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MILK THISTLE SUPPLEMENTATION AND EXERCISE TO INFLUENCE BILIRUBIN AND BODY WEIGHT OUTCOMES

Don Arthur Matutina
University of Kentucky, arthurdonleto@gmail.com
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Don Arthur Matutina, Student

Dr. Kyle Flack, Major Professor

Dr. Dawn Brewer, Director of Graduate Studies

MILK THISTLE SUPPLEMENTATION AND EXERCISE TO INFLUENCE BILIRUBIN AND BODY WEIGHT OUTCOMES

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in Nutrition and Food Systems in the College of Agriculture, Food and Environment at the University of Kentucky

By

Don Arthur Matutina

Lexington, Kentucky

Director: Dr. Kyle Flack, Professor of Dietetics and Human Nutrition

Lexington, Kentucky

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ABSTRACT OF THESIS

MILK THISTLE SUPPLEMENTATION AND EXERCISE TO INFLUENCE BILIRUBIN AND BODY WEIGHT OUTCOMES

Background: Traditional obesity treatment is ineffective as rates are still on the rise, thus necessitating novel treatments. Plasma bilirubin, once thought as only a clinical marker of liver disease, is now considered an important hormone, correlated with better outcomes within certain metabolic disease states such as cardiovascular disease and diabetes. This is largely due to bilirubin's role in promoting fatty acid oxidation via its interaction with PPARα and its antioxidant capacities to reduce lipid peroxidation. Possible methods of mildly increasing plasma bilirubin may provide a novel obesity treatment. Objective: To evaluate if Silmarin supplementation via milk thistle, exercise, or a combination of the two can increase plasma bilirubin. This study also tested the hypothesis that increases in plasma bilirubin are correlated with decreases in body fat mass. Methods: Male and female adults with obesity (BMI > 30.0, N=19) were split into 4 groups and participated in a 12-week intervention. Participants were assigned either Milk Thistle or a Placebo supplement condition, and either an exercise (participate in a 12-week aerobic exercise program) or sedentary control (engage in no structured exercise) condition. Fasting blood samples were collected and body composition assessments (BodPod) were performed pre and post intervention. Results: Trends were observed for the Silmarin supplementation + Exercise group for increasing plasma bilirubin and decreasing fat mass. Increased levels of bilirubin were moderately correlated with decreasing fat mass. Conclusions: In this small sample size, there are trends demonstrating ways to increase bilirubin through supplementation and that increasing bilirubin promotes decreases in body fat mass.

KEYWORDS: Bilirubin, Exercise, Milk Thistle, Obesity Treatment

Don Arthur Matutina

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Date

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By
Don Arthur Matutina

Dr. Kyle Flack
Director of Thesis

Dr. Dawn Brewer
Director of Graduate Studies

06/30/2023
Date

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CHAPTER 1 Introduction

1.1 Bilirubin and Chronic Diseases

Obesity has been on a steady rise the last few decades. Through 2017 – March 2020, US obesity prevalence increased from 30.5% to 41.9%. During the same time, the prevalence of severe obesity increased from 4.7% to 9.2% according to the Centers of Disease Control and Prevention (CDC) [1]. Obesity has been linked to increased risk in developing cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), stroke, osteoarthritis, and some cancers. However, it is not the actual excess amount of adiposity that is to blame for all these comorbidities. In fact, metabolically healthy obese (MHO) individuals show no adverse signs in glucose levels and insulin sensitivity, lipid metabolic markers, and hypertension[2]. The true culprit of unhealthy obesity is excess visceral fat deposits that harmfully change metabolic processes. For instance, cardio metabolic risk factors are present in those with greater visceral adiposity[3], which steadily progresses to metabolic syndrome. Since obesity is still on the rise, novel interventions must be developed and examined. New information regarding bilirubin offers promise for novel obesity treatment strategies due to its anti-oxidative and hormone-like properties. Bilirubin is a byproduct of hemoglobin breakdown in the spleen[4] and recent findings have shown that there may be possible metabolic implications, such as increasing fatty acid oxidation.

Bilirubin synthesis is the result of hemoglobin breakdown and heme release. Heme oxygenase then catalyzes the conversion into biliverdin. Biliverdin, by the way of biliverdin reductase produces bilirubin. Bilirubin is further glucuronidated into bile to be secreted out of the body. Traditionally, bilirubin has been utilized as a marker for liver disease or progression, normal levels being 0.2 to 2.0 mg/dL and liver diseases values being above 3.0 mg/dL, but there is currently evidence demonstrating physiologically elevated levels of bilirubin is indicative of positive metabolic outcomes[5].

A study published in Diabetic Medicine uncovered a positive correlation between blood serum levels of bilirubin and C-Peptide, an indicator of insulin synthesis[6]. The prevalence of Diabetic Neuropathy in regard to total bilirubin levels has also been investigated, demonstrating a strong negative correlation between total bilirubin and prevalence and severity of Diabetic Neuropathy[7]. However, it is not clear if it is diabetes that causes low bilirubin, or vice versa. Diabetes is not the only chronic disease linked to plasma bilirubin levels.

Heart disease is a common comorbidity associated with obesity and, according to the CDC, is the leading cause of death in the United States[8]. A longitudinal study demonstrated a significant negative relationship between baseline bilirubin and CVD[9]. An interesting study also looked into the protective effects of Gilbert's syndrome, a disorder characterized by mild hyperbilirubinemia, demonstrating that elevated bilirubin has beneficial effects on platelet aggregation and better CVD outcomes[10]. The literature is clear that elevated bilirubin has a negative association with CVD and T2DM.

In regards to obesity, a strong relationship between bilirubin and BMI has been established[5.] Jenko-Praznikar et. al. performed a cross sectional study that looked at serum bilirubin levels; and body composition, demonstrating lower amounts of bilirubin in those that exhibited abdominal obesity[11]. Another study focused on both obesity and T2DM demonstrated a negative association between serum bilirubin vs obesity and T2DM[12]. Elucidating the functions of bilirubin may be a way to describe the relationship between disease states and bilirubin, starting with its most common function, that of an antioxidant.

1.2 Bilirubin as an Antioxidant

Bilirubin has been a known antioxidant with its effect mainly in lipid peroxidation. Lipid peroxidation, in short, is the chemical change of fat through oxidation within the body generating highly reactive free radicals. Not only does lipid peroxidation change the way that fat works, it also stimulates the immune system and thus promotes a low grade-inflammatory response[13]. In fact, obesity's root cause in developing comorbidities stems from the chronic low-grade

inflammatory response often accompanying obesity. Not only is the body now in a state of inflammation, but fat cells are also compromised and fatty acid oxidation is more difficult. This becomes a repeating cycle of increased fat accumulation and decreased fat oxidation[14]. Bilirubin effectively changes this by being an electron donor, quenching free radicals that promote lipid peroxidation. By nullifying said oxidation, fatty acid oxidation can occur in normal circumstances. This is a possible pathway that bilirubin plays an important role in that may influence obesity. By taking the excess electron, bilirubin prevents the cascade of negative effects that occur with unmitigated fat oxidation[15]. This has been directly tested, demonstrating when bilirubin is decreased, an increase in oxidant induced cell death occurs[16, 17]. However, bilirubin's effect on fat does not only fit within the context of an antioxidant, but of that of a hormone.

1.3 Bilirubin as a Hormone and PPARα

A hormone is a molecule that is transported and acts upon a cell, specifically by binding to target receptors. Bilirubin is transported through the blood and activates proliferator-activated receptor alpha (PPARα) inside the cell[5, 18]. PPARα is a nuclear receptor that when activated creates a cascade effect that increases fatty acid oxidation by induction of certain key enzymes that promotes β-oxidation in the mitochondria and releases Fibroblast Growth Factor 21 (FGF21)[19]. FGF21 is a hormone that helps with insulin sensitivity within adipocytes which promote glucose uptake. Therefore, increasing the amount of bilirubin within the system may help with increasing the amount of expression of PPARα, FGF21, and thus fatty acid oxidation.

1.4 Bilirubin Synthesis and Buildup

Bilirubin, Heme Catabolism, and Exercise

As stated before, bilirubin is synthesized within the spleen by the breakdown of hemoglobin. Heme, a product of hemoglobin lysis, is further broken down into iron, carbon dioxide, and biliverdin by heme oxygenase. Biliverdin is reduced into bilirubin by biliverdin reductase. When donating an electron, bilirubin is turned back into biliverdin. Bilirubin is in an unconjugated state which means that it is not water soluble. Conjugation occurs when bilirubin in

the liver is acted on by uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) by binding glucuronic acid to the bilirubin[5]. This allows the conjugated bilirubin to be able to be excreted into the small intestine which is excreted further through feces. Exercise has been shown to increase bilirubin through the increase of heme oxygenase. Furthermore, exercise has shown to also increase the amount of hepatic biliverdin reductase A, a type of biliverdin reductase found in the liver, and reduces the expression of UGT1A1[20]. Finally, exercise is also known and has been shown to increase red blood cell catabolism through various means, such as elevated core temperatures, repeated foot strikes, and skeletal muscle breakdown[21], thus increasing heme availability and thus bilirubin synthesis[22].

This has a few implications. The most obvious is that increasing energy expenditure through exercise increases fatty acid oxidation and glucose uptake through various pathways involving insulin, epinephrine, and insulin like growth factor 1. But the effect of increasing plasma bilirubin may offer another benefit independent of exercise[22]. By increasing heme oxygenase and biliverdin reductase, the amount of new bilirubin increases through increasing heme breakdown and biliverdin reduction. UGT1A1, the enzyme that conjugates bilirubin and thus allows it to be turned into waste, is reduced with exercise, thus reducing bilirubin clearance to increase plasma bilirubin accumulation[23]. These pathways are consistent with the elevated bilirubin levels among leaner populations that have greater levels of physical activity. Exercise, however, is not the only method that may promote greater bilirubin levels.

Bilirubin and Milk Thistle

Another way that has been shown to increase the amount of bilirubin in the system is through supplementation of Milk Thistle (*silybum marianum*), which decreases UGT1A1[5]. Milk thistle contains silymarin flavonoids, which are composed of various other subsets of molecules that include silydianin, silybin A, silybin B, isosilybin A, and isosilybin B traditionally used as a supplement to help with liver disease[24]. These flavonolignans are also prime candidates of conjugation. In other words, these molecules use up the available UGT1A1 and glucuronic acid,

as shown in study done by Mohamed et. al. [25], which gives it less chances to act upon bilirubin to conjugate it. This in turn increases the amount of bilirubin within the blood.

Bilirubin and the Gut Health

Looking back at the life cycle of bilirubin detailed in the "Bilirubin, Heme Catabolism, and Exercise" section, bilirubin ends its life conjugated, in the small intestine and ready to be excreted out through feces. This process begins in the liver where bilirubin is conjugated by the addition of glucuronic acid by the actions of UGT1A. The newly conjugated bilirubin is then released from the liver to join bile within the biliary system[26]. This system puts bile, which contains the conjugated bilirubin, into the gut where the bilirubin feeds the gut microbiota and turns it into urobilinogen. These urobilinogens are either reabsorbed through biliary flow, or is excreted out through feces[26, 27]. Depending on the available bacteria within the gut, the amount of urobilinogen increases[28].

1.5 Bilirubin for Obesity Treatment

As stated, bilirubin has been shown to have a plethora of functions and effect on various metabolic processes and so can be considered as a viable way of reducing the negative impacts of obesity[29] Currently, bilirubin is used as a biomarker to indicate liver health. However, with the current line of new research being done on bilirubin, it can be utilized as a novel treatment or in conjunction with traditional obesity treatment. The research noted truly starts with bilirubin having a negative correlation with obesity [12], CVD [9], and T2DM [6], prompting additional research into the molecular mechanisms at play.

The effect of bilirubin as an antioxidant is the first step in utilizing this as a possible synergistic treatment for obesity. Obesity is characterized by low levels of inflammation due to unmitigated oxidation. Biliverdin and bilirubin serve as the two molecules that effectively make bilirubin an antioxidant with bilirubin being the electron donor thus turning back into biliverdin. The importance of antioxidant function in the body cannot be understated. The metabolic pathway of ROS and natural occurring antioxidants are a necessary part of everyday bodily function.

Increasing the amount of bilirubin will provide the body with a natural way for ROS and oxidative stress to be eliminated or diminished. Within obesity, the amount of inflammation through ROS is increased and increasing bilirubin may be a helpful way to improve the metabolic health of those classified as obese.

The other way that bilirubin treatment can affect the body is its use as a hormone. PPARα is a nuclear receptor that promotes transcription of genes responsible for increasing energy expenditure and is activated by bilirubin. In fact, PPARα's main purpose is increasing the amount of fatty acid beta oxidation that occurs within liver and muscle cells[30]. The benefit of liver cells increasing beta oxidation is that fat has a smaller chance to accumulate, which helps reducing the likelihood of fatty liver disease. PPARα activation in muscle cells also have a positive effect on insulin sensitivity[29]. This might be a pathway that explains the negative correlation of bilirubin with obesity and diabetes.

Exercise is probably the most natural way to increase bilirubin production through hemoglobin lysis. Breakdown of hemoglobin, as stated above, occurs with exercise and increases the amounts of heme, which increases the amount of bilirubin as heme disposal goes through the bilirubin synthesis pathway[21].

Milk thistle has also shown to be helpful in other ways from increasing the amount of bilirubin. Silymarin compounds increase the amount of bilirubin by decreasing the amount of glucuronic acid able to be used to conjugate bilirubin. However, silymarin compounds also have anti-inflammatory, antioxidant, and antifibrotic[31] natures that make it an additionally positive method for increasing plasma bilirubin.

1.6 Gaps

Currently, there are no randomized, controlled clinical trials evaluating how exercise and/or silymarin supplementation can influence plasma bilirubin levels among individuals classified as obese. It is also uncertain if changes in plasma bilirubin associated with these

interventions promote decreases in fat mass loss as hypothesized based on bilirubin's interaction with PPARα.

1.7 Conclusion

Bilirubin as a player in obesity treatment, is a complex but intriguing subject. Its relationship with CVD, T2DM, and obesity shows an interaction that point towards mild hyperbilirubinemia being a positive aspect of health. Going forward, looking at the possible ways to safely increase serum level of bilirubin may be a viable treatment for obesity.

CHAPTER 2 Materials and Methods

2.1 Methods

Study Design

Table 1 Groups	
Exercise w/ Placebo (Ex) N=6	No Exercise w/ Placebo (CON) N=5
Exercise w/ Milk Thistle (MT+Ex) N=5	No Exercise w/ Milk Thistle (MT) N=3

This is a randomized double-blind clinical trial. The participants were randomized into one of four groups (Table 1). The University of Kentucky Institutional Review Board approved this study, which is also registered in clincaltrials.gov, NCT 04717726.

Setting

The setting of the study is within the University of Kentucky. The College of Agriculture's Department of Dietetics and Human Nutrition's Performance Nutrition and Body Composition Lab was the site for baseline and post-testing measurements, weekly exercise download meetings, and weekly supplement pickups. Blood draws were performed by trained research nurses of the University of Kentucky's Center for Clinical and Translational Science (CCTS). Participant recruitment efforts included flyers posted in various building within the University of Kentucky Campus and through advertisements through the CCTS. Participants were compensated $250 if they remained in the study and completed all pre and post testing assessments. Due to the pilot nature of this study and the limited funding, the goal sample size was 5 for each group or 20 total.

Participants

The inclusion criteria for the study are those with a BMI of 30 or greater between the ages of 18 to 50. The BMI of 30 was set as the study aims to evaluate obesity treatment. The minimum age of 18 was set as it is the minimum age of adult permission, and the max age of 50 was set to limit any potential effects advanced age may have on our outcomes. Participants were also not engaging in any exercise, assessed by a 7-day acti-graph accelerometer assessment prior to beginning the intervention. Additional exclusion criteria are as follows:

- Taking medication that affect energy expenditure (thyroid medication)
- Have a chronic cardiovascular, pulmonary, or endocrine disease (CVD, diabetes, COPD, cystic fibrosis)
- Use tobacco
- Any conditions that prevent safety in exercising
- Are currently taking probiotic or other herbal supplements.
- Pregnant or lactating

2.2 Interventions

The non-exercise groups were provided a one-week supply of the assigned supplement to be taken morning and night with a log to help them keep track. These were returned weekly and exchanged for a blank log sheet and a new bottle of the supplement. Participants were placed on either the milk thistle or placebo supplement. The Placebo pill consisted of 600 mg of maltodextrin in gelatin capsules assembled within the Department of Human Nutrition Performance Lab utilizing a tampering pill assembly. The Milk thistle supplement consisted of 1300 mg of a milk thistle (GNC Herbal Plus) standardized for 50% silymarin, the active compound of milk thistle responsible for increasing bilirubin.

The two exercise groups followed the same intervention, performing 5 independent aerobic exercise sessions weekly, totaling 2500 kcals or 500 kcals per session. All exercise sessions were required to be aerobic based (running, walking, cycling, etc.) and not resistance training based. Exercise sessions were recorded via Polar A300 watch, that estimates energy expenditure taking heart rate and individual characteristics (height, weight, age, sex, training status) into account. The Polar watch was chosen for its availability of heart monitors as well as its ease of use. The A300 connects to a computer via USB to download metrics of each exercise session (duration, intensity, energy expenditure).

2.3 Measurements

After informed consent was obtained, participants were provided an Actigraph accelerometer to objectively assess physical activity, specifically Moderate to Vigorous Physical Activity, and Vigorous Physical Activity (MVPA and VPA) for 7 total days. Participants were cleared to begin the intervention if their 7 day VPA did not reach the threshold of 90 minutes. The participant then completed the Dietary Health Questionnaire (DHQ-III), a validated food frequency questionnaire to evaluate habitual dietary behaviors [33, 34, 35]. Outcomes of interest were servings of fruits and vegetables as various polyphenols from these foods could be considered a confounding variable.

Baseline and post-intervention assessments of body composition were performed via air displacement plethysmography (BodPod) utilizing the Siri model of equation with a thoracic gas volume measurement. The BodPod calculates total body density to ascertain fat free mass and fat mass, while the Siri equation was used due to its accuracy with the obese population[32]. Measuring thoracic gas volume was done for a more accurate measurement (opposed to equations calculating thoracic volume) and needed as thoracic cavity must be taken out of the total body density equation. Baseline and post-intervention blood draws yielded plasma and serum samples that were frozen for future analysis in addition to immediate processing that included a full Hemogram without differential to assess red blood cell count and a hepatic function panel (plasma bilirubin, ALT and AST enzymes levels).The post-intervention BodPod and blood draw were done 36-48 hours after the final exercise session of the intervention.

2.4 Statistical Analysis

Statistical analysis was performed with IBM SPSS statistical software. Group by time interaction for each outcome primary (Fat Mass, Body Fat Percentage, and Bilirubin) and secondary outcomes

Table 2 Secondary Measured Outcomes	
MCV	Mean Corpuscular Volume
HCT	Hematocrit
RBC	Red Blood Cell
HGB	Hemoglobin
MCH	Mean Corpuscular Hemoglobin

(table 2) of the 4 groups was performed with pre and post analysis of variance (ANOVA) analysis with LSD Post Hoc Analysis. Non-paired t-tests were used to test for differences between groups at baseline and if there was any need to account for these differences in ANOVA models. Percent change scores were calculated for the primary and secondary outcome measures and compared across groups and to zero (if these percent change scores were significantly different from 0 and each other) via 1-way ANOVA. Bivariate correlation analysis was performed between percent change variables.

CHAPTER 3 Results

2-way ANOVA

There was no group by time interactions observed for any outcome variable, including those listed in table 2.

Non-paired T-Tests

There were no differences in any outcome measure between groups at baseline. This included dietary analysis of servings of fruit, dark green vegetables, red or orange vegetables, and legumes (table 3).

Percent Change, 1-way ANOVA

There was a group effect for the FM percent change (p =.04) while percent body fat percent change trended towards significant (p = 0.06). For FM percent change, MT+Ex decreased 4.3 percent (p=0.12) , which was significantly different from the control (p=0.09) which increased 11.4 percent and the MT group (P=0.14) that increased 4.2 percent.

Table 3 – Baseline Values for Measured Outcomes

	MT	MT+Ex	Ex	CON
BMI	37.03	34.30	34.72	37.77
Bilirubin (mg/dL)	0.38	0.51	0.12	0.12
Fat Mass (kg)	51.23	39.79	37.72	47.25
Body Fat %	45.63	42.98	36.98	42.90
Fat Free Mass (kg)	59.19	52.69	64.09	61.91
MCV (fL)	85.67	89.20	86.40	87.67
HCT (%)	40.75	41.80	42.74	40.17
RBC (10*6/uL)	4.76	4.70	4.95	4.60
HGB (g/dL)	13.30	14.24	13.34	13.53
MCH (pg)	28.03	30.30	28.92	29.47
ALT (U/L)	18.33	39.80	23.80	43.00
AST (U/L)	17.00	25.00	19.80	27.00
VPA (minutes)	0.17	1.6	7.4	0
Fruit (Cups)	0.99	0.29	0.48	0.73
Dark Green (Cups)	0.24	0.12	0.33	0.13
Yellow/Orange (Cups)	0.21	0.11	0.23	0.10
Legumes (Cups)	0.11	0.07	0.04	0.02

The MT+Ex group demonstrated trends for increasing bilirubin levels (48.10%, p = 0.31). The Ex group demonstrated a trend for decreasing bilirubin percent change, Ex (-16.67%, p = .07), while control decreased bilirubin percent change (-31.00%, p = .03).

Percent change values for all primary and secondary outcomes are listed in appendix 1.

Bivariate Correlation

Moderate correlations were observed between bilirubin percent change versus fat mass percent change and versus percent body fat percent change β -.329 and -.326 respectively. This indicates increasing bilirubin is associated with decreases in body fat.

CHAPTER 4 Discussion

Bilirubin

The present study aimed to increase bilirubin and determine if increasing bilirubin promotes body fat loss. This is the first human study to attempt at increasing bilirubin through exercise and sylamarian supplementation and assessing fat mass loss, with prior research conducted in animal models or using a cross sectional design. The degree of change shows a trend of increasing bilirubin within the MT+Ex. However, MT, or Ex alone was not able to increase bilirubin levels. The control group showed greatest trends at decreasing bilirubin.

The Ex group most likely did not increase in bilirubin level because the level of exercise did not coincide with the high levels of exercise that may be required to stimulate red blood cell lysis and heme release. This was demonstrated in the lack of change in HCT, RBC, and HGB. This could be due to the much broader instruction of achieving 500 kcals per session in an aerobic setting as these sessions may not have been intense enough to elicit red blood cell lysis. It is possible that intense exercise levels were not achievable in this population and thus not an appropriate method, by itself, to increase plasma bilirubin. This contrasts with some reviews and studies that did see an increase in bilirubin levels within athletes [21, 22]. This difference may be a matter of intensity.

The Milk Thistle not increasing bilirubin is a direct opposite of current published studies. One reason for this may be caused by the diet of the participants, since these participants were a free-living population, and thus may consume foods that contain polyphenols that could interfere with the milk thistle. However, the HHQ demonstrated no differences in fruit and vegetable consumption of the participants between groups. It is possible that supplementation with milk thistle alone was not enough to increase bilirubin due to either an inadequate dose or short study duration. The addition of exercise, however, has a positive effect on bilirubin. It seems silymarin supplementation or exercise alone did not exert increases in plasma bilirubin but when combined, these interventions are enough to promote these changes.

It is important to note that ALT and AST did not increase within the study. Traditionally, bilirubin has been used within clinical diagnosis to determine the status of the liver. Increasing bilirubin through supplementation without increasing ALT and AST was a goal of this study to show that mildly increasing bilirubin is safe.

Bilirubin and Fat Mass

We hypothesized that increasing plasma bilirubin will induce greater fatty acid oxidation and result in greater fat mass loss. This was indeed demonstrated with the overall negative correlation between bilirubin and body fat percentage.

Significant changes in fat mass were observed in the MT+Ex group, which may be largely due to the exercise intervention, however, the same significant changes in fat mass were not observed with exercise alone, thus it appears milk thistle did exert some effect when combined with exercise, although no significant changes in fat mass were observed in the MT group alone.

4.1 Strength and Limitations

The strength of the study lies within the methods of data collection. The Bod Pod is a validated and reliable assessment for body composition while the blood draw panels are used daily for medical examinations. Also, the double-blind nature of the study prevented bias. Limitations include the small sample size of each group. Due to the smaller sample size, getting a significant P-value was not feasible. This was the reason for using percent change to observe trends. Another limitation is the participants that volunteered for this study were a free-living population meaning there are variables that are not totally accounted for. Although this could also be considered a strength as our overall goal is to provide a treatment that can be translated to the general population. There was also minimal structure to the exercise regime other than the minimum 500 kcals and aerobic exercise. This means participants could do different modes (running, walking, elliptical, cycling, fitness class) and at different intensities. Finally, there were

metrics that could not be tested due to the invasive nature or expense of the necessary tests, such as testing for PPARα expression which would have required a biopsy.

Appendix

*Sig indicates 1 sample t-test testing if change score differs from zero

** Indicates change score is different from control (1 way ANOVA)

Bilirubin

	% Change	Sig*	Lower CI	Upper CI
MT	-2.78	0.90	-58.27	52.72
MT+Ex	48.1	0.31	-66.45	162.64
Ex	-16.67	0.06	-35.42	2.09
Control	-31.11	**0.03**	-56.41	-5.81

Fat Mass

	% Change	Sig*	Lower CI	Upper CI
MT	4.18	0.14	-1.87	10.22
MT+Ex	-4.33**	0.12	-10.43	1.77
Ex	-3.6**	0.22	-10.56	3.3
Control	11.4	0.09	-4.65	27.44

% Fat Mass

	% Change	Sig*	Lower CI	Upper CI
MT	.95	0.63	-3.86	5.76
MT+Ex	-3.22**	**0.05**	-6.45	.01
Ex	-2.80**	0.13	-6.95	1.35
Control	6.39	0.14	-5.09	17.87

Fat Free Mass

	% Change	Sig*	Lower CI	Upper CI
MT	1.77	0.06	-.058	3.60
MT+Ex	.89	0.46	-2.14	3.93
Ex	.46	0.46	-1.1	2.01
Control	.260	0.75	-2.77	3.29

MCV

	% Change	Sig*	Lower CI	Upper CI
MT	.92	0.23	-.79	2.64

MT+Ex	-.63	0.57	-3.15	1.9
Ex	.25	0.73	-1.64	2.14
Control	-.39	0.42	-2.09	1.3

HCT

	% Change	Sig*	Lower CI	Upper CI
MT	1.78	0.39	-3.07	6.63
MT+Ex	-.69	0.71	-5.4	4.04
Ex	1.12	0.61	-4.5	6.71
Control	.2.19	0.73	-26.26	21.88

RBC

	% Change	Sig*	Lower CI	Upper CI
MT	1.15	0.61	-4.32	6.62
MT+Ex	-.22	0.92	-5.63	5.2
Ex	.90	0.60	-3.52	5.31
Control	-2.4	0.70	-25.31	20.53

HGB

	% Change	Sig*	Lower CI	Upper CI
MT	.80	0.60	-2.9	4.51
MT+Ex	-1.00	0.54	-5.13	3.14
Ex	1.01**	0.67	-5.05	7.07
Control	-4.97	0.43	-26.91	16.98

MCH

	% Change	Sig*	Lower CI	Upper CI
MT	-.338**	0.75	-2.94	2.26
MT+Ex	-.71	0.32	-2.5	1.04
Ex	.178**	0.90	-3.5	3.85
Control	-2.7	**0.01**	-3.60	-1.79

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VITA

1. Associates Degree of Science in Culinary Arts from Sullivan University
2. Bachelors Degree of Science in Dietetics from the College of Agriculture, Food and Environment at the University of Kentucky
3. Personal Trainer, Chef, Valet Lead Guest Relations
4. Don Arthur Matutina