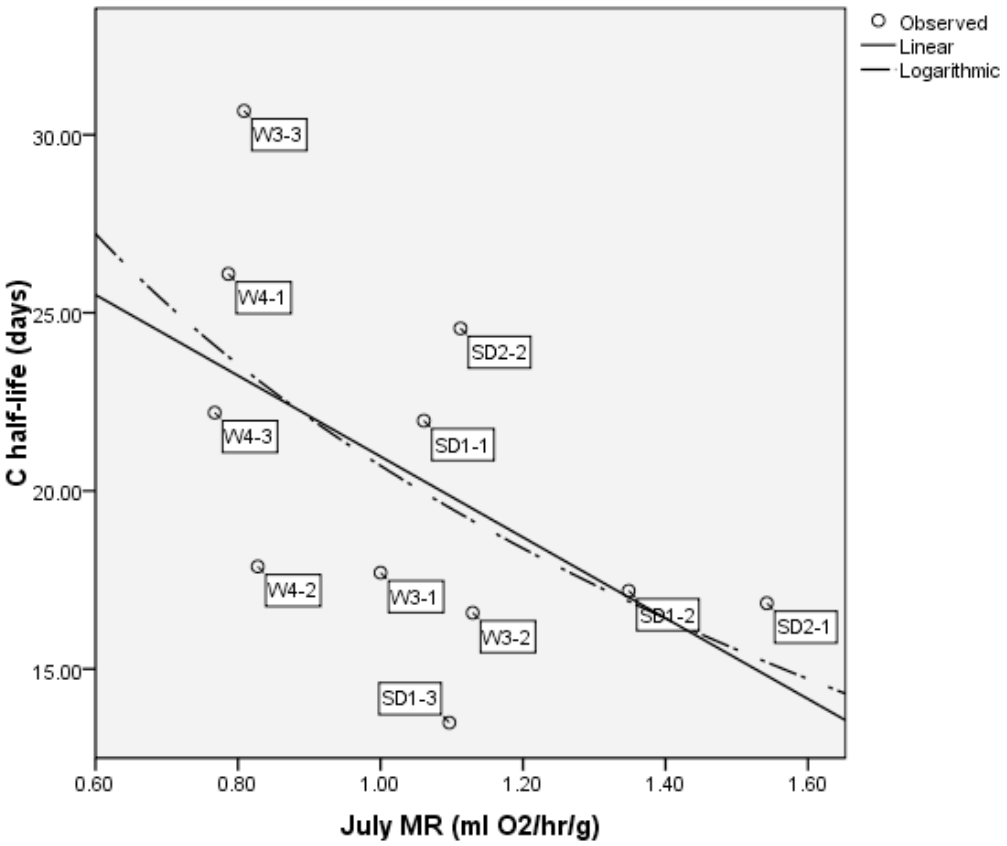

Figure 41: Linear and Logarithmic Relationship between MR Sampled in July, and Tissue Turnover Time for C (Half-life in days).

Table 22
Non-significant R^2 Values and the Equations for the Line of both Regressions of C Half-life on July MR.

	R^2	Sig. of R^2	Equation of line
Linear	0.299	0.082	y = -11.35x + 32.31
Logarithmic	0.325	0.067	y = -12.74*ln(x) + 20.71

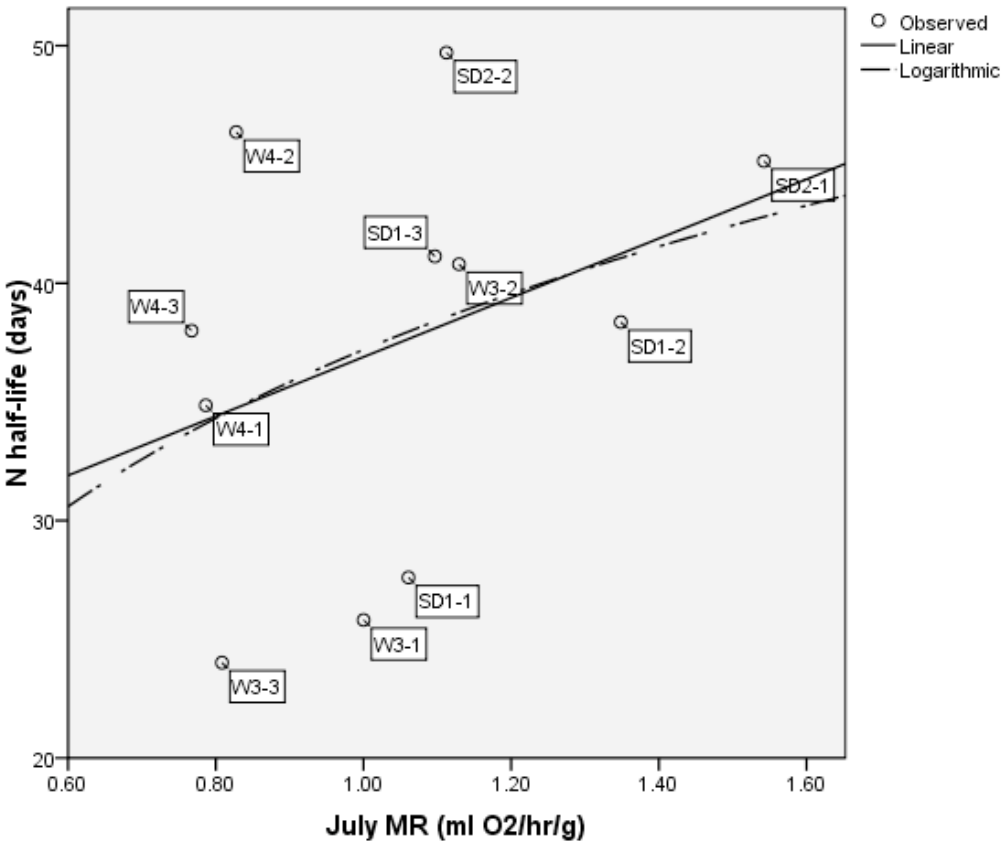

Figure 42: Linear and Logarithmic Relationship between MR Sampled in July, and Tissue Turnover Time for N (Half-life in days).

Table 23
Non-significant R^2 Values and the Equations for the Line of both Regressions of N Half-life on July MR.

	R^2	Sig. of R^2	Equation of line
Linear	0.128	0.280	y = 12.46x + 24.42
Logarithmic	0.344	0.300	y = 12.93*ln(x) + 37.19

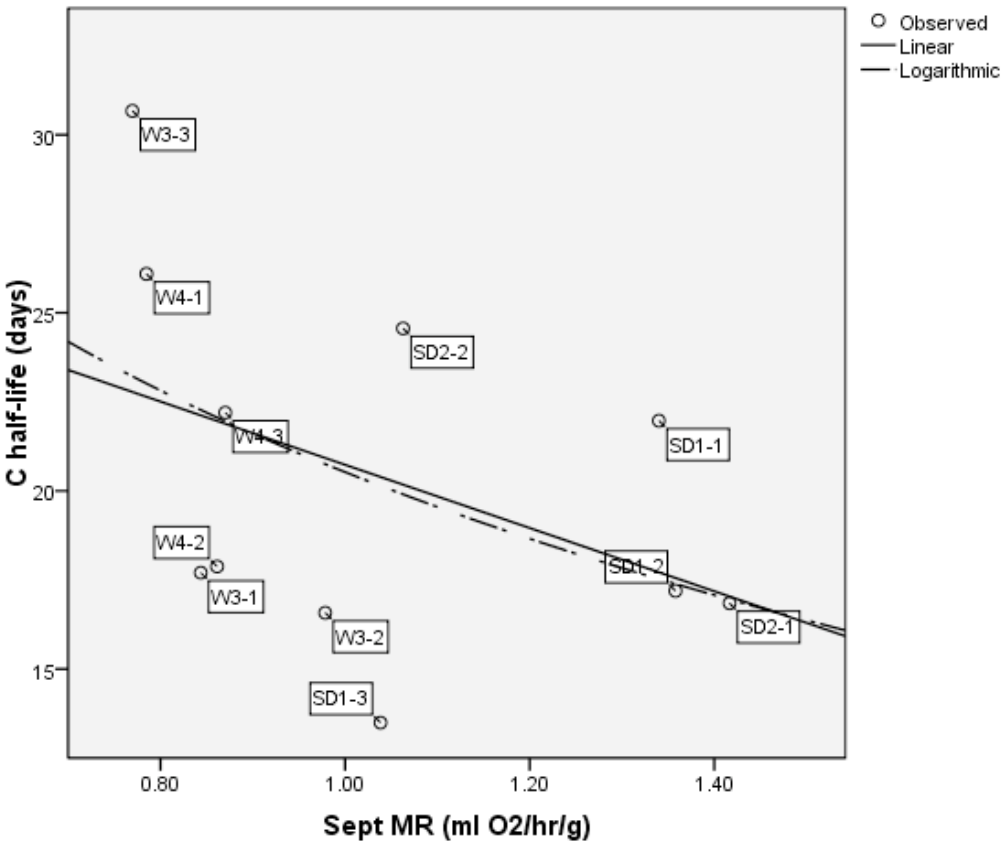

Figure 43: Linear and Logarithmic Relationship between MR Sampled in September, and Tissue Turnover Time for C (Half-life in days).

Table 24
Non-significant R^2 Values and the Equations for the Line of both Regressions of C Half-life on September MR.

	R^2	Sig. of R^2	Equation of line
Linear	0.174	0.202	y = -8.88x + 29.61
Logarithmic	0.204	0.163	y = -10.27*ln(x) + 20.53

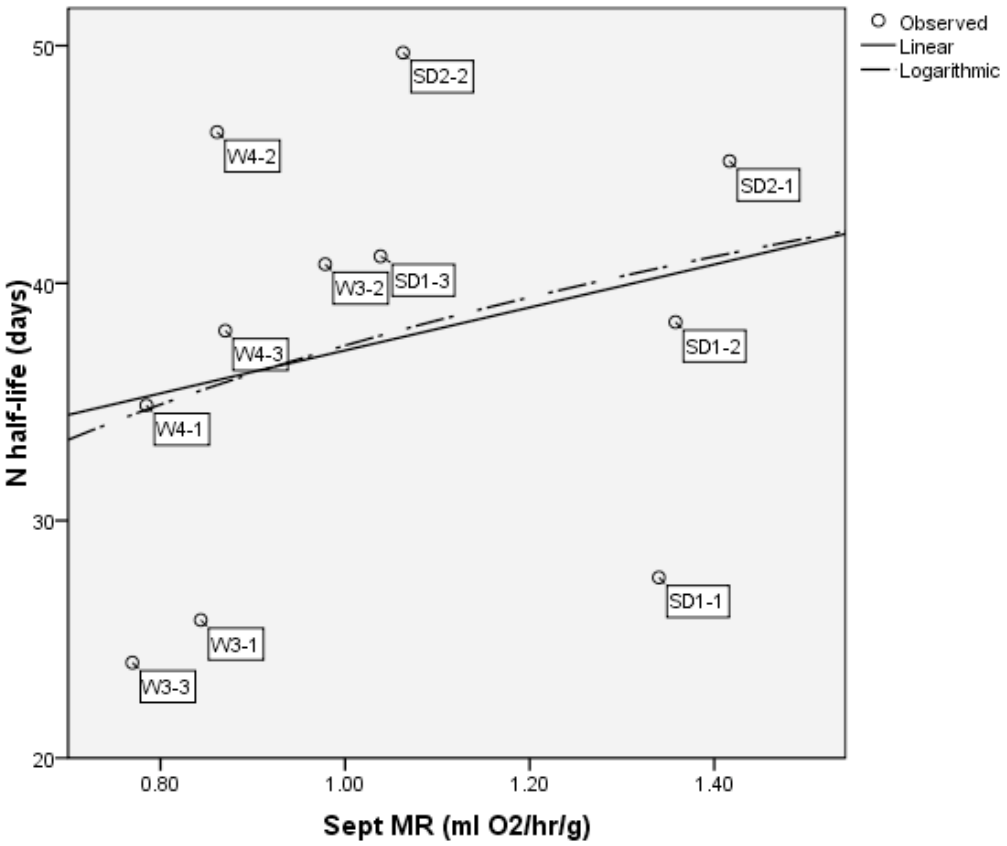

Figure 44: Linear and Logarithmic Relationship between MR Sampled in September, and Tissue Turnover Time for N (Half-life in days).

Table 25
Non-significant R^2 Values and the Equations for the Line of both Regressions of N Half-life on September MR.

	R^2	Sig. of R^2	Equation of line
Linear	0.064	0.453	y = 9.05x + 28.12
Logarithmic	0.085	0.384	y = 11.13*ln(x) + 37.37

Table 26
Pearson's Correlation Values and Significance of Correlation between MR and Half-life of Either Isotope.

	Correlation Coefficient for C ½ life	Sig.	Correlation Coefficient for N ½ life	Sig.
July MR	-0.547	0.082	0.358	0.280
September MR	-0.417	0.202	0.253	0.453

Correlating m with half-life and with mass

Because m is used to calculate half-life (see Equation 7), the two are very highly correlated. Table 27 shows the Pearson's Correlation coefficients and their significance for the correlation between m and half-life for each isotope. Though it is obvious that m and half-life should be correlated, the shape of their relationship is interesting. Figure 45 and Figure 46 show the negative relationship of the regression of half-life on m, for each isotope. Both are fitted with a linear regression and logarithmic regression line to compare their fits. Though both regression lines represent significant relationships, it is interesting to note that the logarithmic relationship gives a slightly better fit than linear. R^2 values, their significance, and the equations for the lines can be found in the Table 28 and : Linear and Logarithmic Regression of N Half-life on m for N.

Table 29
Significant R2 Values and the Equations for the Line of both Regressions of N Half-life on m for N.

	R^2	Sig. of R^2	Equation of line
Linear	0.924	2.53E-6	y = -1691.36x + 68.42
Logarithmic	0.94	8.22E-7	y = -33.17*ln(x) – 96.24

Note. Though both fits are significant, the logarithmic regression gives a slightly better fit. below the corresponding figures.

Table 27
Pearson's Correlation and Significance Values for the Correlation between m and Half-life of both Isotopes.

	Correlation Coefficient for C ½ life	Sig.	Correlation Coefficient for N ½ life	Sig.

m for C	-0.965	1.55E-6	-------	-------
m for N	-------	-------	-0.961	2.53E-6

Note. Because m is used to calculate half-life, the two are significantly correlated.

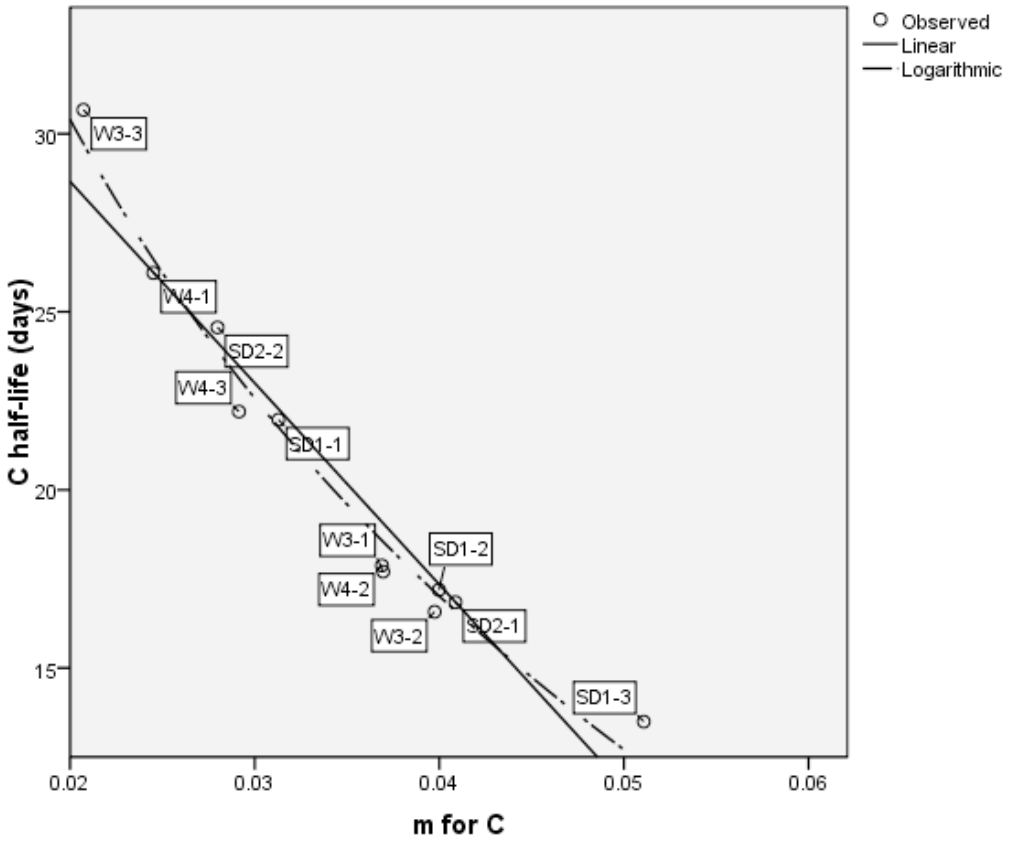

Figure 45: Linear and Logarithmic Regression of C Half-life on m for C.

Table 28
Significant R^2 Values and the Equations for the Line of both Regressions of C half-life on m for C.

	R^2	Sig. of R^2	Equation of line
Linear	0.931	1.55E-6	y = -565.71x + 39.97
Logarithmic	0.979	7.17E-9	y = -19.31*ln(x) – 45.15

Note. Though both fits are significant, the logarithmic regression gives a slightly better fit.

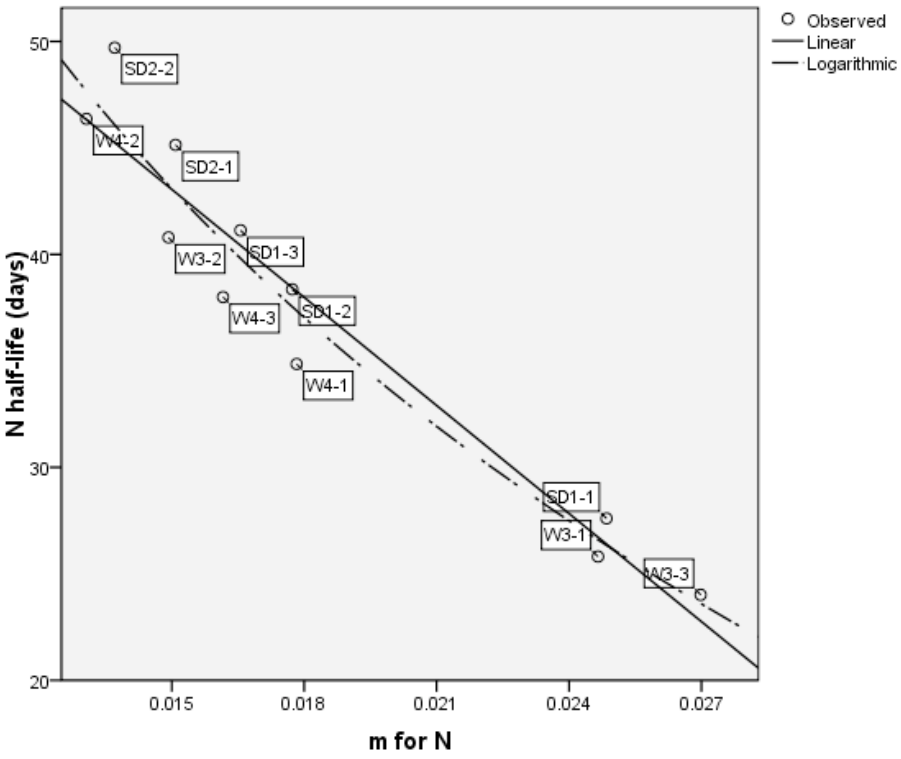

Figure 46: Linear and Logarithmic Regression of N Half-life on m for N.

Table 29
Significant R2 Values and the Equations for the Line of both Regressions of N Half-life on m for N.

	R^2	Sig. of R^2	Equation of line
Linear	0.924	2.53E-6	y = -1691.36x + 68.42
Logarithmic	0.94	8.22E-7	y = -33.17*ln(x) – 96.24

Note. Though both fits are significant, the logarithmic regression gives a slightly better fit.

Although m significantly influences half-life, m and mass do not appear to be related. The following figures (Figure 48 and Figure 49) show the relationship between m and mass, regressed both linearly and logarithmically. The tables (Table 31 and Table 32) below the figures show R^2 values, their significance, and the equations of the line for each relationship. In this case, mass is again represented as the average mass of each

individual rat from weeks 15-20; but there is no significant correlation between m and mass (see Table 33).

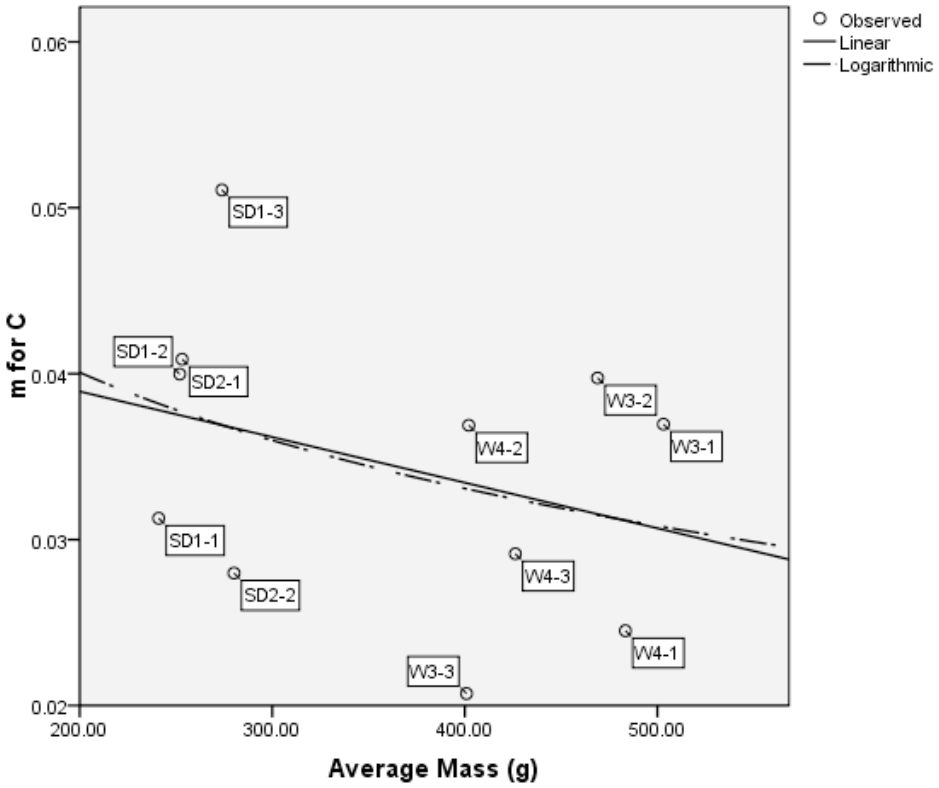

Figure 48: Linear and Logarithmic Regression of m for C on Average Mass.

Table 31
Non-significant R^2 Values and the Equations for the Line of both Regressions of m for C on Average Mass.

	R^2	Sig. of R^2	Equation of line
Linear	0.106	0.328	y = -2.75E-5x + 0.044
Logarithmic	0.116	0.306	y = -0.010*ln(x) + 0.094

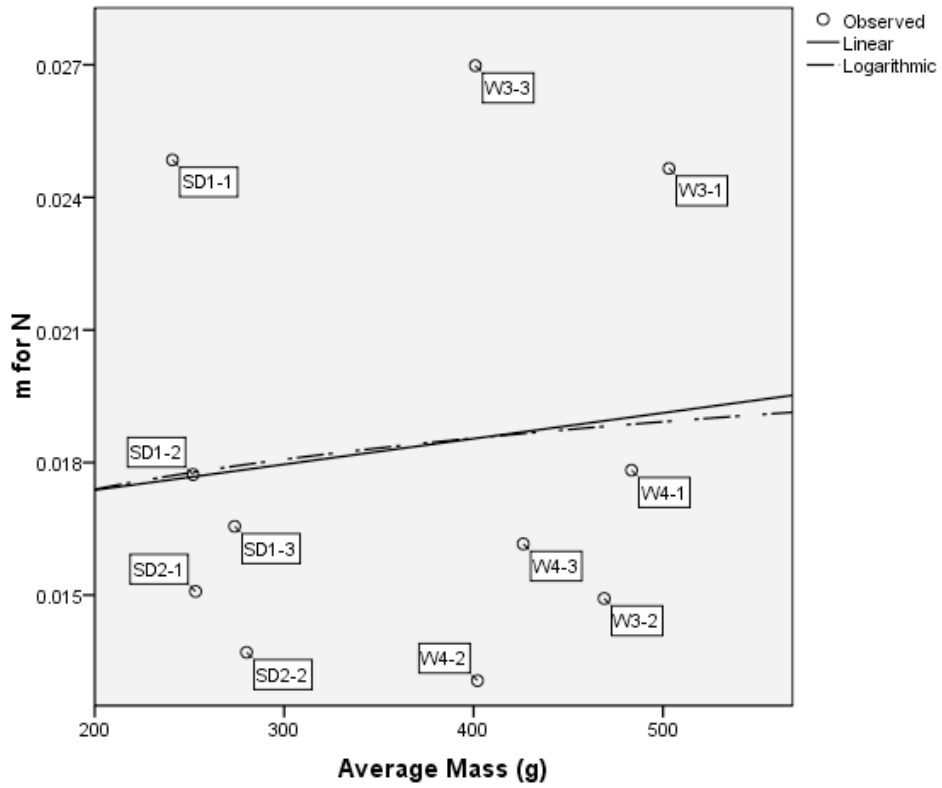

Figure 49: Linear and Logarithmic Regression of m for N on Average Mass.

Table 32
Non-significant R^2 Values and the Equations for the Line of both Regressions of m for N on Average Mass.

	R^2	Sig. of R^2	Equation of line
Linear	0.015	0.718	y = 5.82E-6x + 0.016
Logarithmic	0.010	0.769	y = 0.002*ln(x) + 0.009

Table 33
Pearson's Correlation Coefficients and their Significance for the Correlation Between m and Mass.

	Correlation Coefficient m for C	Sig.	Correlation Coefficient m for N	Sig.
Avg. mass	-0.326	0.328	0.123	0.718

CHAPTER 4

DISCUSSION

Hypothesis

The purpose of this study was to determine if there is a relationship between MR and tissue turnover within two strains of the same species (*Rattus norvegicus*). It was hypothesized that rats with a faster MR/g will also exhibit faster tissue turnover, represented as a shorter half-life of C and N isotopes. As there are two components to turnover, growth and metabolic tissue replacement, this study aimed to see if MR correlated with the metabolic component of turnover. The hypothesis was not supported, as there was not a significant correlation between MR and m in the two strains of rats, or between MR and half-life of the two isotopes. Possible experimental errors that could have led to this result are mis-calibrations with the MR equipment, or deviations in the mass spectrometer used to analyze the isotopic signature of the tissue. However, it is more likely that the difference in MR between the two strains of rats was simply not large enough to be reflected in the metabolic component of turnover. Even though the original hypothesis was not supported, the results of this study lend support to previous findings, and are of interest to the fields of isotopic ecology and metabolic rate analysis.

Metabolic Rates

Variation in MR was not only observed between the strains, but within strains individual rats also exhibited differences in their MR. It is possible that some of the variation seen within a strain is due in part to specific dynamic action, which is the term given to the fact that metabolism increases when an organism is digesting food, independent of other activities (Randall et al. 2002). In the current study, rats were not fasted prior to MR sampling, and so are not in the post-absorptive state, and it has been shown that specific dynamic action can account for variations in MR within a species (Randall et al. 2002). Because animals were not post-absorptive, and MR sampling spanned the course of a full day (so that rats were sampled both during their normal time of rest and their normal time of activity), BMR as defined was not obtained. The MR obtained in this study is closer to Resting MR (RMR).

Previous studies have used RMR as a measure of MR when animals have not been previously denied access to food so that they are post-absorptive (Selman C, Lumsden S, Bunger L, Hill WG, and Speakman JR 2001; Speakman JR, Krol E, and Johnson MS 2004). For rodent studies, BMR is not easily obtainable due to the strict requirements. When small rodents are starved, they tend to become hyperactive initially, which violates the requirement that animals be at rest. This hyperactivity is followed by a period when the animal will conserve energy by dropping its body temperature, which violates the requirement that animals be thermoneutral (Speakman et al. 2004). Therefore, it is important to note that MR values obtained in this study do not adhere to the strict

definition of BMR, and would be better described as RMR, which has been shown to be functionally equivalent to BMR in reproducing animals when BMR cannot be obtained (Speakman et al. 2004).

In order to see if the violation of "resting during their normal time of rest" affected the MRs obtained, a t-test was performed to compare rats within a strain that were sampled during their normal time of rest (8am-8pm) against rats of the same strain sampled during their normal time of activity (8pm-8am). Time of day did not significantly influence MR for either strain ($p=0.751$ for SD; $p=0.364$ for W). It is interesting that for the July sampling event, those rats tested during their normal time of rest (during the day) had slightly, but not significantly, lower average MRs than those rats tested during their normal period of activity (see Figure 50). This relationship was not observed during the September sampling event (see Figure 51), and because it was not significant it can be assumed that this violation of the assumptions of BMR did not impact the MR values obtained.

MR correlates with mass because mass will affect the performance of most physiological systems, including energy metabolism. Smaller animals have a higher MR per unit body mass than a larger animal, because smaller animals must respire at higher rates per unit mass (Randall et al. 2002). The two strains of rats had significantly different masses and MRs, but it has been shown that body mass changes only account for a small proportion of total variation in MR within a species, because the range of masses is usually not that large (Spicer & Gaston 1999). It is possible that the variation in

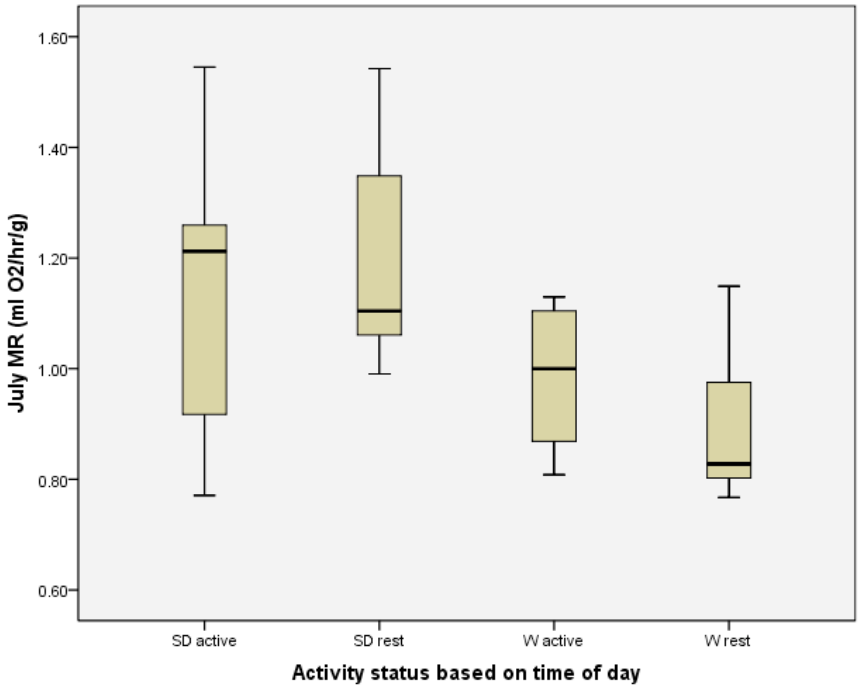

Figure 50: Comparison of July MRs Obtained for Each Strain During their Normal Periods of Activity (8pm-8am) and Rest (8am-8pm). There were no significant differences.

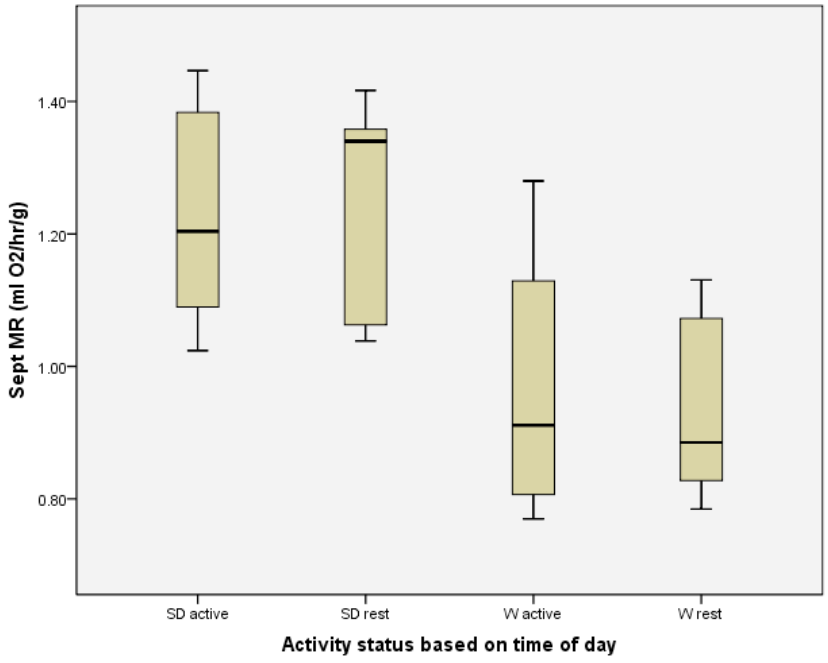

Figure 51: Comparison of September MRs Obtained for Each Strain During their Normal Periods of Activity (8pm-8am) and Rest (8am-8pm). There were no significant differences.

body size between the two strains was not great enough to see the effects of a different MR (ie, to see the correlation with m). For example, in order to see allometric scaling effects within a species, the body masses of the organisms in question must vary by several hundred or thousand fold (Randall et al. 2002).

In the current study, the MR/g of SD rats was approximately 20% higher on average than the MR/g of the W rats (see averaged MR per strain in Table 3). When strains were broken down by experimental grouping, the MR did not vary much for SD (between those rats on control and experimental diets – see Figure 8), but interestingly, the experimental W rats had a lower MR than the control W rats (see Figure 9). This difference was significant for September MR sampling, but not for July. Although at first glance it may seem as though the experimental diet is causing a lower MR over time in the W rats, the average MR of the experimental W rats did not vary appreciably between the two sampling events (0.8864 ml O_2/hr/g in July, and 0.8513 ml O_2/hr/g in September). The average MR of the control W rats was also slightly higher in September (1.04 ml O_2/hr/g) than it was in July (0.98 ml O_2/hr/g). The range of experimental MRs obtained in September is much more narrow than in July, and although the assumption of equal variances was not violated for the Independent t-test between control and experimental W rats sampled in September (Levene's test insignificant; $p=0.113$), it could be that the variation in ranges accounted for the significant difference between experimental groupings in September (see Figure 9). This supports the idea that there was not a component to the experimental beet sugar diet which would have made MRs lower over time in the W rats.

It should also be noted that two of the W rats (W1-2 and W3-2) experienced periodic seizures over the course of the experiment. Studies have been performed to analyze MR changes (especially in the brain) when a seizure is occurring, and they have shown that metabolism increases during a seizure (Meldrum & Nilsson 1976; Duncan 1992). As their MRs were within the normal range during both sampling periods, it is assumed that their medical status did not affect their MRs. No seizures were observed in either rat during the actual collection of MR data, so their MRs were included in the analysis.

Isotopes and Fractionation

In the current study, C isotopes behaved as expected, where the C signature of the experimental rats' blood equilibrated steadily over time to reflect the C signature of the beet sugar diet (see Figure 25-Figure 27). The N signature of the experimental rats, however, had a slightly less steady equilibration with the experimental diet (see Figure 28 - Figure 30). One factor that could influence N fractionation is the amount of protein in the diet. Castellini and Rea (1992) have shown that low protein diets cause protein sparing, in which an organism reserves available protein for tissue maintenance and does not catabolize any for energy, whereas with high protein diets tissues are synthesized using available protein, and the rest is catabolized for energy. However, as both the control 2018 and the experimental beet sugar diets had very similar amounts of protein (18% for the control, 18.3% for the experimental) protein sparing should not be the cause of any patterns of unsteady incorporation rate observed. Both the experimental and control N isotope signatures of both strains had higher variation relative to C as they

equilibrated and reached a plateau, so it is more likely that this variation was caused by the nature of N in the body, as well as the methods of analysis. N is present in very low abundances in the body when compared to C, as N only tracks protein and ammonia, whereas C tracks protein, fat, and carbohydrates. Because of the low abundance of N, it can be harder for the mass spectrometer to gain accurate readings. It is also possible that the mass spectrometer incompletely combusted some samples, leading to a misinterpretation of the isotope abundances. The two weeks (marked as purple dots in the equilibrium figures) were excluded from analysis, but it is possible that other weeks were affected as well, especially since the control rats exhibit similar patterns of variation between weeks even though their signature should not have varied.

Previous studies on tissue turnover

MacAvoy et al. (2006) found SD rats to have a C half-life of 24.8 days, and a N half life of 27.7 days. They also found m for C to be 0.028, and m for N to be 0.025 for SD rats. The results obtained by MacAvoy et al. (2006) differ from those reported here, where C half-life for SD rats was found to be 18.81 (±4.41) days, and N half-life was 40.39 (±8.33) days (see Table 16). Although in both cases, N half-life is longer than C half-life, the difference is more extreme in this study, which could be due to errors in N signature analysis, as discussed above. The values obtained for m for both isotopes also differed from those observed in MacAvoy et al. (2006). Here, m for C was 0.0382 and m for N was 0.0176 (see Table 5). The values obtained for k and m will be discussed in more detail in later sections. MacAvoy et al. also found that C and N tissue turnover rates were similar within a tissue, which is not true for the current study, as C and N half-lives

were very different in whole blood, but again some error could have been introduced for N due to errors in analysis. Fractionation values for SD rats in this study (see Table 6 and Table 7) were very close to those observed in MacAvoy et al., with some variation due to differences in the C and N signatures of the diet.

Growth rate, k and metabolic tissue replacement rate, m

MacAvoy et al. (2005) found that growth contributes approximately 10% to the total tissue turnover rate, whereas metabolic tissue replacement accounts for about 90%. They also found that in adult mice, the isotope turnover due to metabolic tissue replacement was at least ten times as great as the turnover that was due to an increase in mass (MacAvoy et al. 2005). Similarly, Tarboush et al. (2006) found that in the zebrafish *Danio rerio,* between 68-80% of the observed isotopic signature changes were due to metabolic tissue replacement. Likewise, in this study it was shown that growth accounts for only a small fraction of tissue turnover in adult SD and W rats. When measuring turnover of C in the tissues of SD rats, on average m accounted for 99 ± 0.24% of the turnover. Likewise, in W rats m accounted for an average of 93 ± 1.55% of the C turnover. Similar results were obtained for N turnover, where m accounted for 98 ± 0.75% in SD rats and 89 ± 2.67% in W rats. Because the average growth rate of the W rats was an order of magnitude higher than k for the SD rats (0.0020 g/day vs 0.00028 g/day, see Table 5), the fraction of turnover accounted for by k is slightly higher in the W rats, for both isotopes. When broken down by treatment, control and experimental SD rats had very similar growth rates, but the k for experimental W rats was significantly higher than the controls (see Table 2). The experimental W rats may have had a

significantly faster growth rate than the controls because of the nature of the experimental diet. The beet sugar diet is 58.8% beet sucrose whereas the control diet only contains 4.93% sugar (but is still compatible as carbohydrates [including ground wheat, ground corn, wheat middlings, and soybean meal] account for 57.33%). The higher sugar content of the experimental diet could account for the greater mass gain (see Figure 15 and Figure 16) and thus higher k (see Table 2) and lower MR/g (see Figure 9). Because this difference in mass, k, and MR/g between experiment and control animals was not observed in SD rats, further studies would be necessary to see what caused the difference to be apparent in W rats. The W rats consumed more food on average than the SD rats, which could have led to the more pronounced differences between the experimental and control rats.

The W rats gained more adipose tissue than the SD rats throughout the course of the experiment, and because lipids are depleted in ^{13}C, the remaining C pool becomes enriched in ^{13}C as the animal ages and lipid accretion increases. If lipid accretion were to affect the $\delta^{13}C$ signature of the blood, then this increase would also be reflected in the control animals to a certain extent (controls would obviously not account for any extra adipose storage due to the experimental diet). One-way ANOVA analyses on the C signatures of the controls of both strains from weeks 1-15 (odd weeks only) showed that there was not a significant difference in the $\delta^{13}C$ signature of SD ($p=0.781$) or W ($p=0.448$) rats over time, showing that any increase in adipose tissue in the controls did not result in significant changes to the C signature over time.

Correlating turnover with MR, mass, and half-life

Though the two strains had significantly different MRs, m was not significantly different between strains, nor was m significantly correlated with MR during either sampling event or for either isotope, though the correlation was nearly significant for the July MR sampling event where p=0.063 (see Table 21). Though the correlation between m and MR was not significant, there are still trends in the data. MR versus m for C had a positive relationship, where a logarithmic regression explained slightly more of the variation (R^2=0.365) than a linear regression (R^2=0.334) during July (see Figure 38 and Table 17). These values were slightly higher during the July MR sampling event than during the September event, where the logarithmic R^2 was 0.216 and the linear R^2 was 0.185 (see Figure 39 and Table 19). Yet in both cases the logarithmic fit seemed to be just slightly better, which was observed for the majority of regressions, even though in many cases the regression was nevertheless insignificant. The only significant relationship (besides the relationship between mass and MR, and m and half-life) was observed for the logarithmic regression between m for C and July MR, where there was a slight significance (p=0.049, see Table 17). This significance was not observed during the September sampling event, where the logarithmic relationship between m for C and MR was insignificant (p=0.15). It is possible that if MR were reliably obtained more than two times over the course of the experiment, a more conclusive relationship could have been obtained between MR and other variables. It is known that there is variation in MR even within a species, and tissue turnover time also varies among individuals, so repetition of sampling would lead to more robust results. It is interesting to note that the relationship

between m and MR was slightly more meaningful than the relationship between MR and half-life for C (see Figure 41 and Table 22).

The SD and W rats did not group separately when comparing MRs and m, but instead represent a range of values for both variables. It is probable that there is a relationship between m and MR, but the variation in MR within a species is not great enough to be significantly reflected in m. Previous studies have shown that plotting several species together (mice, rats, and birds) gives a stronger relationship between m and MR, as well as between MR and half-lives, than the relationship observed in this study (MacAvoy et al. 2006). MacAvoy et al. (2006) also state that either a linear or logarithmic model can be used to predict the relationship between tissue turnover and MR within a limited range of sizes, as turnover and MR/g are positively related. Therefore, it is probable that the range of sizes observed in this study was not great enough to result in an appreciable difference between the two models.

The relationship between C half-life and MR is negative (see Figure 41 and Table 22) whereas the relationship between N half-life and MR is positive (see Figure 42 and Table 23). Though neither relationship is significant, they should be in the same direction. As a higher MR should give a higher value of m, and thus faster tissue turnover, it would make sense that this relationship should be negative (as MR increases, turnover increases, so half-life decreases). It is probable that the N values are skewed, as N signatures for SD did not fit well with previous studies on SD tissue turnover time, and it is known that N values are not as reliably obtainable as are C values. There was a very high standard deviation for the N half-life of the SD rats, so it could be that the data is skewed due to one or more individual SD rats, and this is throwing off the relationship. It

could also be due to the dip observed in the N signature of the blood, which was likely caused by incomplete combustion during analysis. When the two most extreme weeks (the purple dots in Figure 28 and Figure 29) were excluded from analysis, the N half-life of SD rats decreased from 47 to 40 days, suggesting that these values skewed the calculations. The range of N half-lives observed for individual rats was much larger than the C half-lives (see standard deviations of C and N half-lives in Table 16). Therefore, calculations with the C isotope probably more accurately reflect the rate of tissue turnover for this study, as the N signatures seemed fallible. For the manuscript written for intended publication of this study, those weeks where N signature seemed off will be re-analyzed whenever possible (that is, when there is enough blood sample left over), and half-lives will be re-calculated for N isotope.

Previous studies have stated that more research is necessary to determine if mammal MR scales logarithmically with tissue turnover, as is the pattern with avian species (MacAvoy et al. 2006). Because m and half-life are related (see Equation 7) they are significantly correlated (see Figure 45, Figure 46, Table 28, and : Linear and Logarithmic Regression of N Half-life on m for N.

Table 29
Significant R2 Values and the Equations for the Line of both Regressions of N Half-life on m for N.

	R^2	Sig. of R^2	Equation of line
Linear	0.924	2.53E-6	y = -1691.36x + 68.42
Logarithmic	0.94	8.22E-7	y = -33.17*ln(x) – 96.24

Note. Though both fits are significant, the logarithmic regression gives a slightly better fit.). They are negatively correlated, which has been shown in previous studies (MacAvoy et al. 2006), because as metabolic tissue replacement rate increases, naturally the half-life of the isotope in the tissue decreases. It is interesting that the logarithmic regression of the variables gives a slightly better fit than the linear regression for both

isotopes. The R^2 value for the logarithmic regression was 0.979 for C and 0.94 for N, where the linear fit was 0.931 for C and 0.924 for N. This lends support to the idea that mammal MR may scale logarithmically with turnover, but as both models were significant, with logarithmic only slightly more than linear, the present data cannot offer conclusive insights into which may be the more appropriate model.

Conclusions

The hypothesis that rats with a higher MR will have faster tissue turnover was not supported, as the half-lives of the two strains did not differ significantly for either isotope. The SD rats did have a slightly shorter C half-life than the W rats, lending support to the hypothesis that, had there been a larger difference in the MRs, it may have been reflected in their tissue turnover rates. However, m was not significantly different between the strains, and m and MR were not significantly correlated, so the original hypothesis cannot be supported. Though MR may not track tissue turnover closely enough to be useful when measuring variations within a species, it does mean that, within a species exhibiting a range of MRs, turnover happens at approximately the same rate. This should simplify ecological field studies attempting to determine turnover rate using MR, as one can be reasonably confident that turnover occurs at the same rate within a species whose sizes and MRs may vary. It has also been shown that demonstrating differences in MR within a species is difficult due to the small range of body masses, and confounding effects from sex, season, and nutrition (Randall et al. 2002). Even though the correlations were insignificant, the general trends observed in this study do reflect those seen in previous studies (MacAvoy et al. 2006), where the rate of C turnover

increases with MR/g, and decreases with mass. Smaller animals have a higher MR/g and a faster rate of tissue turnover, or shorter half-life.

Because many independent t-tests were employed throughout the data analysis, the issue of compounding error justifies brief mention. With each t-test run on the same data set, the chances of committing a type 1 error (false positive) increases. However, the only significant t-tests were those comparing the MRs between strains, which were performed at the very beginning of data analysis, making it unlikely that positive results were due to compounding error. For the purposes of statistical analysis, it is also possible that a sample size of 11 experimental rats (5 SD and 6 W) was not large enough to accurately obtain either a linear or logarithmic relationship, as in most regression analysis there was not much of a difference between the two models. In order to confidently show that one model is better than the other, a larger sample size representing a greater spread of data (which is probably not obtainable within a single species) would be necessary in order to see a clear relationship for either linear or logarithmic models. Because there was such a range of MRs within the species, it was very beneficial to track and report data on each individual rat, rather than averages within a strain, so that relationships could be obtained. In future studies, organisms should be monitored and reported as individuals and not as averages within a strain or species, so as to represent the full range of values. For the purposes of this study, however, it appears as though the differences in MR within two strains of *Rattus norvegicus*, though significant, are not different enough to cause significant variation in the metabolic component of tissue turnover. For the purposes of comparable ecological field studies, it can be assumed that, within a species

of a given range of sizes, turnover happens at approximately the same rate, regardless of different MRs.

APPENDIX A

SUPPLEMENTAL FIGURES

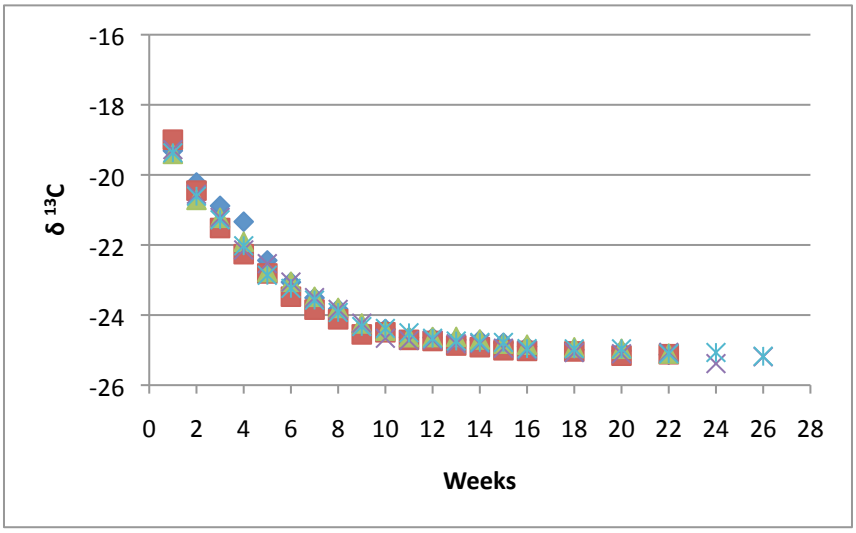

Figure S51: C Turnover in SD Experimental Rats, where Each Line Tracks the Turnover in an Individual Rat.

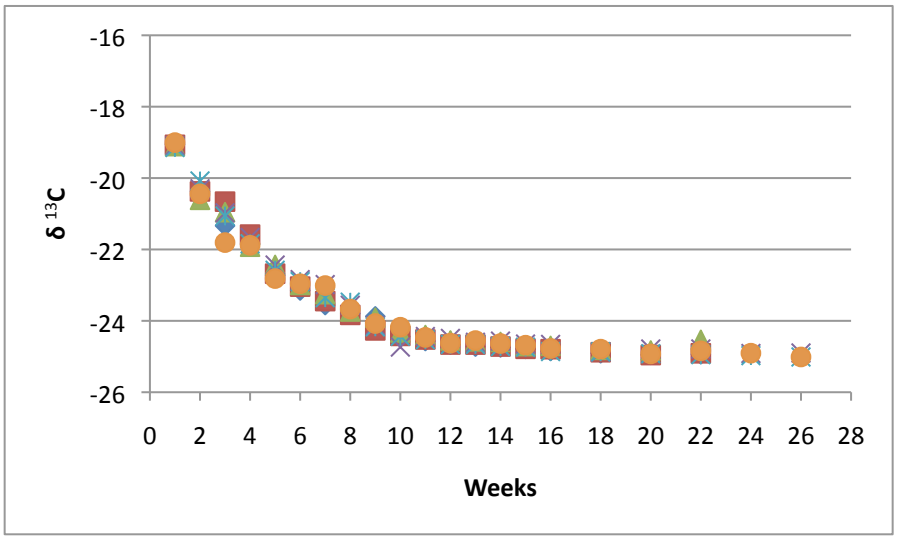

Figure S53: C turnover in W Experimental Rats, where Each Line Tracks the Turnover in an Individual Rat.

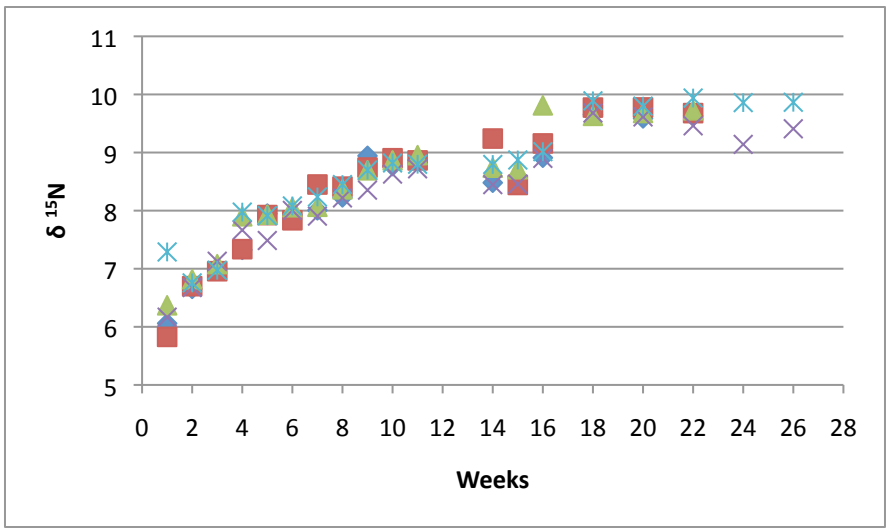

Figure S54: N turnover in SD Experimental Rats, where Each Line Tracks the Turnover in an Individual Rat.

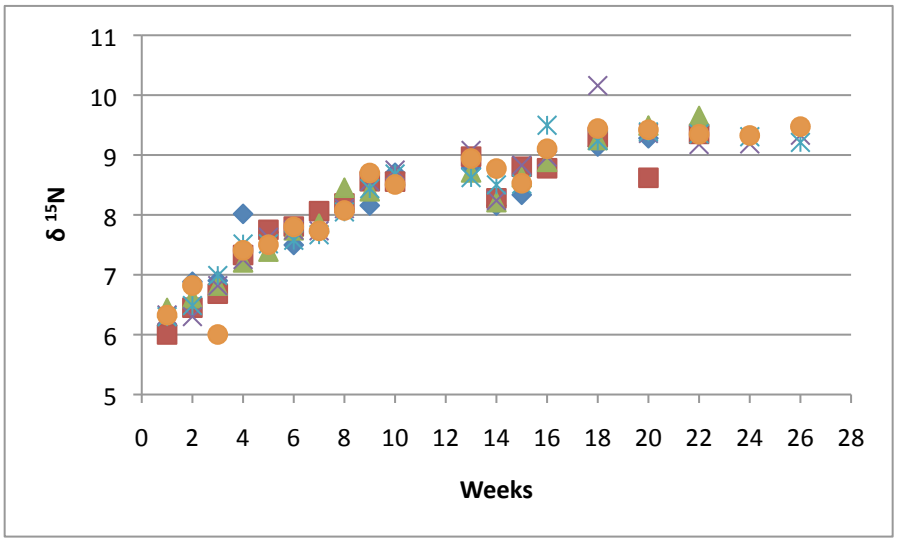

Figure S54: N turnover in W Experimental Rats, where Each Line Tracks the Turnover in an Individual Rat.

Table S33: Standard Deviations Around the Average Carbon Signatures of SD and W Control and Experimental Groups throughout the Study.

(C) Week	1	2	3	4	5	6	7	8	9	10
SD control	0.11		0.09		0.10		0.08		0.07	
SD exp	0.16	0.19	0.23	0.36	0.19	0.18	0.15	0.13	0.15	0.11
W control	0.17		0.37		0.20		0.13		0.06	
W exp	0.05	0.17	0.40	0.14	0.16	0.11	0.23	0.14	0.15	0.18

(C) Wk, cont.	11	12	13	14	15	16	18	20	22	24	26
SD control	0.08		0.07		0.04						
SD exp	0.09	0.05	0.10	0.09	0.10	0.07	0.06	0.09	0.03	0.22	0.01
W control	0.03		0.01		0.03						
W exp	0.06	0.07	0.06	0.07	0.05	0.08	0.04	0.07	0.16	0.04	0.06

Table S34: Standard Deviations Around the Average Nitrogen Signatures of SD and W Control and Experimental Groups throughout the Study.

(N) Week	1	2	3	4	5	6	7	8	9	10
SD control	0.21		0.11		0.15		0.08		0.08	
SD exp	0.56	0.07	0.07	0.31	0.20	0.10	0.21	0.11	0.21	0.11
W control	0.12		0.31		0.40		0.20		0.31	
W exp	0.16	0.23	0.36	0.30	0.14	0.13	0.14	0.15	0.19	0.09

(N) Wk cont.	11	12	13	14	15	16	18	20	22	24	26
SD control	0.05		0.36		0.15						
SD exp	0.09	0.15	0.16	0.32	0.18	0.38	0.11	0.09	0.17	0.5	0.3
W control	0.48		0.20		0.31						
W exp	0.15	0.23	0.17	0.23	0.19	0.25	0.37	0.32	0.16	0.0	0.1

Table S35: k Values, m for C, m for N Values for all Individual Rats (m only for Experimental Rats)

Rat	k	m for C	m for N
SD1-1	0.000263087	0.031285336	0.024849934
SD1-2	0.000341544	0.039975112	0.017728707
SD1-3	0.000294891	0.051073078	0.016557522
SD2-1	0.000271356	0.040871485	0.015083739
SD2-2	0.000238612	0.027981488	0.013706332
SD3-1	0.000319838		
SD3-2	0.000389585		

Table S35 continued.

Rat	k	m for C	m for N
SD3-3	0.000167736		
SD4-1	9.08574E-05		
SD4-2	0.000334897		
SD4-3	0.000254987		
W1-1	0.001664293		
W1-2	0.001307281		
W1-3	0.002041245		
W2-1	0.001442688		
W2-2	0.001718248		
W2-3	0.001301419		
W3-1	0.002199336	0.036955392	0.024655828
W3-2	0.002066614	0.039746607	0.014924602
W3-3	0.001880843	0.02071901	0.026982731
W4-1	0.002060207	0.024506316	0.017828112
W4-2	0.001884597	0.036889992	0.013066087
W4-3	0.00208203	0.029144647	0.016160182

Table S38: C and N Half-lives for all Individual Rats, as well as Averages Within a Strain with Standard Deviations

Rat	C half-life (days)	N half life (days)
SD1-1	21.97	27.60
SD1-2	17.19	38.36
SD1-3	13.49	41.13
SD2-1	16.85	45.14
SD2-2	24.56	49.71
Avg (St Dev):	18.81 (4.41)	40.39 (8.33)
W3-1	17.70	25.81
W3-2	16.58	40.79
W3-3	30.67	24.01
W4-1	26.09	34.85
W4-2	17.88	46.36
W4-3	22.20	38.00
Avg (St Dev):	21.85 (5.60)	34.97 (8.68)

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