Will a Novel Organometallic Complex Mitigate the Effects of Hypertension in Rats Fed a High Fat Diet?

by

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A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved April 2018 by the
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ARIZONA STATE UNIVERSITY

May 2019

ABSTRACT

Background: Nearly 95% of Americans will develop hypertension, and 67% will not seek treatment. Furthermore, hypertension is the leading risk factor for coronary heart disease. While previous studies have increased the use of blood pressure medication among patients that have received hypertension education, medications may not work for everyone. Due to the life-threatening nature of this condition, it is essential to find an effective alternative for treatment. The purpose of this study was to examine the impact of organometallic complex supplementation on hypertension and left ventricular hypertrophy in 6-week old male Sprague-Dawley rats that were fed either standard rodent chow or a high fat diet for 10 weeks at a university in Arizona.

Methods: Forty-two healthy six-week old male Sprague-Dawley rats were randomly assigned to one of three groups: plain water control, 0.6 mg/ml organometallic complex or 3.0 mg/ml organometallic complex as soon as they arrived. Each rat was then housed individually to prevent the sharing of microbiota through coprophagia. Rats in each treatment group were further divided into two dietary groups that were fed either a high fat diet containing 60% kcal fat that was changed every three days or standard rodent chow. Researchers were not blind to which rat was in each group. At the end of the 10-week study, rats were euthanized with an overdose of sodium pentobarbital (200 mg/kg, i.p.). Heart, left ventricle of the heart, liver, and spleen masses were recorded for each animal. Data were analyzed by two-way ANOVA using SigmaPlot 10.0 software.

Results: At the conclusion of this study, the left ventricle mass of the rats in the high fat diet group were significantly larger than those in the chow group. Neither dose of the organometallic complex supplement prevented these effects induced by high fat feeding.

Conclusion: The organometallic complex supplement was not effective at mitigating the effects of a high fat diet on cardiac hypertrophy in rats. Therefore, this supplement should not be used to treat cardiac hypertrophy.

ACKNOWLEDGEMENTS

I would like to begin by thanking my Lord and Savior Jesus Christ for blessing me with the strenghth and wisdom that I needed to complete this thesis. I would not be able to do this without Him.

Second, I would like to thank my wonderful thesis committee. Dr. Sweazea, thank you for your guidance and for reading through my many versions of my thesis. you are a great mentor and I appreciate that you were always willing to answer my questions and provide quick yet substantial feedback. Dr. Whisner, thank you for also reading through my many versions of my thesis and providing extensive feedback that has helped me to become a better writer. I appreciate that you agreed to be a member of my thesis committee. Your expertise in vitamins and minerals was very helpful. Dr. Alexon, thank you for also reading through my many versions of my thesis and agreeing to be on my thesis committee. I appreciate your feedback and expertise in nutrition, they helped me to write the best thesis possible.

Last but not least, thank you to my parents who have always loved and encouraged me and have listened to me talk about my thesis for the past two years.

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CHAPTER 1

INTRODUCTION

Overview

Nearly 95% of Americans will develop hypertension, and 67% will not seek treatment (Wang & Vasan 2005). Furthermore, hypertension is the leading risk factor for coronary heart disease (Yeo et al. 2009). While previous studies have demonstrated the efficacy of blood pressure medication among patients who have received hypertension education (Ho et al. 2016; Saleem et al. 2015), medications may not work for everyone. Due to the life-threatening nature of this condition, it is essential to find an effective alternative for treatment.

There is a novel food grade supplement comprised of mineral powder known as an organometallic complex (OMC). Since it is extracted from soil, it contains minerals such as calcium, magnesium, sodium, potassium, sulfur, protein and fulvic acid. While the combination of minerals in this supplement may be beneficial for hypertension, it has not been tested in this capacity. Therefore, it is unclear whether there will be any effect of the compound on hypertensive symptoms. Furthermore, there are not enough clinical trials to test if nutritional supplements can elicit a decrease in hypertension and left ventricular hypertrophy in rats. A prior study by Deneau et al. (2011) showed that a similar organometallic complex effectively reversed major symptoms associated with diabetes including improved glucose regulation and decreased weight. However, this does not indicate that the organometallic complex used in the present study will prevent the heart, spleen and liver damage that are associated with diet-induced hypertension.

The Study Purpose

The purpose of this study was to examine the impact of OMC supplementation on hypertension, splenomegaly and left ventricular hypertrophy in 6-week old male Sprague-Dawley rats that were fed either standard rodent chow or a 60% kcal high fat diet for 10 weeks at a university in Arizona.

Null Hypothesis

Ten weeks of organometallic complex supplementation in young male Sprague-Dawley rats will not mitigate the left ventricular hypertrophy, splenomegaly, and hypertension that are associated with a consumption of a high fat diet.

Definition of Terms

- Hypertension: High blood pressure measuring 140/90 mm Hg (Yeo et al. 2009).
- Organometallic complex (OMC) – the novel soil-derived test compound used in the current study.

Delimitations

- Rats live in a controlled environment, so these results cannot be generalized for humans or animals not living in a controlled environment.
- Hypertension was associated with a high fat diet, so these results will not apply to people or animals who are genetically predisposed to hypertension.

Limitations

- Cannot measure changes in organ weight over time within intact rats

CHAPTER 2

REVIEW OF LITERATURE

Rodent model of diet-induced hypertension

Sprague-Dawley rats were used in this study due as they are commonly used in studies of diet-induced hypertension. For example, rats fed a high sucrose diet had the same significant impairment in vasodilation as rats fed a high fat diet (Sweazea et al. 2010). An additional study not only repeated these results but also indicated that a high fat diet is associated with hypertension in rats (Sweazea and Walker 2012). Crinigan et al. (2015) also established that renal mass and visceral fat increased in rats following a six-week high fat diet, which may indicate that chronic fat intake is needed in order to induce renal disease as markers of inflammation were not detected. The most recent study by Sweazea and colleagues used Sprague-Dawley rats to determine the effects of white sesame seed oil on renal function and found significant improvement in the health of their kidneys (Aslam et al. 2017). Given these previous results, male Sprague-Dawley rats are an ideal model in which to examine the efficacy of the organometallic complex at mitigating the deleterious effects of chronic high fat intake.

Hypertension

First, it is important to define what hypertension is. A healthy heart and arteries are able to expand to take the flow of blood in, which is often referred to as systolic pressure, and then they contract to push the blood out to the rest of the body, which is referred to as diastolic pressure (Franklin et al. 2008). Hypertension is diagnosed when

the diastolic blood pressure rises to at least 90 mm Hg or higher and the systolic blood pressure rises to at least 140 mm Hg or higher (Franklin et al. 2008). Because there are so many nonmodifiable risk factors for hypertension such as genetic predispositions effecting excretion of electrolytes (Firsov et al. 2012; Solocinski et al. 2015; Stow et al. 2011), dysfunctional renal regulation of vascular tone (Rettig 1993; Rettig and Grisk 2005), and preventing the conversion of angiotensin II to angiotensin III (Carey 2017), and modifiable risk factors such as consuming excessive amounts of sodium (Okamoto et al. 1963), fructose, or fat (Gradel et al. 2018), it is vital to discuss what researchers have already utilized to try to combat this issue.

Dupas et al. (2017) randomly divided 32 three-week-old male Wistar rats into one of two groups: 18 rats in the fructose group, and 14 in the control group (tap water) for twenty-one weeks (Dupas et al. 2017). After a four-day acclimation period, rats had their systolic blood pressure measured through a tail cuff roughly every two weeks throughout the duration of the intervention (Dupas et al. 2017). Fructose drinks were refreshed every few days and the amount of fructose was increased once the rats were ten-weeks-old. The rats were weighed each week (Dupas et al. 2017). Blood was drawn through venipuncture following a 15-hour-long fast and abdominal fat, muscles, hearts, and livers were weighed and frozen following dissection (Dupas et al. 2017). Systolic blood pressure and fasting blood glucose levels were significantly elevated in the fructose group when compared to the control group, despite not having any signs of renal failure or ventricular hypertrophy, but their body mass stayed the same during the course of the study (Dupas et al. 2017). However, rats that consumed fructose had more abdominal fat and greater liver mass even though the heart mass did not change (Dupas et al. 2017).

The rats also did not develop glucose intolerance despite the constant fructose consumption, amount of abdominal fat, and insulin resistance (Dupas et al. 2017). Dupas et al. (2017) concluded that the rapid early onset of hypertension was associated with the high fructose diet, but they could not determine why, especially since they did not measure blood pressure in the rats for the first four weeks (Dupas et al. 2017). The rats developed some hepatic lipid droplets, but not enough to develop fatty liver disease (Dupas et al. 2017). Although Dupas et al. (2017) were trying to find a link between fructose and metabolic syndrome, and this study induced hypertension through a high fat diet, the authors showed that hypertension has a strong correlation with poor diet choices.

Another study by Hou et al. (2017) compared eight-week-old salt-sensitive rats that could not produce nitric oxide with rats that were not salt-sensitive (Hou et al. 2017). Despite the consumption of salt for seventeen consecutive days, the researchers found that aspartate reduced high blood pressure levels in salt sensitive rats after just one week (Hou et al. 2017). The supplements also raised the levels of nitric oxide in the blood and kidneys, which correlated with the decrease in blood pressure (Hou et al. 2017). Since aspartate supplements correlated with an increase in nitric oxide, and this increase correlated with a significant decrease in blood pressure levels (Hou et al. 2017), it would stand to reason that nitric oxide supplementation could lead to an even greater decrease in blood pressure levels.

Organometallic Complex Powder Supplement

While several studies have elucidated the benefits of supplements on hypertension, the only study on a similar Organometallic Complex powder supplement

thus far has involved diabetic mice (Deneau et al. 2011). Deneau et al. (2011) used thirty six week-old male mice that were genetically diabetic. The mice were randomly assigned to one of three groups: a tap water control group, 0.6 mg/mL, or 3 mg/mL of a supplement that was similar to the Organometallic Complex powder used in the present study. It was administered to the animals completely dissolved in water, which is similar to the present study, except the prior intervention lasted for only 31 days (Deneau et al. 2011), whereas the present intervention lasted for ten weeks. Additionally, five mice were housed together, while this study housed rats individually, and mice were only weighed at the beginning and end of the intervention (Deneau et al. 2011). After the intervention, it was observed that the Organometallic Complex powder depressed a major glycation factor in diabetes, similarly to lipoic acid (Deneau et al. 2011). Additionally, Deneau et al. concluded that the Organometallic Complex powder was correlated with more efficient synthesis of adenosine tri-phosphate, upregulation of copper-zinc superoxide dismutase, and cellular respiration (Deneau et al. 2011). Despite consuming the same amount of water, the control group was significantly heavier, the albumin/globulin ratio was vastly lower, especially compared to the 3 mg/mL treated group, the alkaline phosphatase was significantly elevated and they had a massive spike in blood glucose levels during a non-fasted glucose tolerance test when compared to the intervention groups (Deneau et al. 2011). However, the hemoglobin A1C was only marginally higher in the control group, and the blood urea nitrogen levels were elevated, but only in the mice that consumed the 0.6 mg/mL dose (Deneau et al. 2011). However, it is unclear whether these results were from the abundance of minerals that are found in the Organometallic Complex powder supplement (Deneau et al. 2011). Even though these

researchers only looked at this supplement in regard to type 2 diabetes, their study results are of great relevance to the current study since they have exposed some of the ways that the Organometallic Complex interacts with at least one type of animal model. Although the results indicate that this supplement can mitigate some of the effects of type 2 diabetes, and even correlate with weight loss, a similar complex may not relieve the symptoms of hypertension and enlarged left ventricles that develop with chronic high fat intake.

Magnesium

Because one of the main minerals in an Organometallic Complex supplement is magnesium, it is important to discuss the potential health benefits that are associated with it. A study by Li et al. (2017) hypothesized that magnesium supplements could reduce hepatic encephalopathy in rats more than zinc or iron supplements (Li et al. 2017). After randomly assigning forty-eight male Sprague-Dawley rats into five groups of eight (either a magnesium group, zinc group, iron group, rifaximin group, or a hepatic encephalopathy control group), they found that manganese concentrations in the brain and blood ammonia were lower in the eight rats who consumed 50 mg/kg of magnesium supplements when compared to the hepatic encephalopathy group (Li et al. 2017). They also had higher fecal levels of manganese and magnesium (Li et al. 2017). Unfortunately, this study did not examine magnesium supplementation on hypertension, so the health benefits they found may not extend to other disorders.

Magnesium supplements have been shown to have a preventative effect on hypertension. A meta-analysis looked at ten prospective cohort studies involving dietary

magnesium and hypertension (Han et al. 2017). While serum magnesium levels are only slightly associated with the risk of hypertension, consuming 100 mg of dietary magnesium each day significantly reduced the risk of hypertension by 5% (Han et al. 2017).

Unfortunately, it would appear that magnesium may be ineffective once it's combined with other supplements, however. A randomized, double-blind, controlled crossover trial was conducted to try and reduce blood pressure for one day through K Citrate, KCL, or KMG Citrate supplements (Vongpatanasin et al. 2016). Out of the thirty participants who were in the early stages of hypertension, none of the participants who consumed KMG Citrate supplements (which contained 20 meq of Mg) had any change in blood pressure despite having decreased oxidative stress and elevated serum and urinary magnesium excretion levels when compared to the other supplements (Vongpatanasin et al. 2016). While this may have been due to the participants already controlling their hypertension with medication (Vongpatanasin et al. 2016), it seems unlikely that the magnesium in the OMC supplement will have any effect on the hypertensive rats.

Calcium and Potassium

Because this supplement contains appreciable levels of calcium and potassium, it is crucial to elucidate how calcium and potassium have affected hypertensive rats in prior studies. Tolvanen et al. (1998) have studied both of these minerals in twelve spontaneously hypertensive rats compared to a control group for nineteen weeks. At the end of the intervention period, rats who consumed both calcium and potassium supplements had decreased blood pressure levels significantly more than rats that were

given just calcium or potassium alone, but the blood pressure levels were still elevated compared to the non-hypertensive group (Tolvanen et al. 1998).The hearts from the combined supplement group also weighed less than the hypertensive rats who did not receive either supplement (Tolvanen et al. 1998).

In addition, calcium may not work in every case. A prospective cohort study by Wang et al. compared dietary sources of calcium with calcium supplements in over 28,000 middle-aged women who did not have hypertension at the beginning of the study (Wang et al. 2008). Diet and vitamin intake were assessed through a questionnaire, and incidence of hypertension was reported via an annual questionnaire during the decade-long study (Wang et al. 2008). The researchers found that women who had the lowest risk for hypertension consumed their calcium via low-fat dairy, whereas the calcium supplements had no effect (Wang et al., 2008).

Sulfur

Sulfur, another mineral in the OMC supplement, has been used to reduce ventricular hypertrophy in rats. A recent study administered 250 mg/kg sulfur metabolites from garlic paste to six male Sprague-Dawley rats for over two weeks (Khatua et al. 2017). Their hearts and bodies weighed significantly less than rats with cardiac hypertrophy that did not consume sulfur from the garlic paste (Khatua et al. 2017). However, whether sulfur will reduce hypertrophy without garlic remains to be seen. Sulfur has also been tested in pulmonary hypertensive rats. Yu et al. randomly assigned eight male Wistar rats into an 85 mg/kg sulfur dioxide group for three weeks (Yu et al.

2016). By the conclusion of the study, these rats had significantly less collagen clogging their pulmonary artery than the eight rats in the control group (Yu et al. 2016).

Finally, researchers Maia et al. were able to decrease blood pressure in twenty-one-day-old male hypertensive Wistar rats with an amino acid that contains sulfur (Maia et al. 2014). The rats were randomly assigned to a normal diet or one that is low in protein, and only half of them had 2.5% of the supplement administered in their water bottles for three months (Maia et al. 2014). The sulfur supplement only lowered blood pressure levels in rats that received the protein-restricted diet (Maia et al. 2014), and further research is needed to understand the influence of sulfur supplementation combined with other types of deleterious diets.

Conclusion

In conclusion, this literature review has shown that hypertension is so detrimental to one's health that many researchers have dedicated their studies to finding various natural substances that can be used to attenuate this issue. While some minerals, such as magnesium and calcium look promising, they can yield different results in a supplemental form. Furthermore, while potassium chloride has been associated with an indirect decrease in blood pressure in rats by increasing the expelling of sodium through urination (Louis et al. 1971), potassium supplements may not work depending on the type of hypertension (Veiras et al. 2016).

CHAPTER 3

METHODS

Study Design

This study included forty-two healthy male 6-week old Sprague-Dawley rats that were purchased from Envigo (formerly Harlan). Arizona State University IACUC approval was obtained (protocol number: 17-1563R (See appendix B)) before completing this study. Upon arrival, rats were randomly divided into chow and high fat diet-fed groups. The chow diet was considered the reference diet as it was identical to the diet the animals were raised on (3.1 kcal/g, Envigo, Madison, WI). The 60% kcal from fat HFD contained 5.21 kcal/g diet (D12492; Research Diets, Inc, New Brunswick, NJ). The HFD was replaced every three days to prevent spoiling. Rats were fed the respective diets for 10 weeks and had ad libitum access to food and water. Immediately upon arrival, rats in each of these dietary groups were further divided into three treatment groups and administered one of three interventions: vehicle (plain water control group), 0.6 mg/ml organometallic complex (OMC) or 3.0 mg/ml OMC throughout the feeding protocol. Sample sizes in each group were as follows, Chow + plain water (n=10), Chow + 0.6 mg/ml OMC (n=6), Chow + 3.0 mg/ml OMC (n=6), HFD + plain water (n=8), HFD + 0.6 mg/ml OMC (n=6), HFD + 3.0 mg/ml OMC (n=6). Each rat was housed individually to prevent the sharing of microbiota through coprophagia. Rats were kept in a temperature-controlled room with a 12:12 hour light/dark cycle.

Blood pressure was measured every other week through tail cuff plethysmography with a Kent Scientific CODA Monitor (Kent Scientific, Torrington, CT). Handling of the

rats was kept to a minimum in order to reduce stress. At the end of the 10 week feeding trial, rats weighed then euthanized with an overdose (200 mg/kg, i.p.) of sodium pentobarbital. Body length, tail length and abdominal circumference were measured using flexible tension tape to the nearest cm. Epididymal fat pad, heart, left ventricle of the heart, liver and spleen were weighed using a Mettler Toledo analytical balance (model number AB204-S/FACT; Mettler Toledo, Columbus, OH).

Study Design

Forty-two healthy six-week old male Sprague Dawley rats were randomly assigned into one of three groups: plain water control group, 0.6 mg/ml organometallic complex or 3.0 mg/ml organometallic complex as soon as they arrived. Each rat was then housed individually to prevent the sharing of microbiota through coprophagia. Rats in each treatment group were further divided into two dietary groups that were fed either a high fat diet containing 60% kcal fat that was changed every three days or standard rodent chow. Researchers were not blind to which rat was in each group. A minimum sample size of six rats was required for each group and determined through power analysis using SigmaStat 3.0 software and prior research (Sipola et al. 2001, Tanida et al.2005, Rault-Nania et al. 2008). Blood pressure was measured through tail cuff plethysmyogaphy with a Kent Scientific CODA Monitor. Mass of tissues were weighed using a Mettler Toledo analytical balance (model number AB204-S/FACT). Weighing organs is a common procedure, but Kent Scientific references several published papers about measuring tail cuff pressures, but one study in particular by Finne et al. was able to determine that their rats had a particular form of hypertension by using the Kent Scientific CODA blood pressure monitor and cuffs (Finne et al., 2016).

Study Supplement

The independent variable was a proprietary Organometallic Complex supplement in powder form. This supplement was a soil-derived mineraloid isolate which naturally occurs through the oxidation process of coal (Deneau et al. 2011). It is safe for animal and human consumption. The supplement in the present study was obtained through Isagenix International LLC from Gilbert, Arizona (Table 1).

Table 1: Physical, Chemical, and Functional Characteristics of OMC

Component Measured	Concentration or Value	Analytical Methodology or Source
Total Minerals	142391 ppm	ICP
Calcium	49610 ppm	ICP
Sulfur	28040 ppm	ICP
Potassium	15420 ppm	ICP
Sodium	14990 ppm	ICP
Magnesium	12630 ppm	ICP
Fulvic Acids	14.9%	Lamar et al., 2014 [42]
Humic Acids	<0.1%	Lamar et al., 2014 [42]
Protein	23 mg/g	CLG-PRO4 determination by combustion
Nucleic Acids	ND	DAPI (4',6-Diamidino-2-phenylindole)-staining
Total Polyphenols	0.24%	Folin-Ciocalteu
ORAC Score[a]-Hydrophilic	24.92	Brunswick Laboratories
ORAC Score[a]-Hydrophobic	5.44	Brunswick Laboratories

[a]ORAC: oxygen radical absorbance capacity, [b]ICP: Inductively coupled plasma mass spectrometry (Table from Crawford et al., *In Review*).

Statistical Analyses

Study results were represented as mean ± SEM. Two-way ANOVAs were performed to compare differences between groups. SigmaStat 3.0 software was used for statistical analyses, and P-values were considered significant at $P<0.05$. Data were tested for normality and log transformed when necessary. If there was a significant 2-way ANOVA, Tukey post-hoc analysis was used to determine significant differences between dietary treatment groups.

CHAPTER 4

RESULTS

Although we attempted to measure blood pressure by tail vein plethysmography, we were unable to obtain accurate or reproducible measurements using the KODA system. Therefore, these data are not presented.

Morphometrics

After the ten-week-long intervention, epididymal fat pad mass in rats in the 0.6 mg/ml intervention group consuming a high fat diet were significantly increased: 7.44± 1.09 g compared to 6.67 ± 0.65 g in the 3.0 mg/ml intervention group and 6.09 ± 0.32 g in the control group. Waist circumference in rats in the 0.6 mg/ml intervention group consuming a high fat diet were significantly increased: 18.9 ± 0.62 cm compared to 18.3 ± 0.24 cm in the 3.0 mg/ml intervention group and 17.9 ± 0.25 cm in the control group. Naso-anal length in rats in the 3.0 mg/ml intervention group consuming a high fat diet were also significantly increased: 23.2± 0.30 cm compared to 23.1 ± 0.20 cm in the 0.6 mg/ml intervention group and 23.1 ± 0.14 cm in the control group. The high fat diet groups were also significantly increased compared to the chow groups, with the exception of tail length (21.3 ± 0.27 cm in the control group) and Lee's Index of Obesity (327.3 ± 3.3 cm in the 0.6 mg/ml intervention group and 322.9 ± 2.5 cm 3.0 mg/ml intervention group). (**Table 1**).

Table 1: Morphometrics of Sprague-Dawley rats

	Control	0.6 mg/ml OMC	3.0 mg/ml OMC
Epididymal fat pad mass (g)[a]			
Chow	3.70 ± 0.16 (10)	4.14 ± 0.24 (6)	3.89 ± 0.41 (6)
HFD	**6.09 ± 0.32 (8)#**	**7.44 ± 1.09 (6)#**	**6.67 ± 0.65 (6)#**
Waist circumference (cm)			
Chow	16.8 ± 0.28 (10)	17.5 ± 0.17 (6)	17.3 ± 0.20 (6)
HFD	**17.9 ± 0.25 (8)#**	**18.9 ± 0.62 (6)#**	**18.3 ± 0.24 (6)#**
Tail length (cm)			
Chow	21.3 ± 0.27 (10)	21.1 ± 0.29 (6)	21.4 ± 0.51 (6)
HFD	21.3 ± 0.22 (8)	21.5 ± 0.26 (6)	21.5 ± 0.31 (6)
Naso-anal length (cm)			
Chow	22.3 ± 0.15 (10)	22.1 ± 0.14 (6)	22.5 ± 0.24 (6)
HFD	**23.1 ± 0.14 (8)#**	**23.1 ± 0.20 (6)#**	**23.2 ± 0.30 (6)#**
Lee's Index of Obesity			
Chow	321.1 ± 1.7 (10)	327.3 ± 3.3 (6)	322.9 ± 2.5 (6)
HFD	321.9 ± 1.7 (8)	324.7 ± 2.8 (6)	322.0 ± 1.8 (6)
BMI			
Chow	0.74 ± 0.01 (10)	0.77 ± 0.02 (6)	0.76 ± 0.02 (6)
HFD	0.77 ± 0.01 (8)	0.79 ± 0.02 (6)	0.77 ± 0.01 (6)

Data expressed as mean ± SEM (n). Data analyzed by two-way ANOVA. #p<0.02 vs respective chow treated animal. [a]Data were log transformed prior to statistical analyses to approximate normality. Lee's Index of Obesity = (cube root of body mass (g) / nasoanal length (cm)) * 1000 (Bernardis, 1970). Body mass index (BMI) = body mass (g) / nasoanal length $(cm)^2$ (Novelli et al., 2007).

Organ Mass

Six rats in the 0.6 mg/ml intervention group consuming a high fat diet developed a significant increase of 1.39 ±0.05 g in heart mass compared to 1.13 ±0.04 g in the chow group, and 0.97 ±0.04 g in left ventricle mass compared to 0.81 ±0.02 g in the chow group. Rats in the 3.0 mg/ml intervention group had an increase of 1.35 ±0.03 g in heart mass over 1.17 ±0.04 g in the 3.0 mg/ml chow group, and an increase of 0.97 ±0.02 g in left ventricle mass over 0.86 ±0.03 g in the chow group. In addition, eight rats in the control group that consumed a high fat diet had a significant increase in left ventricle

mass (0.96 ±0.04 g) compared to the chow control group. (See **Table 2** for all organ masses). However, all variations in heart and left ventricle masses were explained by diet alone, not the effect of either dose of the organometallic complex supplement. Furthermore, after normalizing to heart mass, left ventricular mass was no longer significantly different. All variations were explained by diet alone and there were no treatment effects since tail length was not significantly different between animals.

Table 2: Organ masses of Sprague-Dawley rats

	Control	**0.6 mg/ml OMC**	**3.0 mg/ml OMC**
Liver mass (g)[a]			
Chow	12.3 ± 0.42 (10)	12.1 ± 0.52 (6)	13.2 ± 0.41 (6)
HFD	12.2 ± 0.71 (8)	12.4 ± 0.35 (6)	12.4 ± 0.36 (6)
Spleen mass (g)			
Chow	0.75 ± 0.02 (8)	0.79 ± 0.04 (6)	0.81 ± 0.04 (4)
HFD	0.79 ± 0.02 (6)	0.87 ± 0.06 (4)	0.77 ± 0.05 (4)
Heart mass (g)			
Chow	1.26 ± 0.05 (10)	1.13 ± 0.04 (6)	1.17 ± 0.04 (6)
HFD	1.33 ± 0.05 (8)	**1.39 ± 0.05 (6)#**	**1.35 ± 0.03 (6)#**
Left ventricle mass (g)			
Chow	0.85 ± 0.03 (10)	0.81 ± 0.02 (6)	0.86 ± 0.03 (6)
HFD	**0.96 ± 0.04 (8)#**	**0.97 ± 0.04 (6)#**	**0.97 ± 0.02 (6)#**
Left ventricle/heart mass (g)[a]			
Chow	0.68 ± 0.02 (10)	0.71 ± 0.01 (6)	0.73 ± 0.01 (6)
HFD	0.72 ± 0.02 (8)	0.70 ± 0.01 (6)	0.71 ± 0.01 (6)

Data expressed as mean ± SEM (n). Data analyzed by two-way ANOVA. #p<0.02 vs respective chow treated animal. [a]Data were log transformed prior to statistical analyses to approximate normality.

CHAPTER 5

DISCUSSION

As hypothesized, rats did not develop a significant difference in epididymal fat pad mass, waist circumference, or growth, and there were no significant differences in heart mass or left ventricle mass in either intervention group when compared to the control groups. This remained true when left ventricle mass was normalized to heart mass. While the present study and Deneau et al.'s intervention were similar, the slight differences in rodent model, duration of treatment, composition of supplements, and disease models may explain why an OMC supplement was beneficial for diabetes (Deneau et al. 2011), but did not attenuate diet-induced cardiac hypertrophy in the present study.

Magnesium supplements have been associated with mediating the effects of L-NAME-induced hypertension in rats (Ozturk et al. 2016). Ozturk et al. (2016) fed 1 gram per kilogram of magnesium to 20 8-week-old rats for 6 weeks. By the conclusion of the study, blood pressure had decreased in the magnesium-treated group to the same level as the control group and reduce ventricular tissue hypertrophy (Ozturk et al. 2016). While magnesium supplements have been shown to decrease the effects of hypertension (Ozturk et al. 2016), it is unknown if the dose of magnesium in the current OMC supplement was too low to notice an effect.

Calcium antagonists have been shown to increase blood pressure in rats, indicating that calcium plays an important role in decreasing hypertension (Kang, Cregor,

& Smith, 1990). A study by Tolvanen et al. (1998) successfully decreased blood pressure in rats through a combination of potassium and calcium supplementation (Tolvanen et al. 1998), and sulfur has been shown to decrease ventricular hypertrophy (Yu et al. 2016). Thus, although the OMC supplement used in the current study contains these minerals, they may not be in sufficient concentration to prevent cardiac hypertrophy as observed in the current study. Wang et al. (2008) has shown that a low-fat diet may be more beneficial for controlling hypertension than using supplements. In addition, the OMC contains fulvic acid, which may have also influenced the results of the study. One of the earliest studies investigated the effect of fulvic acid on ATP production on mitochondria in rat livers (Visser, 1987). The researchers concluded that fulvic acid can inhibit ATP synthesis for a short amount of time and it does not increase oxidative phosphorylation in mitochondria of rodent livers as much as humic acids (Visser, 1987). Fulvic acid has also been studied in rats due to its contribution to certain skeletal diseases (Wang et al. 1996). Wang and colleagues injected 40 Wistar rats with fulvic acid for 130 days and compared them to a control group treated with saline injections (Wang et al. 1996). The rats also consumed radiolabeled fulvic acid, which was found in all organs and bones following dissection, with the largest amounts found in the kidney and liver and the bones of the injected rats were brown, indicating that fulvic acid is a contributing factor of bone disease (Wang et al. 1996).

Since this supplement is derived from soil, it may contain trace amounts of copper. Lind and Glynn (1999) researched the effects of fulvic acid on the absorption of copper. They divided 40 12-day-old Sprague-Dawley rat pups into groups of 10 and administered 0.3 ml of radiolabeled copper, copper, and fulvic acid in infant formula

through intubation (Lind & Glynn, 1999). After 6 hours, the pups were dissected and their organs were tested in a gamma counter (Lind & Glynn, 1999). Pups in the fulvic acid group had less retention of copper in their bodies and absorption in their intestines than the control group (Lind & Glynn, 1999). The researchers concluded that fulvic acid in drinking water may have an impact on copper absorption (Lind & Glynn, 1999). Therefore, it is possible that fulvic acid may have prevented the other minerals in this supplement from being absorbed. Another study found that while fulvic acid is associated with an immune response, it may also lead to hypothyroidism (Vucskits et al., 2010). Two experiments administering different doses of fulvic acid to rats (0.1, 0.2,0.4, and 0.8% in one experiment and 0.4% in the other) were compared to the same doses of humic acid for 26 days (Vucskits et al., 2010). At the end of the first experiment, rats that consumed 0.4% fulvic acid had a greater increase in antibodies than the control group (Vucskits et al., 2010). The higher dose of fulvic acid also increased plasma thyroid stimulating hormone and was associated with a decrease in the T4/T3 ratio (Vucskits et al., 2010). While the amount of fulvic acid in the present study is unknown, it seems that large doses of fulvic acid may have an undesirable side effect. However, fulvic acid can promote wound healing and reduce inflammation in rats and mice when it is applied topically (Sabi et al. 2012). These results were replicated in a later study (Zhao et al., 2015), so it is unfortunate that the same healing and anti-inflammatory properties do not apply to fulvic acid once it has been consumed. Furthermore, high doses of fulvic acid has been shown to decrease iron absorption in rats (Szabo et al., 2017), and it has been associated with lower levels of zinc in the large intestine and kidney (Hullar et al., 2018). For these reasons, further research may be warranted to determine if the dose of fulvic

acid in the OMC supplement is high enough to have impacted the results of the current study.

In conclusion, the OMC supplement did not mitigate the effects of a high fat diet on cardiac hypertrophy in rats. Therefore, this supplement should not be used to treat cardiac hypertrophy.

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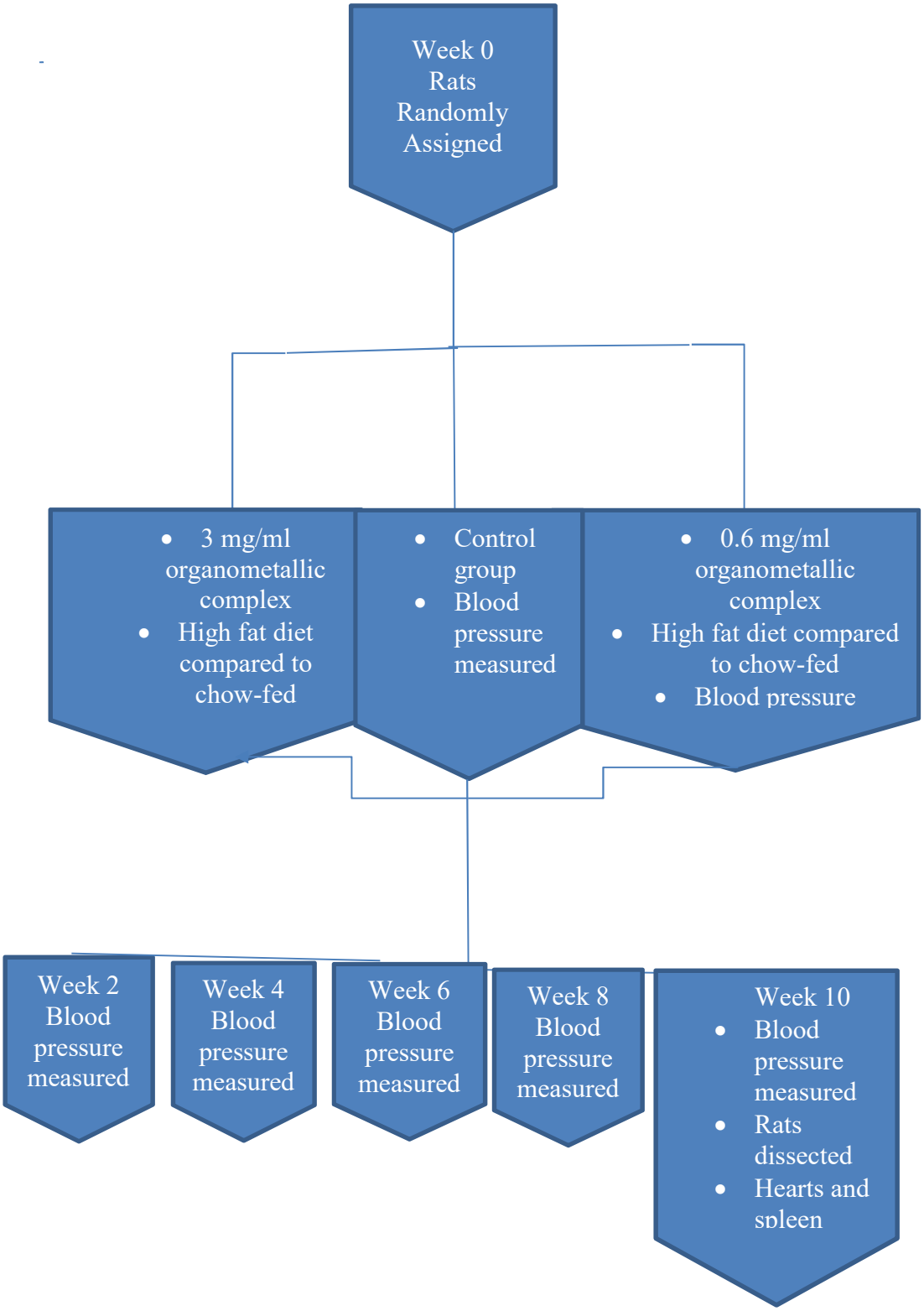
APPENDIX A

OMC STUDY FLOW CHART


Week 0
Rats
Randomly
Assigned
3 mg/ml organometallic complex
High fat diet compared to chow-fed
Control group
Blood pressure measured
0.6 mg/ml organometallic complex
High fat diet compared to chow-fed
Blood pressure
Week 2
Blood pressure measured
Week 4
Blood pressure measured
Week 6
Blood pressure measured
Week 8
Blood pressure measured
Week 10
Blood pressure measured
Rats dissected
Hearts and spleen

APPENDIX B

IACUC APPROVED PROTOCOL

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 312
Tempe, Arizona 85287-6111
Phone: (480) 965-6788 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number:	**17-1563R**
Protocol Title:	**Exploration of the metabolic and vascular protective effects of an organometallic complex (OMC)**
Principal Investigator:	**Karen Sweazea**
Date of Action:	**2/23/2017**

The animal protocol review was considered by the Committee and the following decisions were made:

The protocol was approved.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree.

Total # of Animals:	**60**	
Species:	**Rats**	**Pain Category: C**
Protocol Approval Period:	**2/23/2017 – 2/22/2020**	
Sponsor:	**Isagenix International LLC**	
ASU Proposal/Award #:	**FP00010367; FP00010473**	
Title:	**Exploration of the metabolic and vascular protective effects of an organometallic complex (OMC)**	

Signature: _____ for C. Miller
IACUC Chair or Designee

Date: 2/27/2017

Cc: IACUC Office
IACUC Chair