Summer 8-10-2017

Reduced Immune Investment with Energy Stress: Evidence from a Mouse Model and Human Studies

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Reduced Immune Investment with Energy Stress: Evidence from a Mouse Model and Human Studies

by

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Submitted in partial fulfillment
of the requirements for the degree of
Master of Arts [Anthropology], Hunter College
The City University of New York

2017

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Dedication

For my parents, grandparents, family and friends.

Without you, this wouldn't be possible.

Acknowledgments

I would like to thank my advisor, Herman Pontzer for being a true mentor and helping me through this process with support, encouragement and wisdom. Without his encouragement, this would not have been possible. Thank you to Nesha S. Burghardt for her invaluable guidance, support and insight during this process. Thank you to James Higham and Oluwatoni Sonubi, as well as the Ponzter and Burghardt labs for their help and expertise. Finally, thank you to the faculty and fellow graduate students of Hunter College for imparting their wisdom upon me and helping to shape this thesis.

Table of Contents

List of Figures

List of Tables

Introduction

In order to survive, all organisms must find, use and allocate energy for essential functions, such as growth, homeostasis and reproduction. However, energy availability fluctuates based on limited ecological resources, self-limiting factors (i.e. eating disorders) or political institutions (i.e. forced starvation). Animals respond to low energy availability in a variety of ways in order to survive. Animals have been shown to redirect energy away from non-essential functions (i.e., reproduction and immune function) (Ellison, 2003; Pontzer, 2015b; Wiersma & Verhulst 2005; Lochmiller and Deerenberg, 2000; Perrigo, 1987; Perrigo and Bronson, 1983) toward essential functions (i.e., growth) that are key to survival (Butte, 2000).

Further, animals have been known to decrease their basal metabolic rate (BMR) in attempt to conserve limited energy during low energy periods (Mitchell et al, 2016; Wang et al, 2012; Speakman & Mitchell 2011; Weyer et al, 2000; Rothwell & Stock 1982; Blanc et al, 2003; Ferguson et al, 2007; Cameron et al, 2011; Hambly & Speakman, 2005; Wiersma and Verhulst, 2005, and Vaanholt et al, 2007). These metabolic strategies are believed to be evolutionarily adaptive, as they enable survival by providing a strategy for conserving energy stores and allocating energy to essential processes.

Energy is stored in the fat of most mammals during periods of energy availability (Ottaviani et al, 2011). Total energy expenditure (TEE; kcal/d) is the number of calories burned by all activities of the body that require energy, including basal metabolic rate (BMR; kcal/d), physical activity, growth, thermoregulation, digestion, immune function and reproduction (Pontzer, 2015b). These systems vary in their energetic cost, with systems such as thermoregulation and digestion costing significantly less than physical activity and immune

function (Muehlenbein et al, 2010; Ainsworth et al, 2000; Kinabo and Durnin, 1990; Pontzer, 2015b). Using the energy saving techniques mentioned above, animals show a remarkable ability to survive for long periods of time when energy availability is low. However, research is sparse on how these metabolic adaptations occur and their consequent effects on organs of the body. This is especially relevant for adolescent individuals, where growth is a large portion of TEE (Butte, 2000).

Physical Activity & Food Restriction

Physical activity is a necessity for animals and human populations who must forage for survival. While energy is expended in order to obtain and consume energy, the amount of energy that is invested in foraging is typically matched or exceeded once food is obtained. However, this is not always the case during periods of low energy availability, in which case the energy expended during foraging is not replaced, resulting in an energy deficit. To avoid this deficit, some studies report that animals preserve limited energy by decreasing physical activity (Hambly and Speakman, 2005; Golightly et al, 2012; Wang et al, 2006). However, others report either no change in physical activity (Cameron et al, 2011; Mitchell et al, 2016) or increases in physical activity, due to increased foraging demands (Duffy et al, 1989; Duffy et al, 1997; Overton and Williams 2004; Carter et al, 2009; Vaanholt et al, 2007; Wiersma & Verhulst, 2005). Interestingly, some report both a decrease and an increase in physical activity. Specifically, a decrease in physical activity was found in captive rats in response to the initial stages of food restriction, during which the body is fueled by glycogenolysis and then by gluconeogenesis and ketone body production. Then, as adipose stores become depleted and the need for food

increases, physical activity actually increased (Wang et al, 2006**;** Sclafani and Rendel, 1978 and Koubi et al, 1991).

Energy Stress and Immune System Function

The cost of an activated immune system is high, as demonstrated by various studies in which infection is correlated with increased energy expenditure (Muehlenbein et al, 2010; Lochmiller and Deerenberg, 2000; Martin II et al, 2003; Tocco-Bradley et al, 1987; Demas et al, 1997; Gurven et al, 2016; Long, 1977). Immune activation typically raises BMR due to the increased lipolysis, glucoeogensis, tissue catabolism, and proteolysis that is needed for the increased hormone and cell production (Klasing, 1988; Michie, 1996; Long, 1977; Crouser and Dorinsky, 1996; Lochmiller and Deerenberg, 2000). For example, glucose use has been shown to increase by 68% during the acute phase immune response (Klasing, 1988), while even mild infections can increase gluconeogenesis by 150-200% (Lochmiller and Deerenberg, 2000). Activation of the immune system can also lead to a decrease in body and protein mass (Arturson, 1978; Long, 1977; Biolo et al, 1997). In extreme cases, 15-30% of total body mass can be lost during infection (Long, 1977). Fevers are metabolically expensive as well, as it is proposed that for every 1 F degree increase during a fever, BMR increases by 7% (Roe and Kinney, 1965) and this BMR increase has the ability to rapidly decrease protein stores (Long, 1977). Even mild infections have been shown to increase resting metabolic expenditure (RMR) in children (Fleming et al, 1994). The immune system is sensitive to change even when not fighting an infection, as BMR has been shown to increase during protein antigen vaccinations by 10-15% (Demas et al, 1997).

In addition to the costs of immune activation, daily upkeep of the immune system is believed to have an energetic cost. T and B cells, both important for immune function have a high turn-over rate in healthy individuals (Macallan et al, 2004 and Macallan et al, 2005). In CD4 + T Cells, central memory T cells (TCM) have a proliferation rate of 4.7% per day, and effector memory T cells (TEM) have a rate of 1.5% per day in a healthy non-activated immune system (Macallan et al, 2004). While peripheral blood B cells had a proliferation rate of .46% per day compared to memory B cells with a rate of 2.66% per day (Macallan et al, 2005).

When energy resources are limited, activation of the immune system, even for minor infections, has the potential to deplete energy storage. It has therefore been hypothesized that allocating energy away from immune function during periods of energy shortage may increase long term survival by maintaining energy stores **(**Lochmiller & Deerenberg, 2000**)**. Research investigating calorie restriction and immune system function have commonly focused on anorexic human patients (Cleary et al, 2010; De Filippo1 et al, 2016; Golla et al, 1981; Marcos, 1997) or food restricted animals, such as mice (Christadoss et al, 1984; Gardner, 2005; Martin II et al, 2007), rats (Fernandes et al, 1997) or fish (Martin et al, 2010). Immunosuppression is a well-known and common occurrence associated with calorie restriction (Christodoss et al, 1984; Chandra, 1997**;** Lochmiller and Deerenberg, 2000). Nakamura and colleagues (1990) found that 5 days of calorie restriction decreased T cell counts, spleen weight and thymus weight in mice. Further, Nishida and Sakakibara (2008) found that Japanese women with an average BMI of 16 had reduced leukocyte, neutrophil and lymphocyte cell counts compared to women with a normal BMI. However, many studies report regular immune function after re-feeding, suggesting that immunosuppression is reversible (Allende et al, 1998; Misra et al, 2004).

Further research is needed to characterize how physical activity and energetically expensive systems, such as the immunological system, are affected by low energy availability in adolescent animals. We address this issue using a mouse model and published data in human subjects. Using mice, we tested the predictions that during low energy availability, 1) energy will be allocated away from the immune system and 2) physical activity will be decreased in order to provide energy for more vital functions, such as growth and maintenance. Decreased energy for physical activity and immune function would highlight the metabolic trade-offs that animals face during times of low energy availability. Alternatively, if energy is allocated toward physical activity and immune function instead of away, this may highlight the importance of these systems for survival.

We tested our hypothesis in mice using a combination of food restriction to reduce energy availability and access to a running wheel to monitor physical activity. Energy allocation was assessed by measuring changes in body weight and organ mass. We expected food restriction to decrease wheel running activity and lead to a reduction in body size and organ mass. To evaluate whether the same changes would occur in humans, we conducted a meta-analysis of published peer-reviewed research articles in which immune function was assessed in women with varying body mass index, BMI. BMI was used as a measure of food intake and energy stress. We expected that lower immune cell counts would be found in women with lower BMI's indicating that the immune function is down-regulated by calorie restriction.

Methods

Mouse Experiment

Female 129/SvEv mice (*Mus domesticus*) obtained from Taconic Biosciences (Germantown, NY) were shipped to the Hunter College Animal Facility at postnatal day 21. Upon arrival, mice were group-housed 4 per cage and kept on a 5am/5pm light/dark cycle with food and water available *ad libitium*. At postnatal day 43-45 (middle adolescence), mice were individually housed with a wireless running wheel (MedAssociates, VT) and unlimited access to food. At this point, mice were randomly allocated to one of four groups: Activity-based anorexia (ABA), Food restricted control (FR), Wheel control (WH), and Home cage control (HC). The running wheel was locked for mice in the FR and HC groups and was not locked for mice in the ABA and WH groups. After one day of acclimation to the new housing conditions, baseline measurements (body weight, food intake, water intake) were recorded for three consecutive days immediately before the onset of the dark cycle.

ABA Days 1-10: On Baseline Day 3, all food was removed from mice in the FR and ABA groups, two hours after the onset of the dark cycle. Starting the next day (ABA Day 1), an unlimited amount of food was provided to mice in the FR and ABA groups only during the first two hours of the dark cycle. Mice remained in their respective housing conditions, with (HC and WH) or without (ABA and FR) unlimited access to food for a total of 10 days. Mice continued to have unlimited access to a running wheel that was locked (HC and FR) or able to turn (ABA and WH) throughout the rest of the experiment. Body weight, food intake, and water intake were recorded daily by 5pm. Wheel running was tracked continuously (Wheel Manager Software, MedAssociates, VT) and analyzed during the light and dark cycles. Food anticipatory activity (FAA), defined as running that occurred during the 4-hour period prior to food

availability, was also calculated. Based on limited access to food and/or a free-turning running wheel, the groups were categorized in the following way (Table 1):

- ABA: high activity + calorie restriction
- FR: low activity + calorie restriction
- WH: high activity + high food availability
- HC: low activity + high food availability

Organ Removal: On Baseline Day 3 or ABA Day 10, mice were deeply anesthetized with ketamine and their organs were extracted and immediately weighed wet to 0.001g (Ohaus). Tissue was then stored in a -20°C freezer, later freeze dried in Dr. James Higham's lab at New York University, and weighed again while dry. Final analyses were performed using freeze dried weights. Two cohorts of animals were used, each of which contained the same groups and the same number of mice/group. The heart, liver, kidney and spleen were collected from both cohorts. The brain and blood were also collected from the second cohort.

Human Immune Function Meta-Analysis

To further explore the relationship between low energy availability and immune function, data from published peer-reviewed research articles were analyzed. Criteria for inclusion involved: female subjects, body mass index (BMI) from 13-25, aged 15 years or older, with no illness, known chronic disorders or disease at time of measurement. Only studies that reported measurements of white blood cells (WBC), neutrophils, lymphocytes, red blood cells (RBC), platelet count or hemoglobin values were included the final dataset. Data were extracted from 6 studies (Nishida and Sakakibara, 2008; Takele et al, 2016; Nagata et al, 1999; Lambert et al, 1997; Kim et al, 2013; Misra et al, 2014) and from the CDC National Health and Nutrition

Examination Survey for the year 1998 (Table 6). Immunological function was determined based on WBC, neutrophil, lymphocytes and platelet counts, while RBC and hemoglobin values were used as non-immune-related control measurements. WBC, neutrophil, lymphocytes, platelets and RBC were measured in 1000 cells/μL of blood, and hemoglobin in grams/dL.

Data analysis

Statistical analyses were performed in R studio for both sets of data. P values, degrees of freedom (df) and r^2 was reported for each analysis, with significance level set at $P < 0.05$. Linear regression was used to analyze the effect of BMI, immune parameters and non-immune parameters in our human immune dataset. BMI was further analyzed using the classifications of underweight (<18.5 kg/m^2) and normal (18.5 -24.6 kg/m^2) with immune parameters and non-immune parameters. Multivariate regression was used to analyze the effect of food restriction, physical activity, water intake, organ mass and body mass in our mouse dataset.

Results

Mouse Model Analysis

On the last day of the experiment, ABA and FR groups (the conditions with limited access to food) lost a considerable percentage of their baseline body mass (20 ± 10% and 21 ± 11%, respectively). In comparison, WH and HC groups (conditions fed *ad libitum*) increased their body mass (4 ± 11% and 4 ± 12% respectively) (Table 3) (Figure 6). When pooled into two groups based on feeding regime, mice with limited access to food (ABA and FR) weighed less on day 10 (13.50 ± 1.23g) than mice fed *ad libitum* (HC and WH), (18.07 ± 1.47g). When compared to the organ masses of Baseline mice, the kidneys (-20.5± 10%), spleen

(-51± 19%), and heart (-24± 13%) were smaller (Student's t-test, P <0.001) in Calorie Restricted mice (ABA and FR), while liver (-17± 10%) (Student's t-test, P= 0.095) and brain (+17± 25%) (Student's t-test, P= 0.070) did not differ significantly (Table 2; Figure 1). A similar but non-significant trend was seen in Ad Libitum mice. The kidneys (-3± 12%), spleen (-14± 24%), liver (-1± 14%) and heart (-8± 16%) and brain (+11± 22%) were not statistically different from the organ masses of baseline mice (Student's t-test, P>0.05) (Table 2; Figure 1). Brain mass was just as large or larger for all four treatment conditions compared to Baseline, indicating that the brain was protected against energy stress (Table 2; Figure 1).

Using multivariate linear regressions, with final body mass and condition as independent variables, food intake was correlated with condition (df= 34, r^2= 0.929, P<0.001), especially ABA and FR conditions (df=34, r^2= 0.929, P<0.001) (Figure 4), and body mass (df=34, r^2= 0.929, P= 0.03) (Figure 6). Physical activity was found to have no effect on food intake (df=33, r^2= 0.927, P= 0.881), water intake (df= 35, r^2= 0.886, P= 0.635) or organ size (Table 5). For the organs measured, total body mass did not correlate with the size of the spleen (df= 36, r^2, = 0.692, P= 0.408) or brain mass (df=20, r^2= 0.096, P= 0.195) but was correlated with liver (df= 36, r^2= 0.717, P< 0.001), heart (df= 36, r^2= 0.623, P<0.05) and kidney mass (df= 36, r^2= 0.766, P< 0.001).

Total physical activity (revolutions/day) did not differ between mice in the ABA and WH conditions (df= 14, r^2= -0.071, P=0.978) (Figure 2). However, ABA mice did run significantly more (441.70 ± 435.25 revolutions) than WH mice (33.29 ± 25.58 revolutions) during the 4-hour period prior to food access, consistent with food anticipatory activity (FAA). FAA activity increased dramatically (1704%) throughout the experiment in ABA mice. By day 9, the average FAA in ABA mice was 863 ± 746 revolutions, compared to 68 ± 102 revolutions on ABA day 1

(Figure 5). Consistent with this change in FAA, which occurs during the light cycle, ABA mice ran more during the entire light cycle (3227 ± 3218 rotations) compared to WH mice (1962 ± 1492 rotations) by the end of the experiment (Figure 3). Both groups with limited access to food (ABA and FR) drank less water (33%) than mice fed *ad libitum*. Similarly, water intake was correlated with food intake (df= 38, r^2= .791, P< 0.001).

Human Immune Function

Linear regressions were used to determine the relationship between BMI and immunological parameters. BMI was correlated with WBC (df= 30, r^2 = 0.542, P< 0.001), neutrophil count (df= 22, r^2 = 0.542, P< 0.001) (Figure 7A) and platelet count (df=22, r^2 = 0.154, P= 0.032) (Table 7B). BMI was not a significant predictor of lymphocyte (df= 22, r^2 = 0.073, P=0.107) (Figure 7A), RBC (df= 22, r^2 = 0.035, P= 0.190) or hemoglobin value (df= 22, r^2 = 0.042, P= 0.168) (Figure 7C).

Underweight BMI (<18.5 kg/m^2) was associated with lower cell counts for WBC (df=16, r^2 = 0.502, P< 0.001), neutrophils (df= 10, r^2 = 0.653, P< 0.001) (Figure 8A) and platelet count (Figure 8B) (df=10, r^2= 0.291, P< 0.05), but not for RBC (df= 11, r^2 = 0.104, P= 0.150), hemoglobin value (df= 11, r^2= 0.053, P= 0.221) (Figure 8C) or lymphocytes (df= 10, r^2= 0.161, P= 0.107) (Figure 8A).

There was no correlation found between normal BMI (18.5-24.6 kg/m^2) and cell count for any of the parameters (Figure 9A, B and C). Subjects with normal BMI, only 9% and 12.5% fell below the normal range for WBC and hemoglobin, respectively (Mayo Clinic, 2014), All other measured cell counts were within normal ranges for the normal BMI group.

Discussion

Mice with limited access to food (ABA and FR) lost a significant amount of body weight but did not cease to engage in physical activity. They also allocated energy away from immune function, as indicated by a significantly smaller spleen mass compared to mice fed *ad libitum* (WH, HC and Baseline). These results were consistent with the positive correlation found between BMI and WBC, neutrophil count and platelet count with in the analysis of data collected in humans. Our finding that ABA mice exercised as much as the WH control group indicates that instead of allocating energy away from physical activity, energy was allocated away from other function, such as organ growth. Interestingly, we detected a decrease in the mass of all organs tested except the brain, which appeared to be protected from this downregulation. These findings suggest that during periods of low energy availability, the body adapts by allocating energy away from immune function and organ growth, toward other functions that may be more important for long-term survival, such as physical activity and maintaining the central nervous system.

Our results are similar to other studies that examined energy allocation in conjunction with physical activity and calorie restriction (Vaanholt et al, 2007; Wiersma and Verhulst, 2005; Mitchell et al, 2016). During periods of low energy availability, animals conserve, decrease and reallocate energy usage in a series of ways in order to survive. This is done by decreasing physical activity, BMR and reproduction investment (Cameron et al, 2011; Hambly and Speakman, 2005; Blanc et al, 200; Ellison, 2003). BMR is decreased by reducing body and organ mass, which then reduced the energy needed for biological activities such as cellular respiration and digestion (Wang et al, 2012; Wang et al, 2006). Further, animals reallocate energy from

energetically expensive activities such as immunological function, to more essential ones, such as physical activity. Decreasing BMR and the allocation of energy were observed within our experiment, where mice with limited access to food reduced their body and organ mass, therefore reducing their BMR, and allowing them to function on less energy. This technique would be especially beneficial for our early hunter/gathering hominin ancestors, whose food intake probably varied. Being able to down-regulate BMR in order to function on a reduced calorie intake would have been evolutionarily adaptive.

There are two possible reasons why there is a reduction in immune investment during periods of low energy availability. First, daily immunological maintenance and upkeep may be too energetically expensive during energy stress (Lochmiller and Deerenberg, 2000; Macallan et al, 2005 and Macallan et al, 2004). Alternatively, immune activation against infection may be too energetically expensive (Gurven. 2016; Muehlenbein et al, 2010; Long, 1977). While allocating energy away from immune function may appear to be a risky strategy for an organism, it may actually increase the chance of survival by prolonging energy reserves (Lochmiller and Deerenberg, 2000). Down-regulation of the immune system would then allow for energy to be used for other functions, such as physical activity. Given that our mice with limited access to food ran as much as mice with unlimited access to food, it can be assumed that energy allocated away from immune function was used for physical activity and body maintenance.

Research focused on the relationship between energy stress and physical activity have led to conflicted findings. Some studies indicate an increase (Duffy et al, 1989; Duffy et al, 1997; Overton and Williams, 2004 and Carter and Daniels, 2009), a decrease (Hambly and Speakman, 2005 and Golightly et al, 2012) or no significant difference in physical activity during energy stress (Cameron et al, 2011 and Mitchell et al, 2016). Our results show no effect of food

restriction on physical activity. Energy was not allocated away from physical activity during our experiment as we hypothesized, but rather toward it. This indicates other functions may have been down-regulated instead, and emphasizes the importance of physical activity during energy stressed periods. As with our results, Mitchell et al (2016), found while there was no difference in overall physical activity between ad libitum and calorie restricted mice groups, there was a difference in FAA, light and dark activity.

Increased FAA activity is believed to be an evolutionary adaptive behavior for increasing foraging behavior during low energy availability (Abrams, 1991 and McCue, 2010). Increased FAA activity could be beneficial in the wild, as increased foraging could increase the opportunity for food consumption. Further explanations for increased FAA activity could be the hormone leptin. Studies have shown that calorie restriction leads to high levels of leptin, and hyperleptinemia was found to be associated with increased daily activity, especially FAA (Mitchell et al, 2016). ABA mice also increased their daytime activity, which is interesting as mice are nocturnal animals that forage for food during the night in order to avoid predators (Froy, 2011). Increased daytime activity highlights how energy restriction might influence the circadian rhythm of animals, and change their eating and sleeping schedule.

The lack of effect that physical activity played on our mouse model might be explained by the length of our trials. Many studies conduct calorie restricted mouse models for a month (Mitchell et al, 2016), while ours only lasted 10 days. If our study had continued, physical activity might have been more visibly affected. Our mice might have been still using their fat reserves to sustain themselves even with their reduced calorie intake. While no data was collected on adipose tissue changes in our calorie restricted mice, we noticed decreased fat deposits in the lower abdomen of ABA and FR mice compared to WH and HC. However, fat

deposits appeared to still be present in our Calorie Restricted mice at the end of the experiment, and not completely utilized. This, along with the observed stabilization in body weight and energy intake/g body mass by day 8-10 suggests that Calorie Restricted mice decreased BMR to adapt to their energy stressed environments. These results are in-line with other researchers who have reported weight stabilization during calorie restriction (Yamada et al, 2013) and a decrease in BMR (Mitchell et al, 2016; Wang et al, 2012; Speakman & Mitchell, 2011; Weyer et al, 2000; Rothwell and Stock, 1982; Blanc et al, 2003; Ferguson et al, 2007; Cameron et al, 2011; Hambly & Speakman, 2005).

Future experiments utilizing mouse models should include BMR measurements, as well as a longer period of food restriction in order to better understand energy stress and physical activity. Further research is needed to identify the mechanisms that mediate the down-regulation of the immune system, and consequent effects on the body during low energy availability. Hormones such as leptin, testosterone, cortisol, IGF-I and growth hormone may play an important part in regulating immune function during these periods (Muehlenbein, 2010; Mehler and Brown, 2015), along with energy stressed conditions such as gelatinous marrow transformation (GMT) (Tavassoli, 1976 and Chen, 2004).

Energy Stress in Human Evolution

Research into energy stress has the potential to inform us not only on how our hominin ancestors might have survived and adapted to low energy habitats, but also how we can improve and better understand the dynamics of energy restriction. Our findings indicate that during periods of low energy availability animals respond by allocating energy away from expensive

functions, such as immune function, and toward functions vital to long-term survival, such as physical activity.

Our hominin ancestors likely faced periods of energy stress, and the employment of these energy adaptive techniques would have promoted survival and reproductive fitness. The theory that our energy saving and spending abilities are dynamic and environmentally sensitive was proposed by Pontzer (2015a), in the Constrained TEE Theory. Our results support this theory and show how energy costs for physical activity, growth, immune function, etc., are not fixed, but rather vary based upon energy availability. Energy was allocated away from immune function in our mice, to sustain physical activity. If energy costs were fixed, a change in the allocation of energy would not have been found in our study. The development and retention of a Constrained TEE strategy would have been evolutionary selected for, as it enables long term survival during periods of food restriction by maximizing energy usage. In particular, the ability to allocate energy away from non-essential function towards physical activity would have been beneficial to our hominin ancestors, who as hunter-gathers were more physically active than typical western populations today (Pontzer et al, 2012).

Immune function might have also been impaired in early hominins if a majority of their ingested energy was being allocated towards physical activity. While down-regulation of the immune system appears to be disadvantageous, it potentially evolved as an adaption for long term survival. During periods of low energy availability, survival is dependent upon energy stores. By maximizing energy stores through lowering BMR and the allocation of energy, the survival period during low energy availability can be increased. Therefore, the down regulation of immune function would have been evolutionary selected for, due to the high cost of immune activation (Muehlenbein, 2010; Lochmiller and Deerenberg, 2000; Martin II et al, 2003; Tocco-

Bradley et al, 1987; Demas et al, 1997; Gurven et al, 2016; Long, 1977). Suppression of immune function allows for the conservation of energy stores and therefore increases the chances of surviving starvation. The effect of immune suppression on our hominin ancestors is unknown and requires further research, which could be conducted using current hunter-gather populations facing energy stress.

Limitations

One major shortcoming of this study is that we did not test the consequent effects of a reduction in spleen mass on the functioning of the immune system. Furthermore, we did not distinguish between innate or cellular immunity within the human immune research. We also did not collect or measure the size of other organs important for immune function, such as the thymus.

The limited duration of our mouse model experiment in comparison to other studies (Mitchell, 2016) reduced our ability to see the long-term effects of our conditions on adolescence mice, particularly in organ size. While we were able to see the stabilization of ABA and FR weight throughout the study, indicating they were adapting to their food restricted conditions, ABA's dramatic increase in FAA running towards the end of the experiment may indicate otherwise. If our model had been carried out for 30 days, we might have seen a further decrease in organ size, and seen if physical activity eventually influenced organ size, as well as food and water intake. We also did not record cage activity of FR and HC mice, which may have played a role in our weight data. We did not record fat percentage or distribution, which would have helped to better understand energy allocation and usage in our mice. Finally, we only included female mice, and the inclusion of male mice and a larger sample size would only add to our

results by showing if energy stress effected males differently than females. Further, a larger sample size would help to decrease the margin of error in our results.

Future studies in mice should be conducted for at least 1 month and measurements of, measures humoral and cellular immunity, immune supporting organs (e.g thymus), and fat distribution should be included. Spontaneous in-cage physical activity, in the form of running around the cage, should be reduced or recorded in non-active conditioned mice in order to reduce result errors.

Conclusion

Energy restriction is an issue that many organisms face throughout their lifetime. Survival depends on many components, such as energy usage, energy intake and metabolic adaptations. In order to survive periods of low energy availability, energy is allocated away from non-essential functions and toward essential functions. This study found that during low energy availability, immune function is down-regulated and energy is allocated to physical activity in adolescent female mice. However, the consequences of an altered immune system during energy stress are unknown and require further research. The added stress of physical activity appears to have no effects on food restricted mice, suggesting that the effects of energy restriction on physical activity are not well understood yet, and further research is required.

Figures

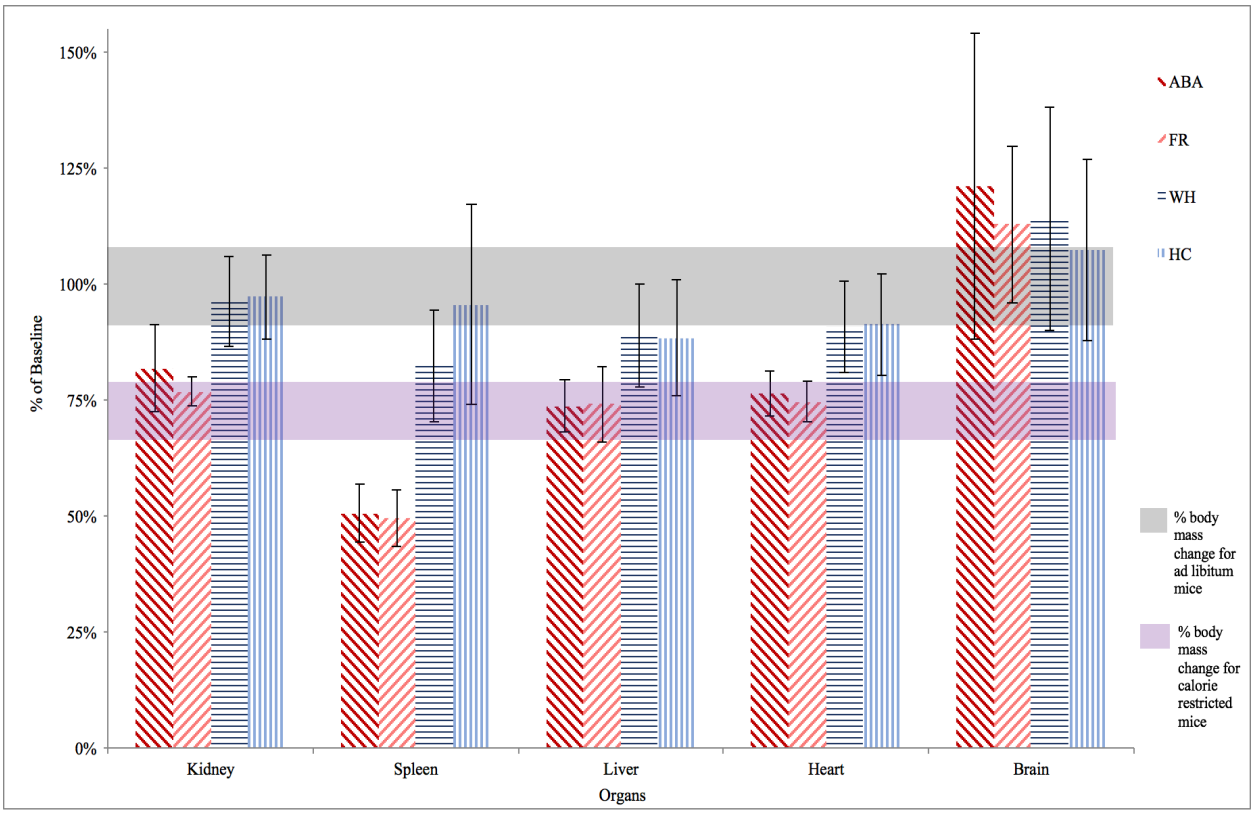


Figure 1. Changes in organ mass following 10 days in respective condition. Presented as final organ size calculated as percent of pre-experiment mass (100%).

All organs decreased for all conditions with the except of the brain, which increased above the baseline weight for brain size. ABA brain size grew the most despite the conditions of calorie restriction and physical exercise. ABA and FR organ size decreased significantly more than WH and HC. The spleen was the most reduced organ for ABA, FR and WH, while the HC spleen was only slightly reduced.

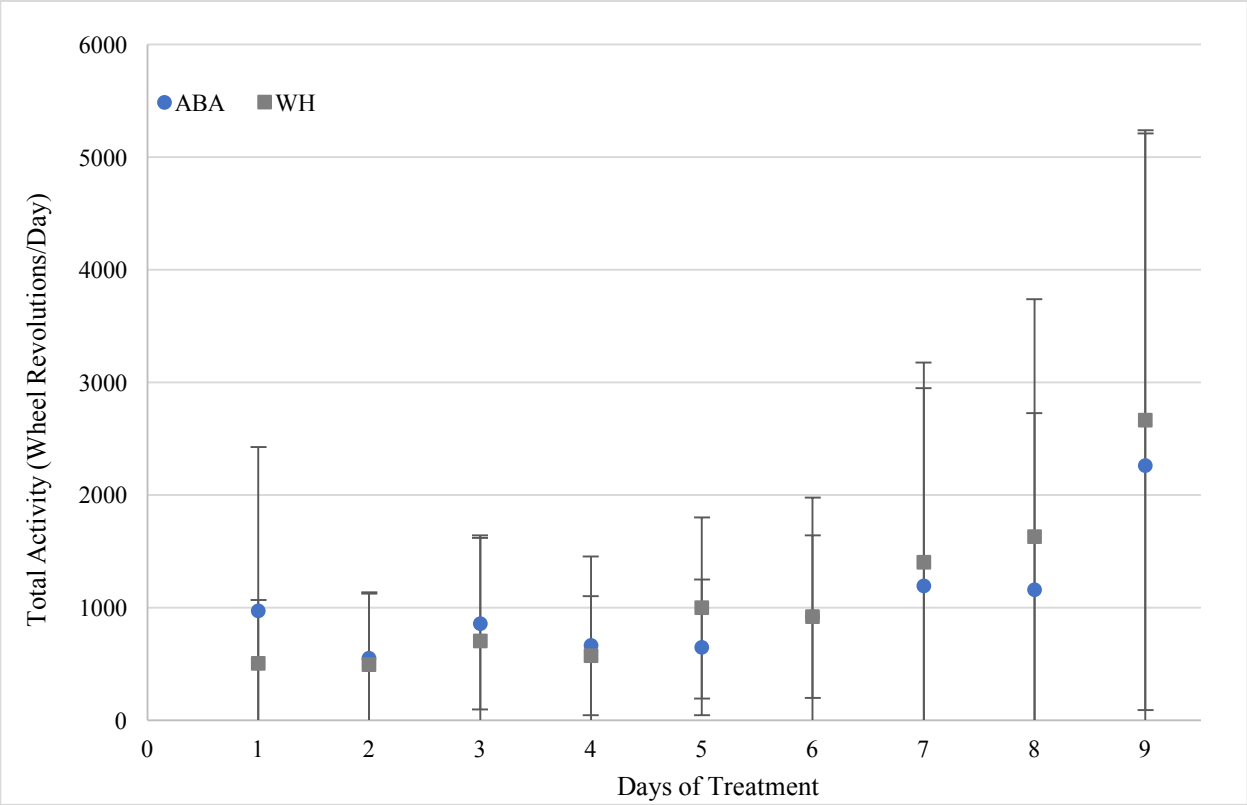


Figure 2. Total Physical Activity (PA) for ABA and WH conditioned mice.

Total running activity for ABA and WH mice over the 10-day mouse model. Running activity increased steadily for both conditions starting on day 5.

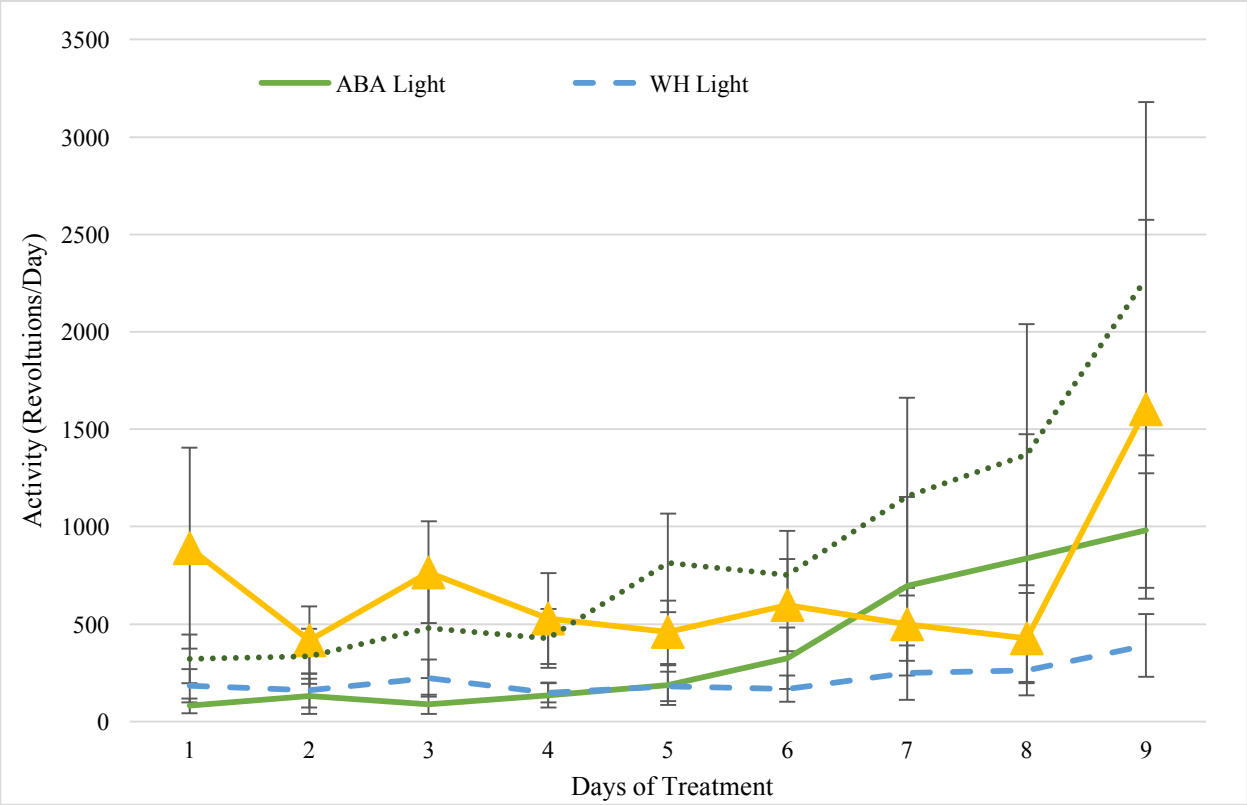


Figure 3. Light vs Dark activity per day of treatment for ABA and WH.

Light and dark activity for ABA and WH mice over 10-day mouse model. Light and dark running activity increased for both ABA and WH throughout the study. ABA light activity increased steadily starting on day 4, while ABA dark activity reached a peak on days 1, 3, and 9. WH light activity stayed the same throughout the experiment and only increased slightly on day 9. WH dark activity increased steadily throughout the experiment.

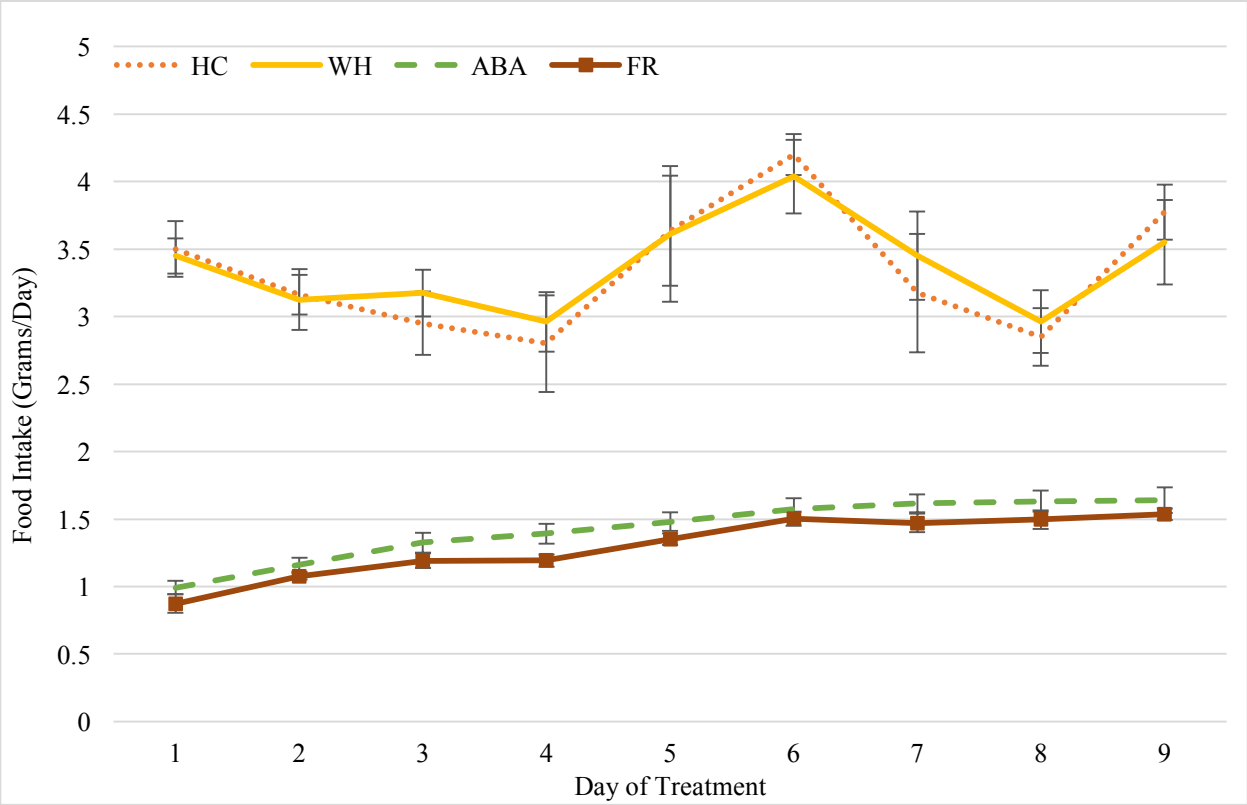


Figure 4. Food intake by condition for length of mouse experiment.

Food intake for all conditions over 9 days of mouse model. Food intake was only recorded for 1-9 for calorie restricted and ad libitum conditions. Food intake steadily increased for calorie restricted mice (ABA and FR), while ad libitum mouse (WH and HC) food intake decreased on days 4 and 8, with a high on day 6.

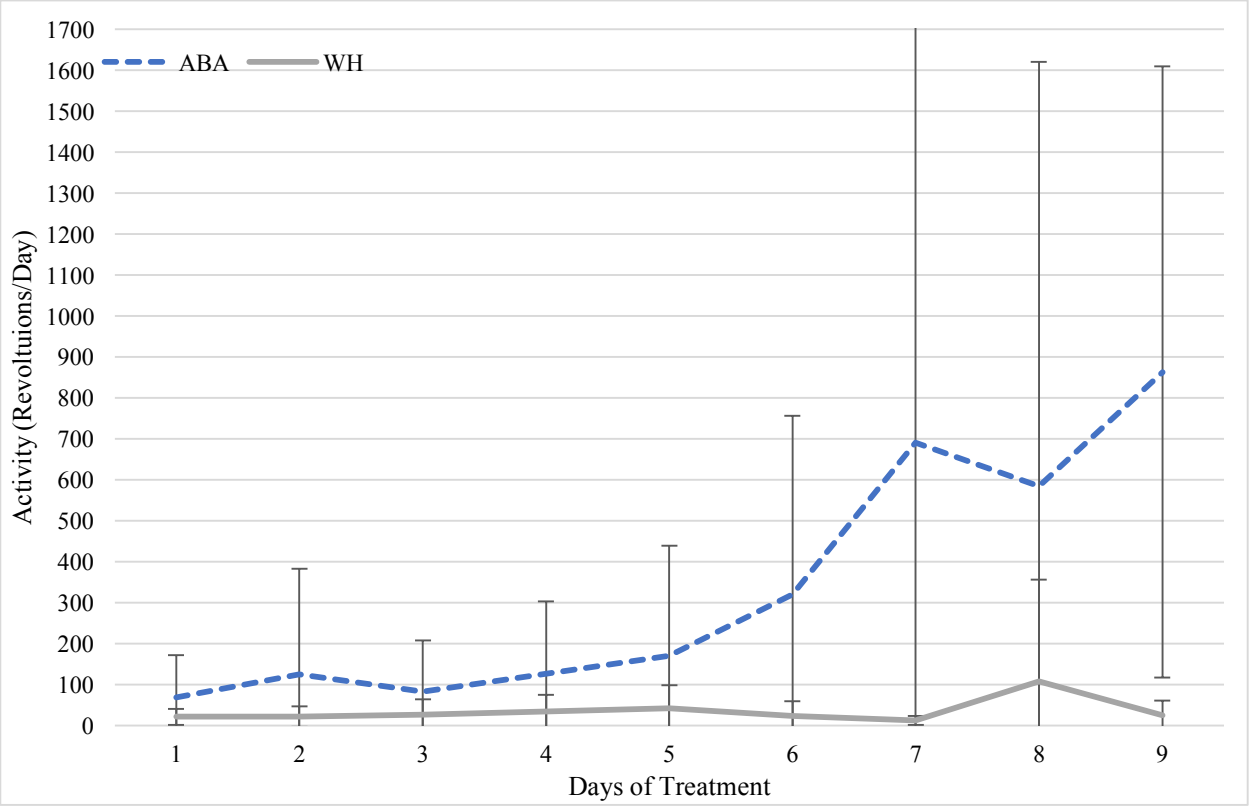


Figure 5. Food anticipatory activity (FAA) measured over length of mouse model.

FAA activity for ABA and WH mice over the 10-day mouse model. ABA increased their FAA activity, starting on day 4, reaching a peak at day 7, decreasing and then steadily increasing until the end of the experiment. WH showed little variation in FAA activity expect on day 8.

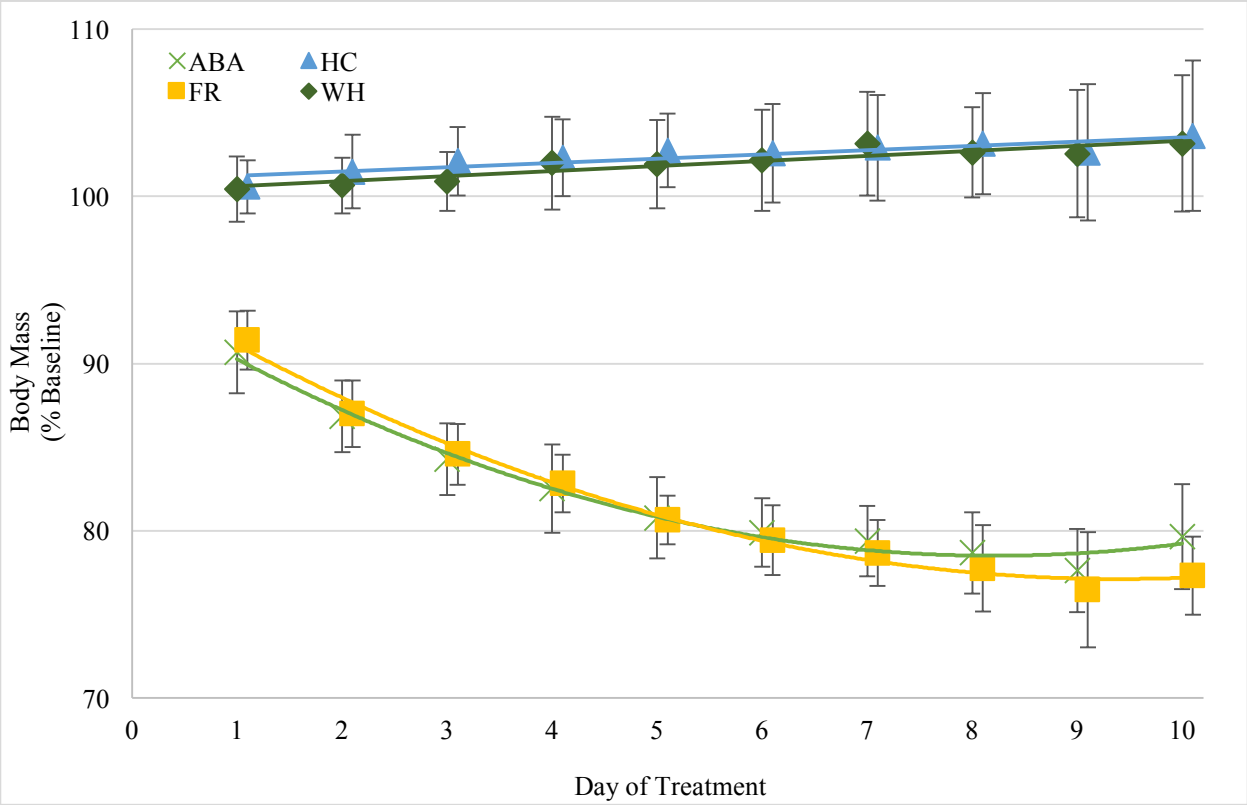


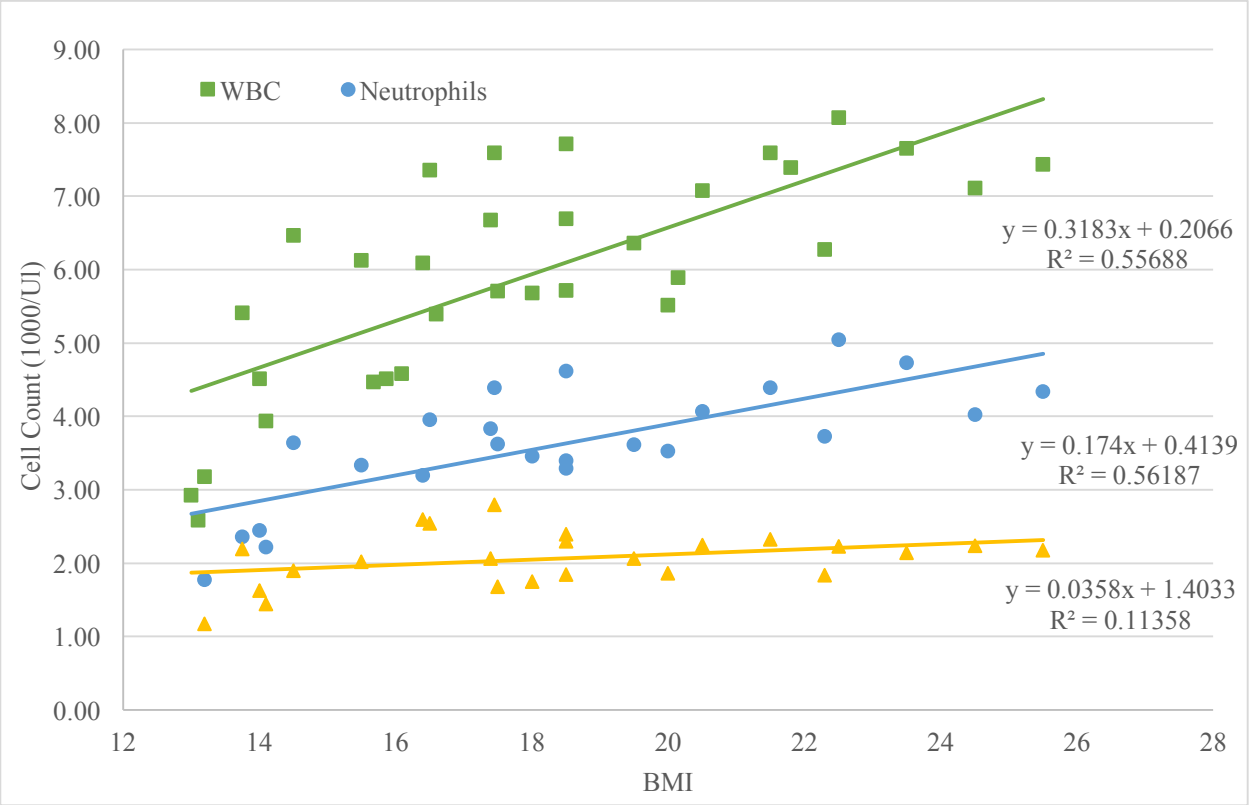
Figure 6. Body mass change over the course of mouse model for calorie restricted and ad libitum mice.

Body mass change in percent of baseline. Baseline was calculated as average body mass for each condition on day 0 of mouse model. Body mass for each conditioned was calculated on day 10 of mouse model and the percent change was found. Calorie restricted mice (ABA and FR) steadily decreased body mass before reaching a plateau around day 9 and 10. Ad libitum (WH and HC) both steadily increased body mass throughout the mouse model.

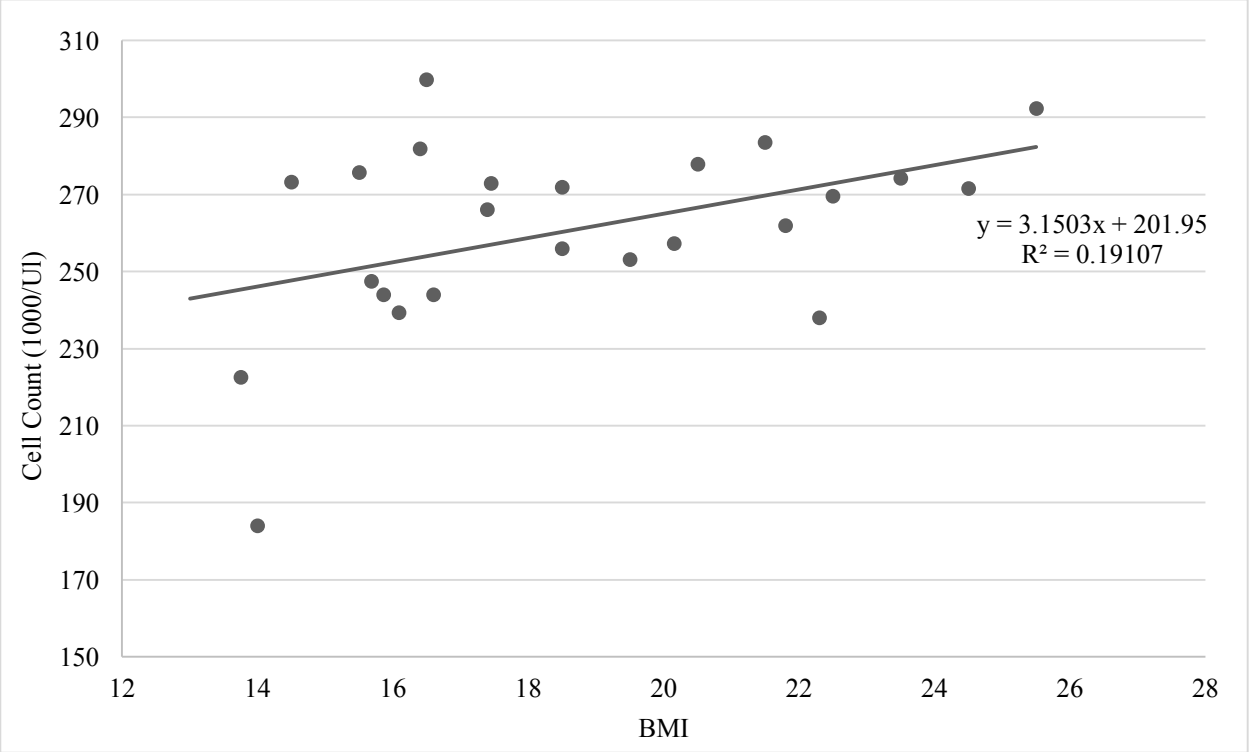
Figure 7. Immune and non-immune parameters plotted against body mass index (BMI).

WBC, neutrophil, lymphocyte, platelet, RBC count and hemoglobin values for subjects with a BMI between 13-25. Figure 7A and B show a correlation between WBC, neutrophil, lymphocyte and platelet count with BMI, but not for BMI and lymphocyte count. Figure 7C shows no correlation found between non-immune parameters, RBC count and hemoglobin value with BMI.

A



B



C

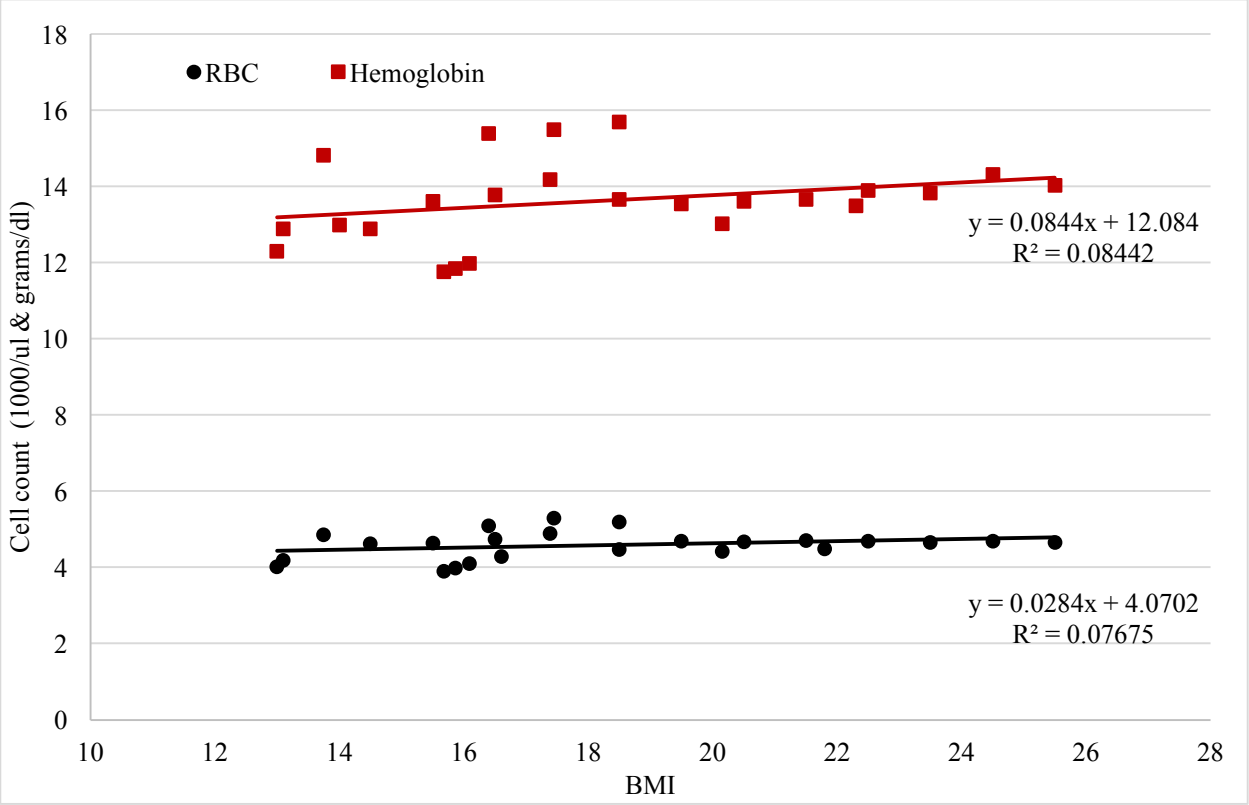
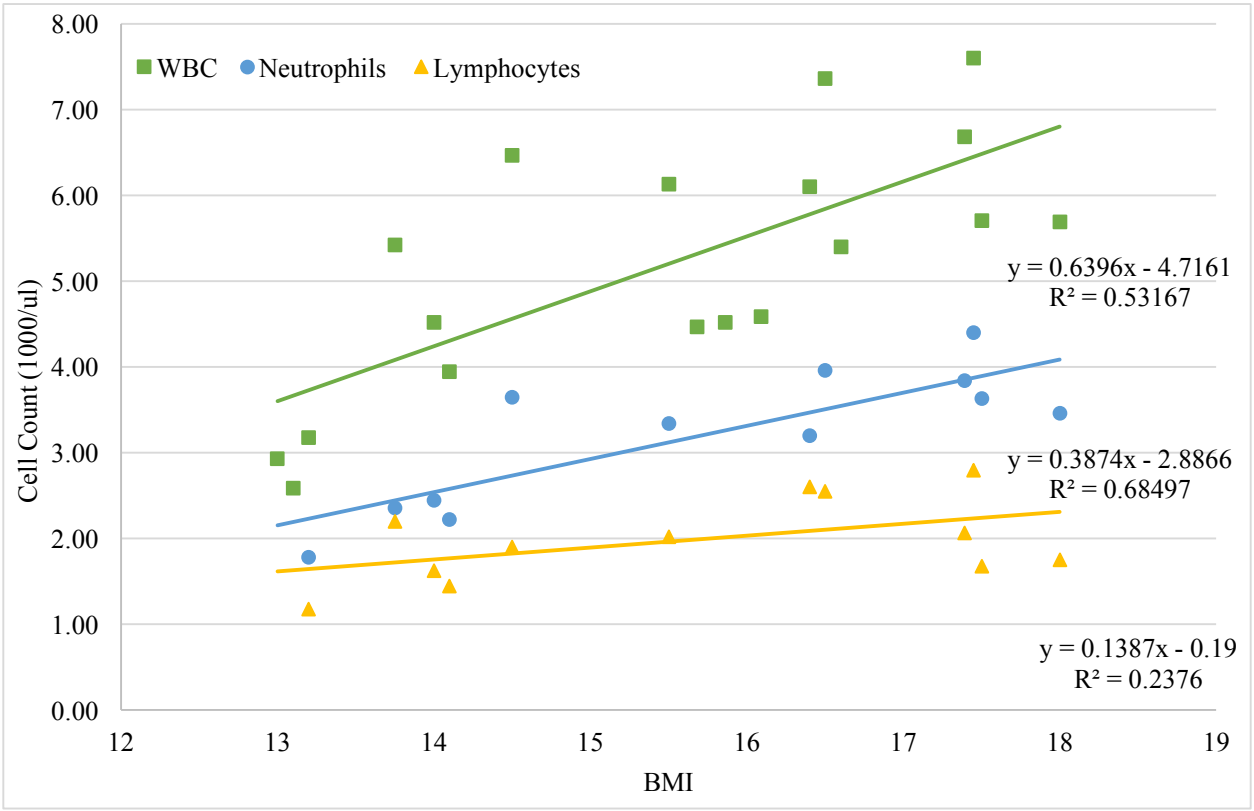


Figure 8. Immune and non-immune parameters plotted against underweight BMI ((<18.5 kg/m^2).

WBC, neutrophil, lymphocyte, platelet, RBC count and hemoglobin values for subjects with an underweight BMI (<18.5 kg/m^2).
Figure 8A and B show a correlation between WBC, neutrophil, lymphocyte and platelet count with underweight BMI. There was no correlation between BMI and lymphocyte count.
Figure 8C shows no correlation found between the non-immune parameters, RBC count and hemoglobin value with underweight BMI.

A



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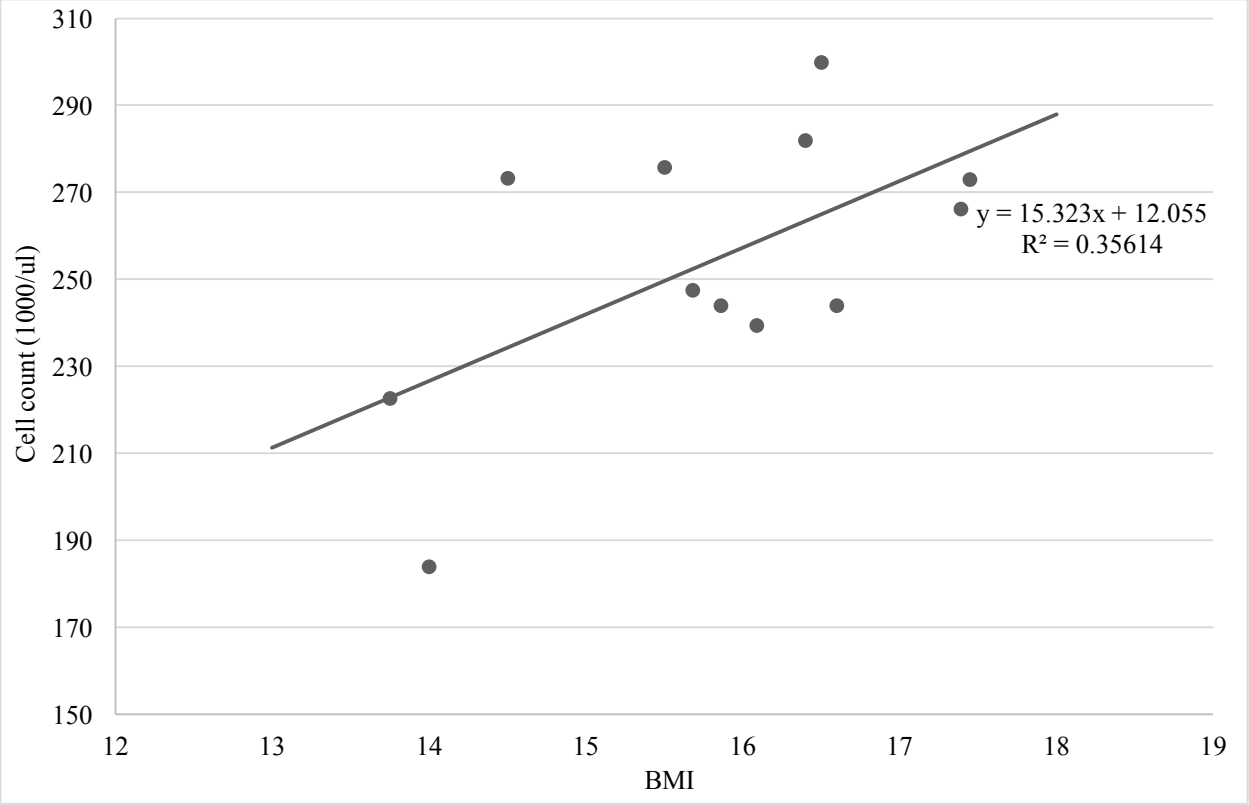


C

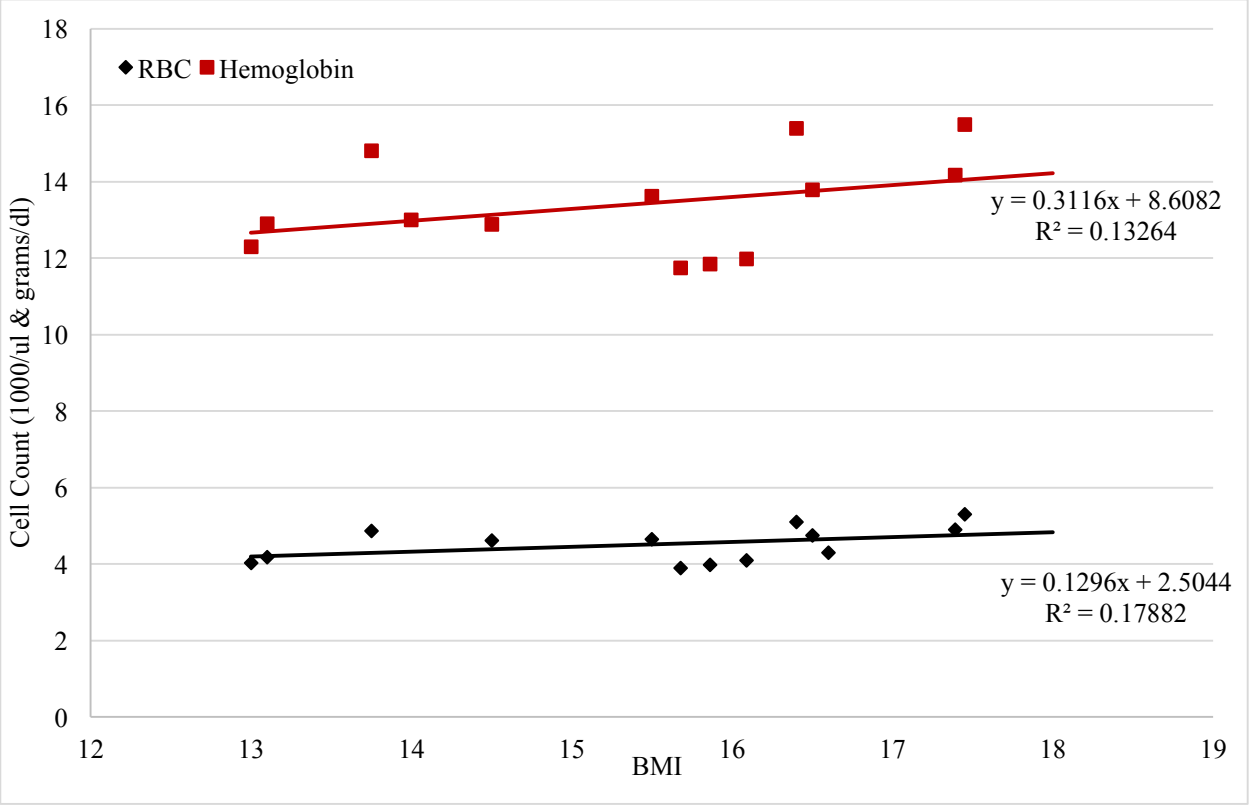
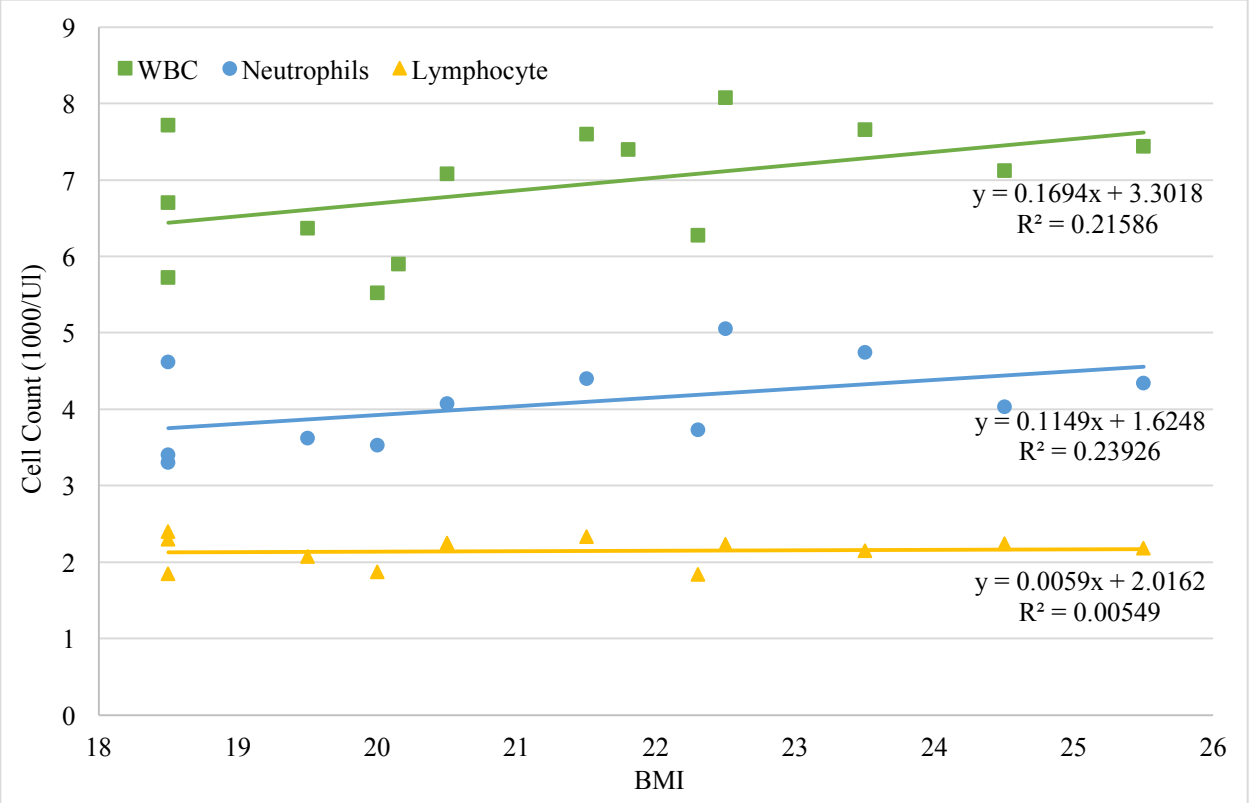


Figure 9. Immune and non-immune parameters plotted against normal BMI (18.5-24.6 kg/m^2)

WBC, neutrophil, lymphocyte, platelet, RBC count and hemoglobin values for subjects with a normal BMI (18.5-24.6 kg/m^2). Figure 8A, B and C shows no correlation between immune and non-immune parameters and normal BMI.

A



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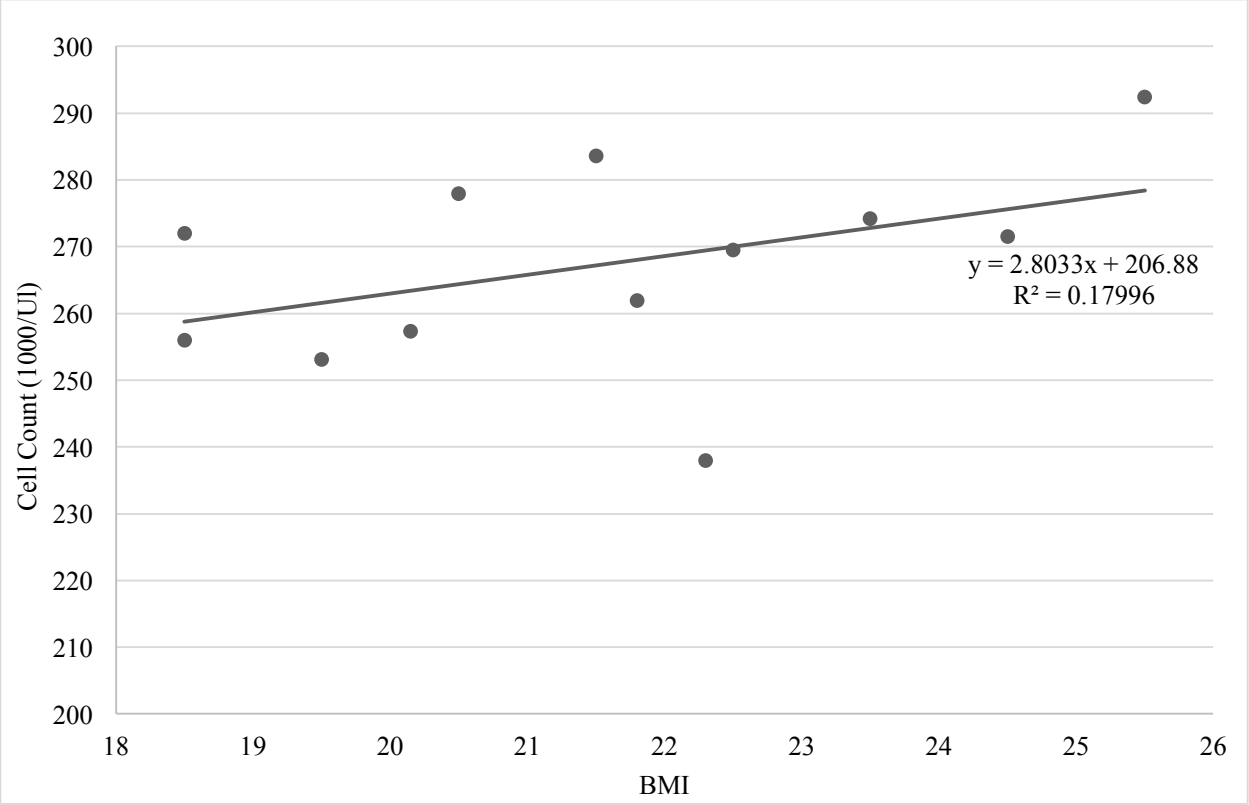
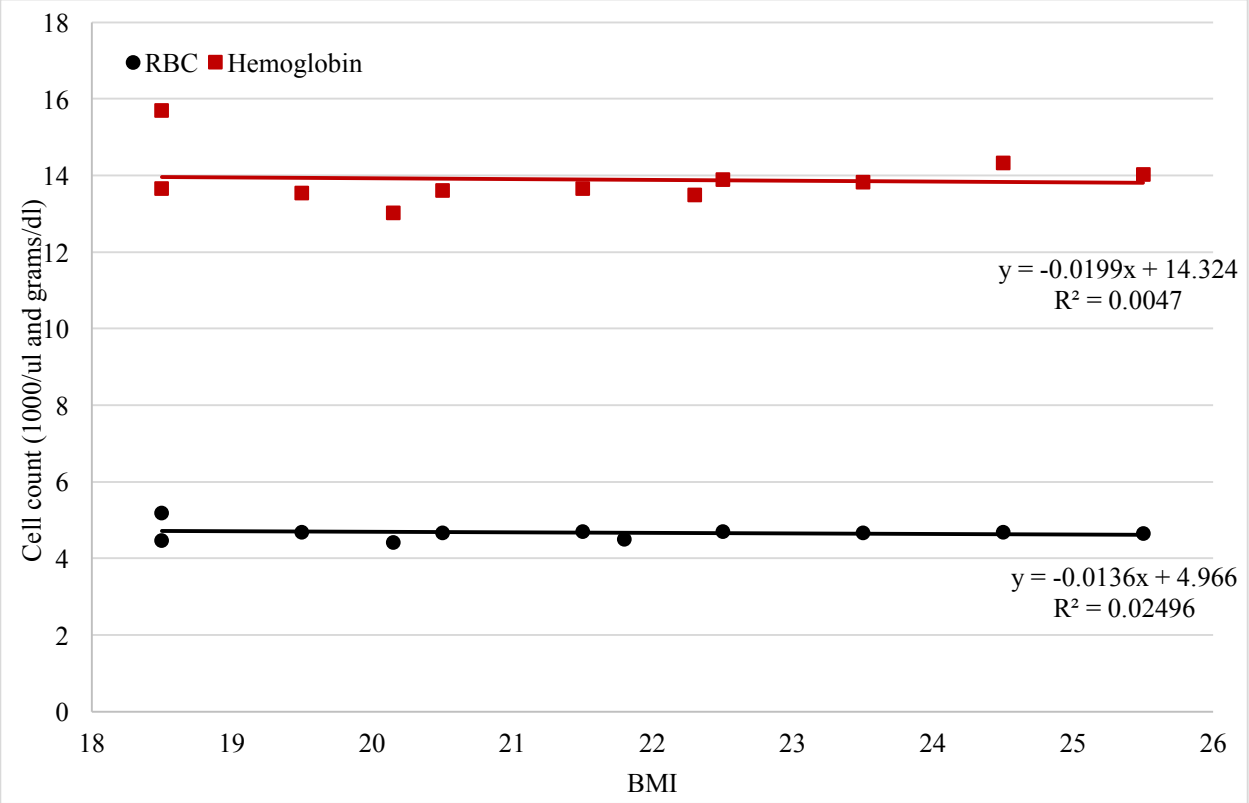


C



Tables

Table 1: Mice conditions by food intake and physical activity.

	Physical Activity	
Food Availability	Low (Locked Wheel)	High (Wheel)
Low (2hrs/day)	Food Restricted - FR	Activity Based Anorexia - ABA
High (Ad libitum)	Home Cage - HC	Wheel Housed - WH

Table 2: Mean dry tissue weights (g) of all organs across conditioned groups on day 10 of mouse model. Values are means ± standard deviations.

	Calorie Restricted		Baseline	Ad Libitum	
	ABA n=8	FR n=8	n=8	WH n=8	HC n=8
Liver	0.1711 ± 0.0129	0.1722 ± 0.0189	0.2072 ± 0.0146	0.2061 ± 0.0257	0.2051 ± 0.0289
Heart	0.0199 ± 0.0012	0.0194 ± 0.0011	0.0259 ± 0.0031	0.0236 ± 0.0025	0.0237 ± 0.0028
Spleen	0.0060 ± 0.0007	0.0059 ± 0.0007	0.0124 ± 0.0022	0.0098 ±0.0014	0.0114 ± 0.0025
Kidneys	0.050 ± 0.005	0.0476 ± 0.0019	0.0619 ± 0.0047	0.0597 ± 0.0059	0.0600 ± 0.0058
Brain	0.108 ± 0.029	0.1016 ± 0.0151	0.0897 ± 0.0039	0.1027 ± 0.021	0.0966 ± 0.0176

Table 3: Descriptive statistics for mouse model per condition.

	Food Restricted (N=16)		Baseline (N=8)	Ad Libitum (N=16)	
	ABA	FR		WH	HC
Body mass Day 0 (g)	17.12 ± 1.06	17.07 ± 1.53		17.68 ± 1.43	17.31 ± 1.51
Body mass Day 10 (g)	13.60 ± 1.34	13.41 ± 1.21	18.33 ± 0.61	18.23 ± 1.53	17.91 ± 1.40
Final body mass percent of baseline (100%)	74.2 ± 7.3 %	73.1 ± 6.1%		99.5 ± 8.3%	98.0 ± 8.0%
Food intake (g/day)	1.41 ± 0.17	1.29 ± 0.12	3.65 ± 0.45	3.37 ±0.34	3.37 ± 0.31
Water intake (µl/day)	2.55 ± 0.24	2.37 ± 0.20	4.75 ± 0.80	3.60 ± 0.30	3.82 ± 0.44
Wheel running activity (rev/day)	536± 344			549± 436	

Table 4: Effect of BMI, Normal BMI and Underweight BMI on immune and non-immune parameters.

	BMI				Normal BMI				Underweight BMI			
Parameter	Mean ± SD	df	r^2	P	Mean ± SD	df	r^2	P	Mean ± SD	df	r^2	P
White Blood Cells	5.93 ± 1.4	1,30	0.542	<0.001	6.89 ± 0.80	1,12	0.150	0.094	5.18 ± 1.45	1,16	0.502	<0.001
Neutrophil	3.63 ± 0.8	1,22	0.542	<0.001	4.06 ± 0.56	1,10	0.163	0.106	3.19 ± 0.80	1,10	0.653	<0.001
Lymphocyte	2.06 ± 0.3	1,22	0.073	0.107	2.14 ± 0.19	1,10	-0.093	0.081	1.98 ± 0.48	1,10	0.161	0.107
Red Blood Cells	4.58 ± 0.3	1,22	0.035	0.190	4.67 ± 0.20	1,9	-0.083	0.643	4.51 ± 0.45	1,11	0.104	0.150
Hemoglobin	13.61 ± 1.0	1,22	0.042	0.168	13.89 ±0.68	1,9	-0.105	0.841	13.38 ± 1.29	1,11	0.053	0.221
Platelet	260 ± 24.6	1,22	0.154	0.032	267.32 ± 14.84	1,10	0.097	0.169	254 ± 31.02	1,10	0.291	<0.05

Table 5: Effect of physical activity on organs mass on day 10 of mouse model.

Organ	df	r^2	P
Spleen	36	0.648	0.964
Kidney	36	0.665	0.414
Liver	36	0.629	0.858
Heart	36	0.573	0.775
Brain	20	0.075	0.503

Table 6: Descriptive statistics of published data.

Authors	Year	Subject Population	Number of subjects	Parameters measured	Method of cell measure	Mean Age	Mean BMI
Nishida and Sakakibara	2008	Aichi, Japan	114	WBC, neutrophil and lymphocyte	Automated, microscopically	33.3 ± 3.8 years	20.7 ± 3.0 kg/m^2
Takele et al	2016	Gondar, Ethiopia	17	WBC, neutrophil lymphocyte RBC, hemoglobin value and platelet count	Automated and flow cytometry	N/A	16.1±0.2 kg/m^2
Nagata et al	1999	Osaka, Japan	13	WBC, neutrophil, and lymphocyte	Flow cytometry	22.05 ± 6.0 years	13.65 ± 0.9 kg/m^2
Lambert et al	1997	Brussels, Belgium	10	WBC, neutrophil lymphocyte RBC, hemoglobin value and platelet count	Automated	17.2 ± 0.7 years	14 ± 0.5 kg/m^2
Kim et al	2013	Seoul, South Korea	67	WBC, RBC, hemoglobin value and platelet count	Automated	25.62 ± 6.02 years	15.86 ± 1.93 kg/m^2
Misra et al	2014	Massachusetts, USA	60	WBC, RBC and platelet count	Automated	15.8± 1.6 years	16.6 ± 1.4 kg/m^2
CDC: National Health and Nutrition Examination Survey	1998	USA	303	WBC, neutrophil lymphocyte RBC, hemoglobin value and platelet count	Automated	34.82± 14.49 years	20.50± 3.50 kg/m^2

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