Change in Circulating Klotho in Response to Weight Loss, with and without Exercise, in Adults with Overweight or Obesity

by

Katherine A. Collins

BS, Pfeiffer University, 2015

MS, University of Pittsburgh, 2016

Submitted to the Graduate Faculty of

School of Education in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2019

UNIVERSITY OF PITTSBURGH

SCHOOL OF EDUCATION

This dissertation was presented

by

Katherine A. Collins

It was defended on

July 18, 2019

and approved by

Fabrisia Ambrosio, Ph.D., MPT, Associate Professor, Department of Physical Medicine and Rehabilitation

Kelliann K. Davis, Ph.D., Assistant Professor, Department of Health and Physical Activity

Renee J. Rogers, Ph.D., Assistant Professor, Department of Health and Physical Activity

Dissertation Advisor: John. M. Jakicic, Ph.D., Distinguished Professor, Department of Health and Physical Activity

Change in Circulating Klotho in Response to Weight Loss, with and without Exercise, in Adults with Overweight or Obesity

Katherine A. Collins, PhD

University of Pittsburgh, 2019

The increased risk in aging-related comorbidities associated with obesity is a significant public health concern. Weight loss via lifestyle intervention has been shown to improve negative health outcomes; however, the mechanistic pathway by which this occurs is unknown. One biomarker of importance is Klotho, with higher levels being associated with slower aging processes. Evidence has shown Klotho to be lower in obese adults compared to normal-weight adults. Exercise may independently increase levels of Klotho; however, whether the effects on Klotho are enhanced when exercise is included within the context of a weight loss intervention is not understood. **Purpose:** The primary aim was to examine changes in Klotho concentration in response to a behavioral weight loss intervention, by intervention condition, and weight loss response. **Methods:** Three hundred and eighty-three overweight and obese adults (age: 45.0±7.9 years; BMI: 32.4±3.8 kg/m2) were randomized to DIET, DIET+PA150, and DIET+PA250. One hundred fifty-two of 383 participants provided blood samples at all time points, and were classified as "responder" or "non-responder" for this study. The intervention contained a prescribed calorie restricted diet (1200-1800 kcal/day) and increased physical activity (0-, 150-, 250 min/week). Intervention groups attended weekly in-person group sessions for months 1-6, with combined in-person and telephonic sessions for months 7-12. **Results:** Participants had significant reductions in weight, BMI, and body composition, and increased cardiorespiratory fitness across the 12-month intervention. Klotho concentration also significantly, increased across the 12-month behavioral weight loss intervention (p=0.010); however, there was no

difference by intervention group or weight loss response. Although non-significant, participants who performed physical activity had greater changes in Klotho concentration across the 12-month intervention. Further, Klotho concentration was found to be inversely associated with lean body mass (p=0.021). **Conclusion:** This study provides evidence within the context of a weight loss intervention, that Klotho concentration significantly, but modestly, increases with weight loss. However, despite sustained weight loss, this increase in Klotho was not sustained throughout the intervention. Results do not support weight loss drive Klotho response. However, non-significant results support physical activity to influence Klotho, within the context of obesity and weight loss. Further investigation to examine how weight loss and physical activity may alter biomarkers of aging in adults with obesity is warranted.

Table of Contents

List of Tables

List of Figures

1.0 Introduction

1.1 Background

Overweight and obesity are associated with major health risks and increase in premature death.[1,2] Overweight is defined as having a body mass index (BMI) $\geq$ 25 kg/m^2 to <30 kg/m^2, and obesity is defined as having a body mass index of $\geq$30 kg/m^2.[1,3,4] The current prevalence of obesity is estimated to be 35.5% among adult men and 35.8% among adult women,[3,5] with obesity having an increase in prevalence compared to levels of a few decades ago among adults in the United States.[3,4] The increased prevalence of obesity is of significant public health concern due to its association with many chronic diseases such as cardiovascular disease, diabetes, certain cancers, sleep apnea, osteoarthritis, musculoskeletal problems, and others.[5-10] The health-related consequences of obesity are enhanced at higher levels of obesity.[5,6]

The health-related concerns of obesity may be a result of excess body weight promoting accelerated aging by affecting the maturation of adipose tissue, influencing inflammation and glucose homeostasis, promoting insulin resistance, oxidative stress, DNA damage, telomere dysfunction, increased vasomotor tone, increased sympathetic drive, and more.[1,3] The accelerated aging that occurs with obesity may serve as the pathway for increased risk in diabetes and cardiovascular disease. Of the many biomarkers that have been investigated, very few have been identified to play a role in the longevity pathway. Vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and insulin-like growth factor (IGF) – 1 have been identified as potential biomarkers that lie along the molecular pathway of aging.[11] VEGF-A has been identified for its role in angiogenesis, BDNF plays a part in neurogenesis, and IGF-1

promotes growth and maintenance of muscle. While these biomarkers may provide a better understanding to human longevity, their influence on the molecular pathway of longevity appears to be associated with downstream effects rather than being markers of early detection in accelerated aging.

One biomarker that may be provide insight to accelerated aging is Klotho, as it has been found to be associated with longevity, as well as, cardiovascular and neuroprotective effects with higher levels of Klotho being associated with a slower aging process and fewer negative-health outcomes.[12,13] Thus, Klotho may be an important biomarker to understand the pathways of accelerated aging that may be present with overweight and obesity (*Figure 1*); however, there is limited data available on this relationship, with even less data available on how Klotho responds to weight loss using lifestyle intervention.

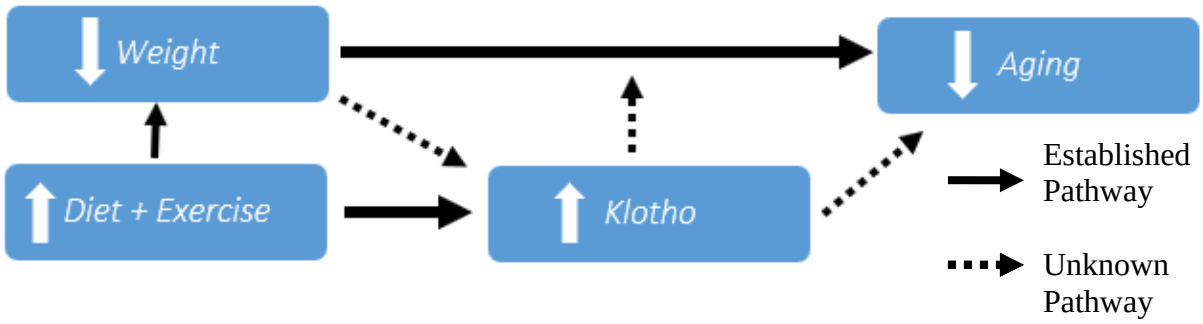

Figure 1. Potential Pathway to Explain the Effect Weight has on Aging

Klotho was first discovered by Kuro-o et al. in 1997, who found an insertion mutation on the 5' flanking region of α-Klotho in mice, that was associated with several symptoms of premature aging including soft tissue calcification, arteriosclerosis, skin atrophy, gonadal dysplasia, infertility, hypoglycemia, server hyperphosphatemia, osteoporosis, emphysema, and an overall shorter life span.[12,13] Human Klotho is primarily expressed in the kidney, but is also

detectable in the placenta, prostate, and small intestines.[13] The majority of circulating Klotho results from proteolytic cleavage of the intracellular domain of the full-length protein by secretases. [14] The current understanding of Klotho is based on its ability to exert both fibroblast growth factor-23 (FGF23) dependent and FGF-23 independent, pleiotropic actions with soluble Klotho acting as both a paracrine and endocrine factor.[12,14,15]

Secreted Klotho acts as a humoral factor that is linked to nitric oxide production in the endothelium, regulation of calcium channel activity, suppression of oxidative stress, transforming growth factor-β1 signaling, and insulin/IGF-1 signaling.[16] Klotho has been identified as having two homologs, β-Klotho and α-Klotho. α-Klotho is the primary homolog that has been studied and is associated with anti-aging factors, whereas β-Klotho is less understood but thought to be associated with inflammation and fat oxidation.[11]

1.2 Clinical Significance

There is a limited evidence that has examined the association between body weight, or measures of adiposity, and Klotho. Of the published data that are available, lower Klotho levels have been observed in adults with obesity compared to those who are of normal body weight.[17] Animal studies also link Klotho to obesity and suggest that Klotho may play a role in energy metabolism, white adipose tissue accumulation, reductions in the consequences of consuming a high fat diet, and insulin resistance.[18,19] However, the effects of weight loss in adults with obesity on Klotho has not been examined, and therefore it is unclear if intentional weight loss in adults with obesity would result in favorable improvements in Klotho and thereby potentially provide a mechanistic pathway by which health improvements are obtained in response to weight

loss. Thus, key contributions of this proposed study examined the relationship between Klotho and body weight, body composition/lean mass, and cardiorespiratory fitness in adults who are overweight or obese prior to initiating a weight loss program. Moreover, this study examined whether intentional weight loss using lifestyle intervention resulted in favorable changes in Klotho (*Figure 2).*

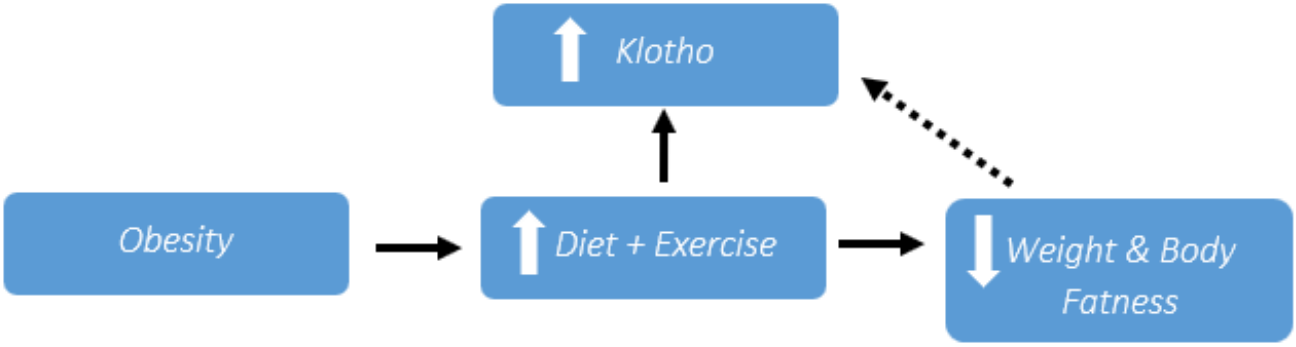

Figure 2. Potential Pathway to Explain the Effect of Diet, Exercise, Weight and Body Fat on Klotho Level

Another key aspect in the study of Klotho is the influence of exercise, [11] which also appears to be an important lifestyle behavior for body weight regulation, prevention of lean muscle mass loss, and prevention and treatment of numerous health-related conditions.[20] It has been suggested that similar anti-aging effects that are linked with exercise and physical activity are also linked to Klotho.[11] There is evidence to support performing exercise may result in an increase in Klotho. Following a 16-week exercise training program, individuals with a BMI $\geq$30 kg/m^2 showed a significant increase in Klotho concentration in response to an acute exercise bout.[11] Dalise et al. demonstrated that following a 30-minute bout of exercise there was significant upregulation of Klotho in mice.[21] However, particularly related to obesity, studies have not examined whether exercise coupled with dietary restriction to induce weight loss enhances the Klotho response compared to dietary restriction alone. Thus, an additional contribution of this proposed study was to examine Klotho response to weight loss induced by

dietary restriction compared dietary restriction combined with exercise in adults with overweight or obesity. Moreover, given the design of this study, we were able to examine whether the dose of prescribed exercise has any additional influence on change in Klotho within the context of a behavioral weight loss intervention.

This study examined Klotho in stored blood samples from a completed randomized clinical trial to determine: 1) the association between Klotho and level of overweight or obesity prior to weight loss, and 2) the change in Klotho across a 12-month behavioral weight loss program that included diet alone or diet plus exercise in high and low responders to the intervention. The behavioral weight loss intervention included participants randomized to one of three interventions: 1) Diet Alone [DIET], 2) Diet + 150 minutes of moderate-to-vigorous physical activity [DIET+PA150], 3) Diet + 250 minutes of moderate-to-vigorous physical activity [DIET+PA250] (Figure 3). Using this design, the following specific aims were examined.

Figure 3. Conceptual Framework of 3 Different Weight Loss Strategies on Circulating Klotho Levels

1.3 Specific Aims

1. To examine the association between circulating Klotho and BMI, parameters of body composition (fat, lean mass), and cardiorespiratory fitness in sedentary adults who are overweight or obese prior to engaging in a behavioral weight loss intervention.
2. To compare the effect of three intervention conditions (DIET, DIET+PA150, DIET+PA250) on circulating Klotho levels across a 12-month intervention.
3. To examine whether change in Klotho differs between individuals classified as high responders to the behavioral weight loss intervention (>10% of baseline weight) and those individuals classified as low responders to the behavioral weight loss intervention (<5% of baseline weight).

1.4 Hypotheses

1. It is hypothesized that there will be in inverse association between BMI and circulating Klotho levels, with individuals at a lower BMI having higher levels of circulating Klotho. A similar inverse association will be observed when examining the association between body fatness and Klotho, with a lower level of body fatness reflecting a higher level of circulating Klotho. However, there will be a positive association between lean body mass, cardiorespiratory fitness, and circulating Klotho.
2. It is hypothesized that there will be a significant difference in circulating Klotho between intervention conditions across the 12-month intervention with both the DIET+PA150 and

DIET+PA250 resulting in higher levels compared to DIET. Moreover, DIET+PA250 will result in higher levels of circulating Klotho compared to DIET+PA150.

3. There will be a significant difference in circulating Klotho across the intervention between individuals classified as high responders and low responders, with circulating Klotho being significantly higher in high responders compared to low responders.

2.0 Review of the Literature

2.1 Overweight and Obesity

2.1.1 Prevalence

Overweight, defined as a body mass index (BMI) $\geq$25kg/m^2 to <30kg/m^2, and obesity, defined as a BMI of $\geq$30kg/m^2, are prevalent in 33.9% and 35.1% of US adults respectively.[3] According to the National Health and Nutrition Examination survey the prevalence of overweight and obesity is 68.8% overall, and 73.9% among men and 63.7% among women.[4] The overall prevalence of obesity is higher among middle-aged adults compared to their younger counterparts. Also, there is a higher prevalence among non-Hispanic black and Hispanic adults compared to non-Hispanic white and non-Hispanic Asian adults.[1]

2.1.2 Consequences of Obesity

Over the past decade obesity has become a global epidemic, with dramatic increases in both adults and children. With this, both men and women who are overweight or obese are at an increased risk of developing numerous health conditions.[7] These including a number of comorbidities such as, metabolic syndrome[2,6], cardiovascular disease[2,6,8,22], type 2 diabetes[2,6,8,22], hypertension[2,6,8,22], certain cancers[6,8,22], and sleep apnea[6,9,10]. Obesity has been estimated to be associated with an excess of 112159 deaths from cardiovascular disease alone.[22]

Along with increased mortality rates, overweight and obesity are associated with increased health service use which has led to an economic burden among this population. Abdominal adiposity assessed by waist circumference has been linked to increased total healthcare expenditures, independent of age, gender, ethnicity, and smoking status. [6] In 1998, it was found that the medical cost of obesity was estimated to be roughly $78.5 billion, with almost half of the cost funded by Medicare and Medicaid.[23] In 2006, Finkelstein et al. found that overweight and obese patients were spending $1,429 more, or about 42% higher, on healthcare costs compared to their normal weight counterpart. This annual medical burden has increased from 6.5% to 9.1% of annual medical spending and could be as high as $147 billion per year based off a National Health Expenditure Accounts estimate.[23] The increased prevalence of overweight and obesity has not only causes physical burden on individuals, but also emotional and financial burden.

2.1.3 Factors Affecting the Etiology of Obesity

Overweight and obesity are complex multifactorial disease states, with their origins stemming from an imbalance between energy intake and energy expenditure. Overweight and obesity are affected by both environmental and genetic factors, with the interaction between the two leading to increases in body weight and fat accumulation.[24] Figure 4 displays four primary factors that have been shown to lead to overweight and obesity modeled from Weinsier et al., Ravussin et al., and others; genetic predisposition, metabolic, appetite and dietary and physical inactivity.[25-27]

Figure 4. Factors Effecting the Etiology of Overweight and Obesity

Genetic predisposition is thought to be one of the multiple factors that can cause an individual to develop obesity. There have been multiple hypotheses developed in order to attempt to understand what role genetics exactly plays. The "thrifty genotype" hypothesis developed by Neel et al., discusses how throughout evolution, humans had to go through periods of time where they would feast and overeat in order to survive and this over time made individuals more susceptible to fat accumulation. However, even though at one point in time this was a means to survive, in the current obesogenic environment, this adaptation has become a liability.[26,28,29] Speakman et al., developed the "predation release" hypothesis arguing that millions of years ago the act of predation was removed from the humans everyday life with the development of weapons, fire and social behaviors. This shift in lifestyle led to a modification in the population distribution of body fatness due to genetic drift and random mutations. Speakman et al., believes

that obesity is due to this random genetic drift, rather than direct selection. Meaning individuals still have the ability to remain thin, even if they are living in an obesogenic environment.[29,30]

Metabolic factors including energy expenditure and fuel utilization are key contributors to becoming overweight or obese. Energy expenditure is comprised of three components, resting energy expenditure, thermic effect of food, and activity expenditure.[25-27,29] Although each components' contribution to total energy expenditure is variable, typically the smallest contribution comes from the thermic effect of food and the largest from resting energy expenditure.[25,31] Resting energy expenditure is highly dependent on body mass, in particular fat-free mass, as it is considered more metabolically active compared to fat tissue. When considering absolute resting energy expenditure, individuals who are overweight or obese typically have a higher resting energy expenditure because of the increased body size, but when this is adjusted for lean body mass, resting energy expenditure is comparable among normal, overweight, and obese individuals.[25] Resting energy expenditure, however, has not been shown to predict weight gain, and has only a small impact on an individual's ability to gain weight. Conversely, total energy expenditure has been shown to be inversely correlated with rate of weight gain. This suggests it may be important to increase activity in order to increase total energy expenditure as a potential way to prevent the onset of obesity.[25-27,29]

Substrate utilization via respiratory quotient has also been found to be associated with weight gain. In both the Pima Indians study and The Baltimore Longitudinal Study, it was found that a high respiratory quotient, meaning low fat oxidation, is suggested to be a metabolic index as a predictor of weight gain.[25,26,32] However, there have been findings to contradict these studies, therefore more research is needed to understand the fuel utilization mechanism and its relation to weight gain.

Appetite and dietary factors have also been found to be associated with weight gain. Leptin is a gene primarily expressed in adipose tissue, known to regulate body weight. Contradictory evidence has been found with leptin concentrations and the impact it has on weight gain. Some report low plasma levels of leptin to be predictive of weight gain.[25,26,33] Others have found higher plasma leptin levels to be associated with weight gain.[25,33] There have been many dietary factors found to be associated with those who have a higher body mass index. These include, a dietary intake of higher energy density foods, high ethanol intake, frequent intake of sugared beverages, high intake of meat and processed meat, larger portion sizes, absence or bad location of supermarkets, and "fast food," which have all been found to be associated with weight gain.[24] Lifestyle factors including low physical activity, alcohol use, smoking, too much or too little of sleep, and too much television time have also been found to be associated with increased weight gain.[24,25]

Physical inactivity or sedentary behavior have been found to have an inverse relationship to adiposity or weight gain.[25] According to the World Health Organization[24], every year at least 1.9 million people die as a result of physical inactivity. On the contrary, if an individual performs 30 minutes of regular physical activity 5 days a week, their risk for many chronic diseases is decreased, as well as a decrease risk for all-cause mortality independent of risk factors.[24] Chronic diseases influenced by physical inactivity include, hypertension, type 2 diabetes mellitus, musculoskeletal diseases, mental health disorders, ischemic heart disease, and certain cancers, among others. [2,6,8,22,24]

2.2 Weight Loss and Weight Loss Maintenance Intervention Components

2.2.1 Behavioral Therapy Component

Behavioral therapy refers to principles and techniques used to change a participant's behavior and habits.[34,35] The goal of behavioral treatment is for individuals to adopt changes to their lifestyle by reducing caloric intake and increasing energy expenditure. Behavioral techniques are typically used in conjunction with other weight loss components including dietary and physical activity changes. There are various strategies used to help modify participant behavior including self-monitoring, stress management, stimulus control, problem solving, contingency management, cognitive restructuring of thoughts, social support, and relapse prevention training.[34-36] Although the content of behavioral therapy is important, how often it occurs has also been shown to play an important role in an individual's weight loss success. Ross et al., found that when an individual is actively trying to lose weight, group meetings should occur at a minimum of once a month but would have better success if they occurred bi-weekly or weekly, to provide nutrition education, reinforcement, encouragement, and monitoring.[34-37]

2.2.2 Dietary Component

The dietary component of a weight loss intervention is important when an individual is initiating weight loss. To produce weight loss, the calories an individual consumes should be fewer than the amount they expend, producing an energy deficit. Studies show that individuals should reduce their caloric intake to generate a deficit of 500 to 1000 kcal/day to produce a 1 to 2 lb/week weight loss.[34] Low calorie diets are typically recommended for weight loss with an

average calorie intake range from 800 kcal/day to 1800 kcal/day. [34,36,38-40] Low calorie diets have shown to produce an average of 8% weight loss in individuals who are overweight or obese over a 6-month period.[34,36,38,39]

2.2.3 Physical Activity Component

Physical activity is a key component to weight loss interventions and sustaining long term weight loss and maintenance. Physical activity increases total energy expenditure, may inhibit food intake, and may improve quality of life both physically and mentally in individuals[34]. The exercise portion of a weight loss intervention can be supervised or unsupervised, meaning either the participants have to come into the research center and perform their prescribed dose of physical activity, or they perform it on their own outside of the research center.[36,39,41] Physical activity prescriptions are generally based on the American College of Sport Medicine Position Stand[42], which states that individuals should perform 150 minutes/week of moderate or 75 minutes/week of vigorous aerobic physical activity for substantial health benefits and to produce weight loss. For greater health benefits, long-term weight loss, and weight maintenance it is recommended to perform 200-300 minutes/week of moderate to vigorous aerobic physical activity.[42,43] The strategy in which individuals decide to accumulate physical activity minutes does not seem to matter provided that the physical activity period is a minimum of 10 minutes to sum to 30 minutes or more or performing one bout of 30 minutes.[34,42,43] However, evidence included the 2018 Physical Activity Guidelines Advisory Report highlights the potential benefits of performing activity in bouts of less than 10 minutes in duration.[20] If studies include resistance training in their prescribed exercise it is recommended

to perform muscle-strengthening activities that are moderate to high-intensity and involve all major muscle groups on 2 or more days of the week.[44]

2.2.4 Combined Therapy

Studies show that diet only interventions produce weight loss, they have poor long-term outcomes and physical activity only interventions produce poor weight loss outcomes.[34] To produce the best outcome for long-term weight loss success and weight maintenance including both a dietary component and physical activity component in an intervention is important. Long-term follow-up studies have found that patients who are physically active on a regular basis achieve enhanced maintenance of weight loss.[34,45] The inclusion of behavioral therapy has also been shown to be important to improve weight loss success and long-term weight loss. Wing et al., reviewed studies that focused primarily on changing eating behavior, changing physical activity, and motivational strategies to improve weight loss and found that the most effective weight loss program included all three components of diet, physical activity, and behavior modification.[34,36,46,47] The greatest weight loss success was found when all of these components were maintained for at least 6 months.[34,36,46,47]

2.2.5 Long-Term Weight Loss Maintenance

Individuals tend to have success in the short term while still participating in a weight reduction program. However, following the program is when individuals relapse and frequently regain most if not all their lost weight within 1 to 5 years of the program.[34,36] The US Institute of

Medicine found that individuals who participate in a weight reduction program typically lose 10% of their body weight, but within 1 year two-thirds of the weight is regained.[34,37]

The National Weight Control Registry is a self-selected population of adults 18 years or older that have loss 13.6 kg and has kept it off at least 1 year.[48] Three strategies that individuals among this population perform on a regular basis include: consuming a low-calorie diet, performing high levels of physical activity, and weighing themselves frequently.[49] High levels of physical activity have been found to be associated with greater weight loss maintenance success compared to individuals in a diet alone weight loss program.[50] Registry members report an average energy expenditure of 2545 kcal/week in women and 3293 kcal/week in men of physical activity.[48,49] Although it is accepted that physical activity plays an important role in weight loss maintenance, the amount of physical activity that is needed continues to remain uncertain.

In the 2009 American College of Sports Medicine Position Stand it was recommended for long term weight loss maintenance that an individual perform 200-300 min/week of physical activity, almost equivalent to the levels of physical activity performed by individuals in the registry. [42,43] Jakicic et al., provides data from a randomized control trial that indicates those individuals who perform greater amounts of physical activity maintain a greater amount of weight loss at 12-month follow-up.[42,51,52] The trials also show very little weight regain in individuals who performed >200 minutes/week of moderate intensity physical activity. [42,51,52] Similarly, Jakicic et al., reported that those individuals who achieved a weight loss of >10% of initial body weight at 24 months, were currently participating in 275 minutes/week of (~1500 kcal/week) of physical activity above baseline levels.[42,53] Jeffery et al.,[54] randomly assigned individuals to either 1000 and 2500 kcal/week of physical activity for 18 months, and found the reported energy expenditure of kcal/week at 18 months was 1629 $\pm$ 1483 and 2317 $\pm$ 1854 for

the 1000- and 2500-kcal/week groups, respectively.[42] They also found no differences for weight loss between groups at 6 months, but there were significant differences at 12-, and 18-months of follow-up, with the 2500 kcal/week group indicating significantly greater weight losses.[42,54] In summary, these studies indicate, along with physical activity recommendations, that greater amounts of physical activity result in lower levels of weight regain and improved weight loss maintenance.

2.3 Weight Loss in Relation to Health

Overweight and obesity places individuals at a greater risk for development of many chronic related conditions. Treatment of overweight and obesity through weight loss via lifestyle intervention has been shown to decrease mortality rates, as well as, reduce the risk for these negative health-related outcomes.[55] Health benefits from weight loss using a lifestyle intervention approach appear in individuals with as little as 5% weight loss from baseline, with greater benefits appearing at >10% weight loss.[55,56] It is typically recommended that individuals reduce their calorie intake by 500 to 1000 kcal per week, in order to produce a 1 to 2 lb per week weight loss, resulting in a 10% weight loss by 6 months. This percentage of weight loss has been shown to be clinically meaningful in lowering blood pressure, levels of total cholesterol, LDL cholesterol, triglycerides, blood glucose levels, and increase HDL cholesterol levels, among other health outcomes.[55,57]

The 2013 American Heart Association/American College of Cardiology/The Obesity Society guidelines on the management for overweight and obesity found strong evidence linking weight loss to health outcomes. In overweight and obese adults with type 2 diabetes who

achieved 5% to 10% weight loss in 1 year of lifestyle intervention, were found to have reductions in hemoglobin A1c of 0.6% to 1.0% and a reduced need for diabetic medications.[57] In adults with overweight or obesity who achieve 5 kg to 8 kg of weight loss via lifestyle intervention see reductions in LDL cholesterol by approximately 5 mg/dL and improvement in HDL cholesterol by 2 to 3 mg/dL.[57] In overweight and obese adults with an elevated cardiovascular risk, a weight loss of 5% has been shown to reduce systolic and diastolic blood pressure by 3 and 2 mmHg, respectively.[57]

2.4 Physical Activity in Relation to Health

Physical activity is defined as any bodily movement produced by skeletal muscles that results in energy expenditure.[58] Exercise represents a subset of physical activity that is planned, with a goal of improving or maintaining fitness.[59] The 2018 Physical Activity Guideline Advisory Report states individuals can reach substantial health-related benefits from physical activity by doing 150 to 300 minutes of moderate intensity physical activity or 75 to 150 minutes of vigorous intensity physical activity. To achieve greater health-related benefits individuals should perform > 300 minutes of physical activity per week.[20,59]

The 2018 Physical Activity Guideline Advisory Report states there is strong evidence in adults to suggest a relationship between physical activity and health benefits including lower risk for all-cause mortality and CVD mortality, lower risk for CVD, hypertension, type 2 diabetes, adverse blood lipid profile, certain cancers (bladder, breast, colon, endometrium, esophagus, kidney, lung, and stomach), improved cognition, reduced risk of dementia, improved quality of life, reduced anxiety and risk of depression, improved sleep, slowed or reduced weight gain,

weight loss (typically when combined with a reduced calorie diet), prevention of weight regain following initial weight loss, improved bone health and physical function, lower risk of falls and fall-related injuries.[20]

There are several biological outcomes that are associated with the health benefits of performing physical activity. Regularly participating in physical activity has been shown to improve body composition[60-65], enhance lipid lipoprotein profiles[60,66-73], improve glucose homeostasis and insulin sensitivity[60,61,74-77], reduce blood pressure[74,78,79], improve autonomic tone[80,81], reduce systemic inflammation[80], decrease blood coagulation[60,66], improve coronary blood flow[60,66], augment cardiac function[60,66], and enhance endothelial function[82-85].[60]

Physical activity has been found to produce a decreased risk for all-cause mortality and cardiovascular disease. An increase in energy expenditure by 1000 kcal per week or increase in physical fitness of 1 MET has been shown to be associated with a mortality benefit of 20%.[60] Conversely, physically inactive women whom engage in 1 hour or less of physical activity per week, are shown to have a 52% increase risk for all-cause mortality, doubling in risk for cardiovascular disease related mortality, and a 29% increase in cancer related mortality. Similar risks have been found among men.[60,79,86,87] Wessel et al., studied 936 women who had undergone a coronary angiography for myocardial ischemia, and found that individuals who had higher physical fitness scores had significantly less coronary artery disease risk factors, less angiogenic coronary artery disease, and lower risk for cardiovascular adverse events.[86,88]

Physical activity has also been shown to reduce risks associated with type 2 diabetes. Helmrich et al., examined in a large prospective cohort study, that each 500 kcal increase in energy expenditure per week was associated with a decreased incidence of type 2 diabetes by 6%. This finding was prominently found in those whom were at a high risk of developing

diabetes (i.e. individuals with a high BMI).[60,89] Interventions on physical activity have also shown to be effective in management of type 2 diabetes. Wei et al., found that physically inactive men with diagnosed type 2 diabetes had a 1.7-fold increased risk of premature death compared to their physically active counterparts.[60,90]

A study examining physical activity levels among breast, colorectal, and prostate cancer survivors found that those who participated in physical activity levels recommended by the American Cancer Society, (i.e. 30 minutes of moderate intensity physical activity on at least 5 or more days a week) reported significantly greater health-related quality of life compared to individuals who just adhered to dietary recommendations.[88] Studies in cancer patients of both breast and colon have looked as physical activity levels, and those individuals whom self-reported being physically active were found to have a decreased recurrence of cancer and risk of death from cancer.[60,91]

Physical activity has also been shown to provide health benefits regardless of BMI in adults. The fitness-fatness hypothesis suggests a higher level of cardiorespiratory fitness can substantially reduce adverse effects of obesity on morbidity and mortality, potentially making obesity not as important of a risk factor as generally believed. It has been found in multiple studies and meta-analyses that un-fit individuals have twice the risk of mortality regardless of BMI, compared to normal weight-fit individuals. Overweight and obese-fit individuals have a similar mortality risk as normal weight-fit individuals.[92-96] Again, suggesting performing physical activity and improving cardiorespiratory fitness is important for reducing negative health-related outcomes regardless of BMI.

2.5 Background on Klotho

The accelerated aging that occurs with obesity and the mechanism by which this occurs is of importance in order to better understand the increased risk for many negative health-related outcomes. One biomarker that may provide insight into to accelerated aging is Klotho. It was first discovered by Kuro-o et al., in 1997, who found an insertion mutation in the 5' flanking region of α-Klotho in mice that was associated with several symptoms of premature aging, including soft tissue calcification, arteriosclerosis, skin atrophy, gonadal dysplasia, infertility, hypoglycemia, severe hyperphosphatemia, osteoporosis, emphysema, and an overall shorter life span.[12,13] Human Klotho is primarily expressed in the kidney, but is also detectable in the placenta, prostate, and small intestines.[13] Circulating Klotho results from either direct secretion by the cell or from cleavage of the intracellular domain of the full-length protein by secretases. Both processes lead to 'soluble' Klotho, which is found in blood, urine, and cerebrospinal fluid.[14] It was originally thought that Klotho functioned primarily as a coreceptor for FGF23 binding to FGF receptor 1, and that their cooperativity function was restricted to the ectodomain of membrane Klotho. However, the current understanding is that Klotho exerts both FGF23-dependent and FGF23-independent, pleiotropic actions, while soluble Klotho acts as both a paracrine and endocrine factor.[12,14,15]

Klotho is thought to enter into the circulation system through three different potential pathways, (1) alternative RNA splicing, (2) proteolytic cleavage, (3) movement of Na+, K+-ATPase (adenosine triphosphatase). (1) Klotho genes can directly generate the secreted form of klotho protein by alternative RNA splicing, liberating klotho into the extracellular space and subsequently, the circulation.[97,98] (2) The transmembrane form of the klotho protein could also be cleaved by members of the ADAM (a disintegrin and metalloproteinases) family, and released

into the circulation system (*Figure 5*).[98,99] (3) Klotho is primarily expressed in kidney cells, but is not present on the cell surface, rather it is diffusely expressed in the cytoplasm, and overlaps on the endoplasmic reticulum and Golgi apparatus. It has been reported that in the endoplasmic reticulum and Golgi apparatus, the premature and mature form of klotho can bind to Na+, K+-ATPase. In this process, the klotho - Na+, K+-ATPase complex are potentially going up to the cell surface where klotho can be cleaved and secreted into the extracellular space and eventually the circulatory system.[98,100] With research leaning towards proteolytic cleavage as the primary pathway of secreted Klotho, the major source of secreted klotho still remains yet to be confirmed.

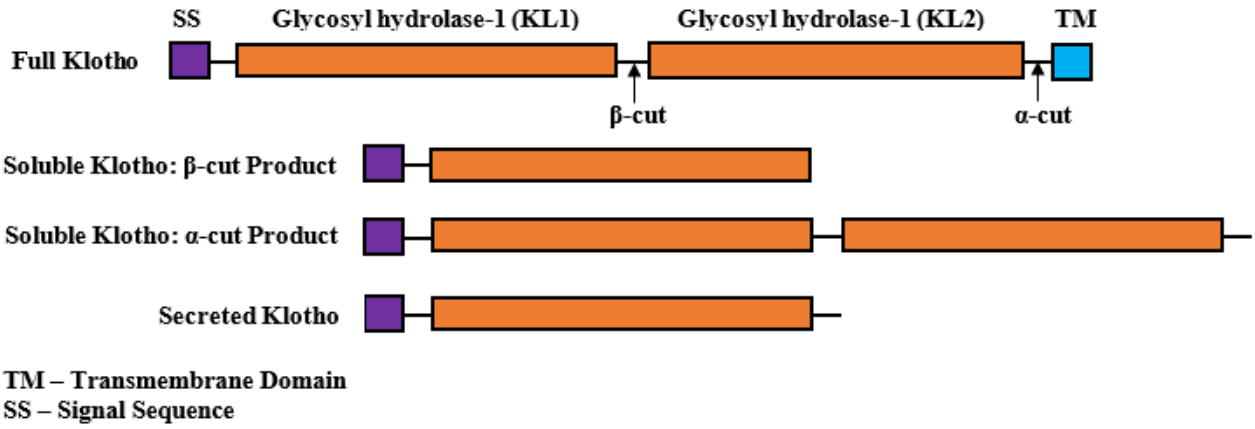

Figure 5. Primary Pathway Klotho Enters the Circulation, Proteolytic Cleavage.

Two Primary Cleavage Sites have been Identified in the Klotho Protein, One between the KL1 and KL2 Domains (β-cut) Cleaved by ADAM10, and the Second near the Transmembrane Region (α-cut) Cleaved by ADAM17. Adapted from Xu et al with Copyright permission.

As a hormone, Klotho targets multiple tissues and organs that result in anti-aging and cardiovascular protective properties. It reduces oxidative stress by inhibiting insulin and insulin-like growth factor-1 signaling pathways. It enhances endothelial nitric oxide production leading

to an improvement in endothelium-dependent vasodilation. It is an endogenous inhibitor of vascular calcification and it decreases cell-surface abundance of the Ca2+ channel TRPC6. This calcium channel is expressed in cardiac, glomerular, and vascular smooth muscle cells, and plays a vital role in the regulation of vascular resistance and blood pressure. The upregulation of TRPC6 leads to cardiac hypertrophy and glomerulosclerosis, therefore when Klotho downregulates the channel there is a potential protective effect.[14,101] Klotho may also influence many other pathways including the p53/p21 signal pathway, cAMP signal pathway, PKC signal pathway, and Wnt signal.[98]

Normal serum Klotho levels have been inconsistently studied, with results varying. Pedersen et al., analyzed Klotho on two different immunoassays to establish an assay specific reference interval for healthy adults. It was found that serum Klotho levels were significantly higher in time-resolved fluroescecne immunoassay (TRF) compared to an ELISA (IBL), with no correlation found between the assays. For the TRF immunoassay the reference interval for serum Klotho in males was 11-181ng/mL and for females 19-316ng/mL, respectively. In the ELISA immunoassay the reference interval was 204-741pg/mL, and no differences were found between genders. It was also found that serum Klotho levels were independently associate with estimated glomerular filtration rate and body weight with the TRF immunoassay, but with ELISA serum Klotho was only found to be associated with age.[102]

Klotho and its association to aging and longevity, has been found to be associated with other aging biomarkers including VEGF-A, IGF-1, and BDNF.[21] BDNF has been found to be involved in the process of neurogenesis, which is defined as the formation of new neurons from neural stem cells and progenitor cells which occurs in various brain regions such as the subgranular zone of dentate gyrus in the hippocampus and the subventricular zone of lateral

ventricles.[21,103] VEGF–A has been found to be involved in the process of angiogenesis, which is defined as the generation of new capillary blood vessels.[21,103] IGF-1 has been linked to the promotion of growth and maintenance of muscle.[21] While these biomarkers provide insight to human longevity, their influence on the molecular pathway of longevity appears to be associated with downstream effects rather than markers of early detection in accelerated aging.

2.6 Associations Between Klotho and Health-Related Outcomes

Low Klotho concentration has been linked through several studies to negative health-related outcomes including heart disease, cognition and brain health and musculoskeletal health. Klotho has been identified as the "anti-aging hormone," and so understanding where Klotho falls in these negative health-related outcome pathways is important in understanding how Klotho functions.

The Invecchiare in Chianti (Aging in Chianti study) "InCHIANTI" study, is an epidemiological research study primarily examining older adults and biomarkers, risk factors, and physiological subsystems of adults in order to provide better insight into precision medicine. This study has identified a relationship between low plasma Klotho levels and health outcomes including cognitive decline, poor grip strength, and cardiovascular disease.[104,105]

Shardell et al.,[105] examined 833 participants aged 55 years and older without dementia that were enrolled in the InCHIANTI study. Cognition was measured using the Mini-Mental State Examination (MMSE) and Trail-Making Tests A and B at baseline, 3-years, and 6-years. Plasma Klotho levels were measured at 3-year and 6-year visits. It was found that for every additional increase in the natural logarithim of Klotho (pg/mL) there was a 35% lower risk of

meaningful decline in MMSE. However no statistically significant associations were found between Klotho and Trail-Making Tests A or B. It is thought that there was no relationship detected in Trail-Making Tests A and B because they solely test executive function and motor skills, compared to the MMSE that tests global cognition.[105]

Semba et al.,[104] analyzed the relationship between plasma Klotho levels and grip strength in 804 adults who were 65 years or older enrolled in the InCHIANTI study. Grip strength and plasma klotho levels were measures at 3-year, 6-year, and 9-year visits. It was found in the unadjusted model that grip strength was positively correlated with plasma Klotho at a threshold of <681 pg/mL. Once adjusted for age, sex, education, smoking, physical activity, cognition, and chronic disease, plasma Klotho was still found to be significantly associated with grip strength ($p=0.0009$) in adults with plasma Klotho <681 pg/mL, suggesting lower plasma Klotho levels are associated with poor musculoskeletal strength.[104]

Similarly, Semba et al.,[106] examined whether plasma Klotho levels were related to prevalent cardiovascular disease in 1,023 adults aged 24 to 102 years using a cross-sectional design. Plasma Klotho levels were found to be associated with age, C-reactive protein, cognition, HDL cholesterol, and cardiovascular disease. Researchers then adjusted for cardiovascular risk factors (age, sex, smoking, total cholesterol, blood pressure, and diabetes) and found the log of plasma Klotho was significantly associated with prevalent cardiovascular disease with an odds ration per 1 standard deviation increase equaling 0.85 and a 95% confidence interval of 0.72 to 0.99. [106]

Plasma klotho concentrations and knee strength were also analyzed by Semba et al.,[107] in the Health, Aging, and Body Composition (Health ABC) Study, which is a community-based prospective study investigating the impact of changes in weight and body composition on age-

related physiological and functional changes. Plasma klotho concentrations were measured in 2,734 older adults, aged 71-80 years, with knee extension strength measured using isokinetic dynamometry at baseline, 2-year, and 4-year visits. Participants in the highest tertile of plasma Klotho levels were found to have higher knee extension strength at baseline compared with those in the lowest tertile, when adjusted for age, sex, race, smoking, study site, C-reactive protein, interleukin-6, and diabetes. Participants in the highest tertile of plasma Klotho at baseline were also found to have less deterioration in knee strength over the 4 years of follow-up assessments compared to those in the lowest tertile.[107]

When analyzing the gene sequence of Klotho, there have been single nucleotide polymorphisms (SNPs) on exons 2, 3, and 4, as well as in non-coding regions such as the promoter region and introns.[108,109] These SNPs have been found to be associated with many negative health-related outcomes including low bone mineral density, osteoarthritis, high systolic blood pressure, atherosclerotic coronary artery disease, cardiometabolic stroke, and high fasting glucose.[109-119] The greatest understood polymorphism of the Klotho gene is known as KL-VS, which contains six SNP sequence variants.[109,120] This allele has been observed in association with healthy aging in a recent meta-analysis from Di Bona et al.[109,121] It has been found to be associated with a heterozygous survival advantage over the age of 75, but intriguingly a homozygous disadvantage after 75 years due to negative signs and symptoms of coronary artery disease, HDL cholesterol, blood pressure, and stroke.[109,114,120] KL-VS has also been found to be associated with an increased risk of age-related decline of memory, and a lower incidence of the genotype in type 1 diabetics, as well as, retinopathy and low levels of inflammatory markers, pro-antigenic factors, and adhesion molecules.[109,114,115,120] However, the mechanistic pathway by which these SNPs display their effects are not fully understood.

2.7 Klotho, Obesity, and Aging

A number of epidemiological studies have linked elevated Klotho levels to improved health outcomes and longer lifespan in humans.[104-107] These outcomes highlight Klotho as a marker of biological age.[109] Klotho deficiency has been linked to a number of debilitating age-related illnesses, suggesting Klotho functions as an integrator of organ systems. Left ventricular myopathy has been linked to an imbalance in the FGF23/Klotho complex in patients with chronic kidney disease.[109,122] Circulating Klotho levels have been found to be inversely related to atrial fibrillation in individuals undergoing hemodialysis.[109,123] However, the exact mechanism by which these outcomes occur is in question. There is potential for Klotho levels to directly affect the pathway or phenotype which results in an excessive increase in FGF23 levels leading to a decrease in Klotho levels. As mentioned, Klotho levels also impact pathways including insulin/insulin-like growth factor-1, endothelial nitric oxide production, TRPC6, p53/p21, cAMP, Wnt, and PKC. [14,98,101] Studies have also linked Klotho to rapamycin (TOR), transforming growth factor-β pathways, as well as, mediating anti-inflammatory actions and maintenance of calcium balance. [109,124-126] Many of these pathways link Klotho and preservation or deterioration of longevity in older adults. However, a potential connection between Klotho and aging, is the weight status of an individuals because of the accelerated aging that occurs when an individual is overweight or obese.

Due to the last century's economic and scientific advances, aging is not the most frequent cause of death after the age of 28.[127,128] Because individuals live to an average age of 80 years old, this leads to a longer physiological aging process promoting changes in cellular function, metabolic rearrangements, and structural changes in organs.[127,128] Obesity has been recognized to promote the aging process by affecting the maturation process of adipose tissue, influencing

inflammation and glucose homeostasis, promoting insulin resistance, oxidative stress, DNA damage and telomere dysfunction, increased vasomotor tone, increase sympathetic drive, and more.[127,129] A major concern with the rise in prevalence of obesity, is the health of children. Children are presenting with diseases such as arterial hypertension and diabetes mellitus, typically found in middle-to-older age individuals. Children who are overweight or obese who do not present with disease, still prematurely develop abnormal endothelial cell dysfunction and arterial intima-thickening, almost mimicking the aging process in certain physiological aspects.[11,127,128,130-132]

The pathway that causes accelerated aging in overweight and obesity, may be similar to the anti-aging pathway that occurs with increased levels of Klotho. With this, there is evidence that weight status affects Klotho levels, with obese and underweight individuals both presenting with approximately half the levels of Klotho in the blood compared to those of normal weight,[17] linking obesity with Klotho levels and longevity. Therefore, it is important to investigate whether weight loss in individuals with overweight or obesity will reverse this accelerated aging process, as well as increase levels of Klotho.

2.8 Effect of Physical Activity and Weight Change on Klotho

As Klotho is linked to longevity as well as cardiovascular and neuroprotective effects, its' relationship to obesity and exercise is important to understand. Amitani et al.,[17] investigated Klotho's role in human metabolism by examining the association between plasma Klotho levels and BMI (normal weight, underweight/anorexia nervosa, obese). The average BMI for participants was 21.84 $\pm$ 0.36 kg/m^2 for normal weight individuals, 13.12 $\pm$ 0.26 kg/m^2 for

underweight/anorexia nervosa individuals, and 35.72 ± 3.17 kg/m^2 for obese individuals. These results show mean Klotho levels for participants to be 1391.62 ± 144.96 pg/mL for normal weight control participants, 764.64 ± 65.43 pg/mL for underweight/anorexia nervosa individuals, and 847.09 ± 111.31 pg/mL for obese individuals, with Klotho levels for underweight and obese individuals significantly differing from the normal weight control group. Individuals among the underweight/anorexia nervosa group were then put through a weight recovery program, resulting in an average BMI of 20.35 ± 1.38 kg/m^2, which was significantly greater than pre-weight recovery program BMI, as well as, in normal BMI range. This weight change also impacted Klotho levels, increasing them to 1004.96 ± 103.36 pg/mL, which was significantly greater than pre-weight recovery Klotho levels, and similar to normal weight control group levels.[17]

Similarly, Ohnishi et al.,[19] observed Klotho to be an important mediator of glucose metabolism, obesity, and hepatic steatosis. Mice whom had a deficiency in leptin were used because of their tendency to develop obesity and hyperglycemia. Klotho function was eliminated from certain mice which resulted in reduction of fat accumulation, reduced body weight, and suppressed fatty changes in the liver which in turn affected glucose metabolism. However, they concluded more research needs to be done to determine the molecular pathway between Klotho and obesity to provide insight into the pathobiology of obesity.[18,19]

The influence of exercise on Klotho is important to understand because it has been shown to play a key role in lifestyle behavior change for body weight regulation, as well as the prevention and treatment of numerous health-related conditions.[11,20] Avin et al.,[11] suggests an overlap in the physiological and functional response between Klotho expression and exercise. This was examined in sedentary, young (36.0 ± 7.0 years) and old (68.3 ± 3.0 years) females, by evaluating change in circulating Klotho levels before and after completion of an acute exercise

bout, as well as whether completion of an exercise training protocol affects the Klotho response to an acute exercise bout.[11] Prior to training, no significant changes in the circulating levels of Klotho in response to an acute exercise bout was observed. The younger participants then completed a 16-week training program consisting of four to six exercise sessions weekly, with the older participants only completing 12-weeks of the same exercise prescriptions. Following completion of the training program, there was a significant increase in circulating Klotho levels in response to an acute exercise bout in young individuals. Although there was an increase in circulating Klotho in response to an acute exercise bout following completion of the training program in older individuals, the effect of training was attenuated.[11]

Dalise et al.,[21] analyzed a similar relationship between exercise and Klotho in mice. Mice were allocated to a low (15 minute), medium (30 minutes), or higher (60 minute) running condition at 80% speed from their maximal capacity test. They performed a single running session per day, 5 times a week for 4 weeks. This resulted in a significant dose-dependent relationship between aerobic exercise and Klotho levels, with the medium condition containing double the amount of Klotho in the blood compared to controls, demonstrating a potential up-regulatory effect cause by exercise training on Klotho levels.[21]

When examining the effect of both obesity and exercise on circulating Klotho levels, there appears to be an association. Avin et al.,[11] stratified young participants by low BMI (<30 kg/m^2) and high BMI (>30 kg/m^2). Individuals in the higher BMI group demonstrated no change in circulating Klotho levels following an acute exercise bout at baseline. Following the 16-week training program, individuals in the higher BMI group trended towards a significant increase in Klotho levels (~30% increase; $p=0.06$) following an acute exercise bout.[11]

3.0 Methods

3.1 Methods from Parent Study

3.1.1 Subjects

Three hundred eighty-three (383) adults were randomized into 1 of 3 intervention conditions described below. For this study, a subsample of subjects who met the following criteria were selected and included for this ancillary study and secondary analysis.

1. Provided blood samples at the baseline, 6-month, and 12-month assessment periods.
2. Provided all other data at the baseline, 6-month, and 12-month assessment periods.
3. Were classified as an intervention "responder" (achieved $\geq$10% weight loss at both 6 months and 12 months) or "non-responder" (achieved <5% weight loss at both 6 months and 12 months).

Given the above criteria, 182 (responders = 131, non-responders = 51) of the 383 subjects randomized were eligible for this secondary analysis. Additional eligibility and ineligibility criteria for the parent study included the following:

Eligibility Criteria

1. 18-55 years of age.
2. Body mass index (BMI) between 25.0 to <40.0 kg/m^2.
3. Ability to provide informed consent prior to participation in this study.
4. Ability to provide consent from their personal physician to participate in this study.

5. The ability to complete the baseline graded exercise test, and clearance from the study physician to participate in this study after reviewing the results from this study.

Ineligibility Criteria

1. Unable to provide informed consent.
2. Household member on study staff.
3. Females who were currently pregnant, breastfeeding in the past 3 months, currently lactating, or reporting that she was planning a pregnancy within the next 12 months.
4. History of bariatric surgery.
5. Report current medical condition or treatment for a medical condition that could affect body weight. These may include the following: cancer (Note: Persons previously diagnosed with non-melanoma skin cancers, those successfully treated for cancer who have remained disease-free for five years or more were eligible for participation in this study); diabetes mellitus; hyperthyroidism; inadequately controlled hypothyroidism; chronic renal insufficiency; chronic liver disease; gastrointestinal disorders including ulcerative colitis, Crohn's disease, or malabsorption syndromes; etc.
6. Current congestive heart failure, angina, uncontrolled arrhythmia, symptoms indicative of an increased acute risk for a cardiovascular event, prior myocardial infarction, coronary artery bypass grafting or angioplasty, conditions requiring chronic anticoagulation (i.e. recent or recurrent DVT).
7. Resting systolic blood pressure of >160 mmHg or resting diastolic blood pressure of >100 mmHg, taking medication for blood pressure control, or taking medication that can affect blood pressure or heart rate response to exercise (e.g., beta blocker).

8. Eating disorders that would contraindicate weight loss or physical activity.
9. Alcohol or substance abuse.
10. Currently treated for psychological issues (i.e., depression, bipolar disorder, etc), taking psychotropic medications within the previous 12 months, or hospitalized for depression within the previous 5 years.
11. Report exercise >60 minutes per week over the past 3 months. (NOTE: It is important that individuals are sedentary when entering this study to allow for maximal effect of the intervention.).
12. Report weight loss of >5% or participating in a weight reduction diet in the past 3 months.
13. Report plans to relocate to a location not accessible to the study site or having employment, personal, or travel commitments that prohibit attendance to at least 80 percent of the scheduled intervention sessions and all of the scheduled assessments.

3.1.2 Recruitment, Screening, and Informed Consent

Subjects were recruited through advertisements that were approved by the local Institutional Review Board. Individuals responding to the advertisements were instructed to call the investigators by telephone to obtain further information about the study. Upon receipt of a telephone call, staff provided a brief description of the study. Individuals interested in study participation after hearing the description, answered questions to determine initial eligibility based on the criteria listed above. Individuals who appeared to be eligible based on the initial telephone screen were invited to an orientation session where the study was explained to them in

greater detail, components of informed consent were explained, and the individuals was given the opportunity to ask additional questions to the investigators.

Prior to undergoing any experimental procedures for this study, written informed consent was obtained from the potential subject, as well as a medical history and physical activity readiness questionnaire to confirm that no conditions were present that would exclude the participant. Moreover, prior to undergoing any experimental procedures, individuals also provided medical clearance from their personal physician stating that it was safe to participate in a weight loss intervention that included a reduced energy intake diet and exercise.

3.1.3 Research Design and Randomization

Following baseline assessments to confirm eligibility and to collect additional study-related data, eligible individuals were randomly assigned to one of three weight loss intervention conditions. All participants received a behavioral weight loss intervention that included attendance at group-based intervention sessions from weeks 1-24 and then every other week during weeks 25-52. In addition, participants received a brief telephone contact with an intervention staff member approximately twice per month during weeks 25-52. A brief description of the intervention conditions is the following:

1. DIET: This group was prescribed a diet that reduced energy intake of 1,200-1,800 kcal/day. No physical activity recommendations were provided to the DIET group.
2. DIET + PA150: This group was prescribed the same dietary intervention as the DIET group. In addition, the DIET+PA150 condition was prescribed a progression to 150 min/week of unsupervised moderate-to-vigorous intensity physical activity per week.

3. DIET+PA250: This group was prescribed the same dietary intervention as the DIET group. In addition, the DIET+PA250 condition was prescribed a progression to 250 min/week of unsupervised moderate-to-vigorous intensity physical activity per week.

3.1.4 Behavioral Weight Loss Intervention

Intervention Sessions: Subjects in all intervention conditions were instructed to attend weekly weight loss group sessions for weeks 1-24 and approximately every other week during weeks 25-52. These groups were closed to only those participants randomly assigned to a particular intervention condition (DIET, DIET+PA150, DIET+PA250). These intervention sessions focused on behavioral strategies to reduce energy intake and for DIET+PA150/DIET+PA250 to also increase exercise consistent with the intervention protocol. Sessions were led by a variety of professionals that included exercise physiologists and nutritionists. If a group session was missed a brief individual make-up session was offered to allow the content to be shared with the subject. Body weight was measured at each of the intervention sessions to determine responsiveness of the subject to the weight loss program and to provide ongoing feedback. Subjects unable to attend either the group session or the make-up session were mailed intervention materials that were distributed to the other subjects at the intervention session.

In addition to the in-person group sessions, participants received an individual brief (approximately 10 minutes in duration) telephone contact from a member of the intervention staff approximately twice per month during weeks 25-52 on weeks when an in-person session was not scheduled. This telephone contact was intended to provide an opportunity for individual interaction with the intervention staff to assist in understanding of the intervention content and to

address barriers to engagement or adherence to the intervention components. The interventionist used a standard script to direct the approach and content of this telephone contact.

Diet Intervention: The identical dietary intervention was provided to all subjects regardless of randomized intervention assignment (DIET, DIET+PA150, DIET+PA250). This included prescribing subjects to consume 1,200-1,800 kcal per day, and to reduce their dietary fat intake to 20-30 percent of their total daily energy intake. Initial energy intake as determined based on baseline body weight and then adjusted based on weight loss response across the intervention. Meal plans, which were developed by registered dietitians, were provided to facilitate adoption and compliance with these dietary recommendations. In addition, participants self-monitored their dietary intake in a food diary that was returned to the intervention staff at each intervention session, with the intervention staff reviewing these diaries and providing written feedback relative to self-reported eating behavior.

Physical Activity Intervention: By study design the DIET group did not receive information about physical activity nor was physical activity prescribed. The physical activity prescription differed between randomized intervention conditions (DIET+PA150 and DIET+PA250) as described below.

Subjects in the DIET+PA150 intervention were instructed to engage in moderate intensity physical activity 5 days per week. The total duration per day began at 20 minutes per day and gradually progressed to at least 30 minutes per day. Physical activity was progressed in a gradual manner (5 min/d in 4-week intervals) to maximize adherence and minimize the onset of musculoskeletal injuries. Moderate intensity was prescribed was set using the Borg 15-point Rating of Perceived Exertion (RPE) scale, with the range set at 13-15 on this scale.

Subjects in the DIET+PA250 intervention were instructed to engage in moderate intensity physical activity 5 days per week. The total duration per day began at 20 minutes per day and gradually progressed to at least 50 minutes per day. Physical activity was progressed in a gradual manner (5 min/d in 4-week intervals) to maximize adherence and minimize the onset of musculoskeletal injuries. Moderate intensity was prescribed was set using the Borg 15-point Rating of Perceived Exertion (RPE) scale, with the range set at 13-15 on this scale.

Participants self-monitor physical activity behaviors (MOD-PA and HIGH-PA) in a weekly diary provided by the study. Participants were instructed to return the diary to the intervention staff at each in-person visit for review, and the intervention staff provided written feedback on the diary prior to it being returned to the participant.

3.1.5 Assessment Procedures

Within the parent study that is providing data to address the specific aims as described in Chapter 1, outcome data were collected at baseline, 6-months, and following the 12-month weight loss intervention. The assessments included measurements of demographic characteristics (e.g. race/ethnicity, age, etc.), height, weight, body mass index (BMI), body composition, dietary intake, physical activity, cardiorespiratory fitness, and collection of fasting blood samples. These data will be used for this ancillary study and secondary data analyses, with the specific procedures used to assess the outcomes described below:

Height, Weight, and BMI: Weight and height were assessed with the subject clothed in a lightweight hospital gown with shoes removed. Weight was assessed using a calibrated digital scale to the nearest 0.1 kg with duplicate measures differing by $\leq$0.2 kg. Height was assessed

using a wall-mounted stadiometer to the nearest 0.1 cm with duplicate measures differing by ≤0.5 cm. Weight and height were used to compute BMI (kg/m^2).

Body Composition: Total body composition (fat mass, lean mass, percent body fat) was measured from a total body scan using a dual-energy x-ray absorptiometry (DXA, GE Lunar iDXA, Madision, WI). For this measurement, participants were clothed in a cloth hospital gown with metal removed (e.g., rings, watch, earrings, etc.). Women also completed a urine pregnancy test to confirm non-pregnancy prior to this measurement.

Cardiorespiratory Fitness: Subjects participated in an assessment of cardiorespiratory fitness. Subjects were requested to abstain from vigorous activity for 24 hours prior to the assessment period. American College of Sports Medicine (ACSM)[133] criteria were used to exclude subjects from this study for whom exercise is contraindicated based on the results of this exercise test.

The speed of the treadmill was kept constant at 3.0 mph (80.4 m/min) with the initial grade of the treadmill being 0% and increasing at 1.0% increments at 1-minute intervals. Heart rate during exercise testing was obtained at one-minute intervals using a 12-lead ECG and immediately upon termination of the exercise test. Blood pressure was obtained during each even minute (2 min, 4, min, 6 min etc.) and immediately upon termination of the exercise test. Rating of perceived exertion was assessed during the final 15 seconds of each minute and at the point of test termination. The test performed to assess cardiorespiratory fitness was a submaximal test that was terminated when the participant first achieved or exceeded 85% of their age-predicted maximal heart rate (HR_{Max} = 220 minus age). A physician evaluated the results of each exercise test to ensure that exercise training was not contraindicated.

Oxygen consumption was measured continuously using a SensorMedics Vmax Encore (SensorMedics Corporation, Yorba Linda, CA) metabolic cart, with gas volumes and concentrations calibrated according to manufacturer specifications prior to each test. Fitness is expressed in absolute (L/min) and relative terms (ml/kg/min). Change in cardiorespiratory fitness is computed as the difference between these values on the baseline test and on the subsequent tests.

Blood Samples: Blood samples were collected in the morning, between 7:30 AM and 10:30 AM, with participants instructed to fast with the exception of water, abstain from exercise, and abstain from alcohol and smoking for at least 12 hours. This was confirmed prior to blood collection. Blood was collected into evacuated tubes, processed in a refrigerated centrifuge, and pipetted into 2.0 mL cryovials prior to storage at -80^{o}C. For this study, the stored serum samples were used for analysis of Klotho.

Klotho was measured in duplicate by solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (Immuno-Biological Laboratories, Takasaki, Japan).[134] Blood samples were analyzed at the University of Pittsburgh McGowan Institute for Regenerative Medicine, with intra- and inter-assay coefficients of variation determined for these specific Klotho assays. Procedures for the analysis can be found in Appendix A.

3.2 Data Analysis

A power analysis was conducted for the parent study that took into account the potential for 20% attrition to the randomized sample. The parent study recruited and randomized 383 participants, which constitutes that overall sample available for this ancillary study. Based on

the criteria for classification as a “responder” or “non-responder”, data from 182 (47.5%) of the randomized sample of participants were eligible for this ancillary study and secondary analyses. Of the 182 participants, 152 provided blood samples at all three timepoints (see Table 1).

Table 1. Number of participants by randomized intervention condition by weight loss response.

	Intervention Condition		
Classification	**DIET**	**DIET+PA150**	**DIET+250**
N=182			
Responder	43	44	44
Non-Responder	20	15	16
N=152*			
Responder	37	35	39
Non-Responder	15	13	13
*Indicates that blood samples were available for Klotho analysis at 0, 6, and 12 months.			

Statistical significance was defined at $p \leq 0.05$. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software, version 25. Data were examined for normality prior to analysis, with non-normally distributed data analyzed using non-parametric statistical techniques.

Descriptive baseline data are expressed as either mean $\pm$ standard deviation or median (25^{th}, 75^{th} percentile) for continuous variables and as frequencies for categorical variables. The analysis plan listed below:

1. To examine Specific Aim 1, non-parametric Spearman rank-order correlations were performed to assess the association between Klotho and measures of weight, obesity status, and cardiorespiratory fitness.
2. To examine Specific Aim 2, a repeated-measures (treatment X time) analysis of variance (ANOVA) was performed to examine change in Klotho concentration by intervention condition across the 12-month weight loss intervention.

3. To examine Specific Aim 3, to examine the change in Klotho concentration across the 12-month behavioral weight loss intervention weight loss response (responder vs. non-responder), and to take into account the randomized intervention group, a three-factor repeated-measures ANOVA (treatment X weight loss response classification X time) was performed.
4. (Exploratory) To examine the association between baseline Klotho concentration and change at either 6 months or 12 months for weight, body composition, or cardiorespiratory fitness, Spearman rank order coefficients were performed.
5. (Exploratory) To examine whether change in weight, change in body composition, or change in cardiorespiratory fitness differed by quartile of baseline Klotho, and to account for initial randomization condition, a series of three-factor repeated-measure ANOVAs (treatment X quartile X time) were performed.

4.0 Results

The primary aim of this study was to examine the change in Klotho concentration across behavioral weight loss interventions in adults who were overweight or obese. This was a secondary analysis of a 12-month randomized weight loss trial with assessments at baseline, 6 months, and 12 months. All blood sample processing was performed at the University of Pittsburgh McGowan Institute for Regenerative Medicine, with all other study procedures (physical assessments, blood collection, and behavioral intervention) being performed at the University of Pittsburgh Physical Activity and Weight Management Research Center.

4.1 Study Participants

One-hundred eighty-two (182) of three-hundred eighty-three (383) adults from the parent study who completed assessments at baseline, 6-, and 12-months and were classified as "responders" or "non-responders" to the behavioral interventions were to be included in this secondary analysis. One-hundred fifty-two (152) individual's completed assessments at baseline, 6-, and 12-months and their serum samples were included in this analysis. Baseline characteristics by sample are shown in Table 2. Figure 6 illustrates participants included in the secondary analysis.

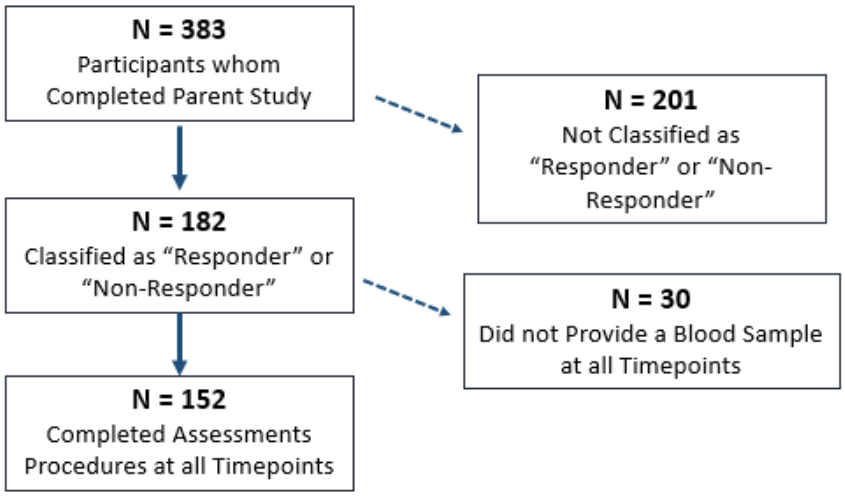

Figure 6. Consort Diagram

Table 2. Baseline Characteristics by Sample

	Total Sample (N=383) Mean ± SD*	**Sample Categorized as "Responder" or "Non-Responder" (N=182) Mean ± SD***	**Sample with Complete Blood Samples for Klotho Analysis (N=152) Mean ± SD***
Age (years)	45.0±7.9	45.5±7.9	45.4±8.0
Weight (kg)	90.9±13.7	91.0±13.6	90.4±13.1
Body Mass Index (kg/m^2)	32.4±3.8	32.3±3.8	32.1±3.7
Lean Mass (kg)	48.6±8.7	48.5±8.9	48.1±8.7
Fat Mass (kg)	39.2±8.2	39.3±7.7	39.1±7.8
Tissue Percent Body Fat (%)	44.5±5.6	44.8±5.5	44.8±5.6
Region Percent Body Fat (%)	43.2±5.5	43.4±5.4	43.5±5.6
Cardiorespiratory Fitness (L/min)	2.1±0.5	2.1±0.5	2.1±0.5
Cardiorespiratory Fitness (ml/kg/min)	22.6±4.4	22.7±4.6	22.9±4.5
Cardiorespiratory Fitness (Termination Time - minutes)	7.7±3.0	7.9±3.0	8.0±3.0
Female (N,%)	304, 79.4%	141, 77.5%	118, 77.6%
Non-White (N,%)	104, 27.2%	44, 24.2%	36, 23.7%
***SD – Standard deviation**			

4.2 Change in Key Variables Across the 12-Month Intervention

Analyses were conducted to examine the impact the behavioral weight loss intervention, and response to the intervention ("responder" vs. "non-responder"), had on key variables that included measures of weight, body composition, and cardiorespiratory fitness. Separate three-factor repeated-measure ANOVA's (treatment X weight loss response classification X time) were performed for each of these variables. Results are presented in Tables 3-10.

Analysis revealed body weight significantly decreased across time (p=0.000), with no significant difference between treatment conditions (p=0.606). However, by definition weight loss was significantly different by weight loss response classification (p=0.001), with "responders" losing a greater amount of weight compared to "non-responders" (Table 3). Similarly, body mass index significantly decreased across time (p=0.000), with no significant difference between treatment conditions (p=0.128). Body mass index was significantly different by weight loss response classification (p=0.000), with individuals in the "responder" classification having a greater decrease in BMI across the 12-month intervention compared to "non-responders" (Table 4).

Table 3. Change in body weight (kg) by treatment condition and weight loss response (responder vs. non-responder)

Treatment	Category	Body Weight (kg)			p-values					
		Baseline	6 Months	12 Months	Treatment	Category	Time	Treatment x Time	Category X Time	Category X Treatment X Time
DIET (N=52)	Non-Responder (N=15)	90.4±9.2	89.6±10.4	91.9±11.5	0.606	0.001	0.000	0.328	0.000	0.355
	Responder (N=37)	91.3±14.3	77.6±11.5	74.9±11.7						
DIET+150 (N=48)	Non-Responder (N=13)	87.9±17.6	86.1±18.0	86.7±18.3						
	Responder (N=35)	91.8±11.0	76.2±9.3	73.8±10.4						
DIET+250 (N=52)	Non-Responder (N=13)	87.5±10.7	86.1±10.1	86.6±9.8						
	Responder (N=39)	89.9±14.6	76.1±13.3	74.0±12.9						

Table 4. Change in body mass index (kg/m2) by treatment condition and weight loss response (responder vs. non-responder)

Treatment	Category	Body Mass Index (kg/m^2)			p-values					
		Baseline	6 Months	12 Months	Treatment	Category	Time	Treatment x Time	Category X Time	Category X Treatment X Time
DIET (N=52)	Non-Responder (N=15)	33.2±3.3	32.9±3.8	33.7±4.0	0.128	0.000	0.000	0.176	0.000	0.274
	Responder (N=37)	32.1±3.1	27.3±3.0	26.2±2.7						
DIET+150 (N=48)	Non-Responder (N=13)	31.1±3.8	30.4±3.8	30.6±4.0						
	Responder (N=35)	32.7±3.5	27.2±3.2	26.3±3.3						
DIET+250 (N=52)	Non-Responder (N=13)	30.9±4.1	30.4±3.8	30.6±3.6						
	Responder (N=39)	31.8±4.1	26.9±3.8	26.2±3.7						

For measures of body composition, lean mass significantly decreased across the 12-month intervention (p=0.000), with no difference by treatment conditions (p=0.724). Moreover, lean mass was significantly different by weight loss response across time (p=0.000), with individuals in the "responder" classification having a greater decrease in lean mass compared to individuals in the "non-responder" classification over the 12-month intervention (Table 5).

Table 5. Change in lean mass (kg) by treatment condition and weight loss response (responder vs. non-responder)

Treatment	Category	Lean Mass (kg)			p-values					
		Baseline	6 Months	12 Months	Treatment	Category	Time	Treatment x Time	Category X Time	Category X Treatment X Time
DIET (N=52)	Non-Responder (N=15)	46.3± 4.5	46.4± 5.0	47.0± 4.5	0.724	0.872	0.000	0.490	0.000	0.087
	Responder (N=37)	48.8± 10.3	46.3± 9.2	46.3± 9.5						
DIET+150 (N=48)	Non-Responder (N=13)	48.5± 1.3	48.8± 1.4	47.9± 12.8						
	Responder (N=35)	49.0± 8.3	46.6± 7.6	46.6± 7.5						
DIET+250 (N=52)	Non-Responder (N=13)	46.8± 8.7	46.6± 1.3	46.3± 8.9						
	Responder (N=39)	47.4± 7.4	45.6± 7.5	45.5± 7.2						

Fat mass significantly decreased across the 12-month intervention (p=0.000) with no difference by treatment conditions (p=0.159). However, fat mass was significantly different by weight loss response (p=0.000), with "responders" losing a greater amount of fat mass compared to "non-responders" over the 12-month intervention (Table 6). Similarly, percent body fat mass significantly decreased across time (p=0.000), with no difference by treatment condition (p=0.187). Percent fat mass was significantly

different by weight loss response (p=0.000), with “responders” losing a greater percent of fat mass across the 12-month intervention compared to “non-responders” (Table 7).

Table 6. Change in fat mass (kg) by treatment condition and weight loss response (responder vs. non-responder)

Treatment	Category	Fat Mass (kg)			p-values					
		Baseline	6 Months	12 Months	Treatment	Category	Time	Treatment x Time	Category X Time	Category X Treatment X Time
DIET (N=52)	Non-Responder (N=15)	41.0± 5.0	40.1±5.9	42.3± 7.1	0.159	0.000	0.000	0.254	0.000	0.536
	Responder (N=37)	39.2± 6.4	28.3±5.9	25.3± 6.1						
DIET+150 (N=48)	Non-Responder (N=13)	36.3± 7.1	34.3± 6.7	35.6± 8.0						
	Responder (N=35)	39.6± 7.2	26.7± 7.7	24.4± 7.9						
DIET+250 (N=52)	Non-Responder (N=13)	37.7± 8.9	36.5± 8.9	37.2± 8.6						
	Responder (N=39)	38.5± 7.2	27.6± 9.5	25.8± 9.3						

Table 7. Change in percent body fat by treatment condition and weight loss response (responder vs. non-responder)

Treatment	Category	Region Percent Body Fat (%)			p-values					
		Baseline	6 Months	12 Months	Treatment	Category	Time	Treatment x Time	Category X Time	Category X Treatment X Time
DIET (N=52)	Non-Responder (N=15)	45.5±2.0	44.9±2.1	45.8±2.6	0.187	0.000	0.000	0.337	0.000	0.823
	Responder (N=37)	43.4±4.8	36.8±6.0	34.2±6.4						
DIET+150 (N=48)	Non-Responder (N=13)	41.9±5.9	40.4±6.0	41.7±6.1						
	Responder (N=35)	43.3±6.1	34.9±8.2	32.8±8.0						
DIET+250 (N=52)	Non-Responder (N=13)	43.2±7.5	42.5±7.9	43.2±7.9						
	Responder (N=39)	43.5±6.0	35.7±8.0	34.2±7.7						

Data for measures of cardiorespiratory fitness are shown in Tables 8-10. There were no significant changes in absolute cardiorespiratory fitness (L/min) across time, by intervention group or weight loss response classification, and no group by time or classification by time effects (Table 8). However, there was a significant increase in relative cardiorespiratory fitness (ml/kg/min) across the 12-month intervention (p=0.000). Relative cardiorespiratory fitness (ml/kg/min) differed by intervention group (p=0.014), with individuals in the DIET+PA250 group having the greatest increase in fitness levels, then the DIET+PA150 group, followed by the DIET group. Further, there was a significant difference by weight loss response (p=0.000), with "responders" having greater increases in cardiorespiratory fitness level (ml/kg/min) across the 12-month intervention compared to "non-responders" (Table 9). A similar pattern of response was observed when test termination time was used to represent cardiorespiratory fitness (Table 10).

Table 8. Change in absolute cardiorespiratory fitness (L/min) by treatment condition and weight loss response (responder vs. non-responder)

Treatment	Category	Cardiorespiratory Fitness (L/min)			p-values					
		Baseline	6 Months	12 Months	Treatment	Category	Time	Treatment x Time	Category X Time	Category X Treatment X Time
DIET (N=51)	Non-Responder (N=14)	2.0±0.5	1.9±0.4	1.8±0.4	0.826	0.147	0.052	0.122	0.720	0.625
	Responder (N=37)	2.1±0.6	2.0±0.5	2.0±0.5						
DIET+150 (N=45)	Non-Responder (N=11)	1.9±0.5	1.9±0.6	1.9±0.5						
	Responder (N=34)	2.1±0.5	2.2±0.5	2.1±0.5						
DIET+250 (N=51)	Non-Responder (N=12)	2.0±0.5	2.0±0.5	2.0±0.5						
	Responder (N=39)	2.1±0.5	2.1±0.5	2.1±0.5						

Table 9. Change in relative cardiorespiratory fitness (ml/kg/min) by treatment condition and weight loss response (responder vs. non-responder)

Treatment	Category	Cardiorespiratory Fitness (ml/kg/min)			p-values					
		Baseline	6 Months	12 Months	Treatment	Category	Time	Treatment x Time	Category X Time	Category X Treatment X Time
DIET (N=51)	Non-Responder (N=14)	21.9±5.1	21.8±4.2	20.0±4.1	0.111	0.000	0.000	0.014	0.000	0.448
	Responder (N=37)	22.8±4.4	25.3±4.4	26.1±4.7						
DIET+150 (N=45)	Non-Responder (N=11)	23.1±5.4	23.6±6.0	23.2±5.4						
	Responder (N=34)	23.3±4.7	28.6±6.8	29.3±6.1						
DIET+250 (N=51)	Non-Responder (N=12)	23.2±4.5	23.0±5.3	22.8±4.9						
	Responder (N=39)	22.9±3.9	27.4±4.8	27.9±4.7						

Table 10. Change in cardiorespiratory fitness (minutes) by treatment condition and weight loss response (responder vs. non-responder)

Treatment	Category	Cardiorespiratory Fitness (min)			p-values					
		Baseline	6 Months	12 Months	Treatment	Category	Time	Treatment x Time	Category X Time	Category X Treatment X Time
DIET (N=52)	Non-Responder (N=15)	6.9±2.3	7.8±2.3	6.4±2.1	0.032	0.001	0.000	0.042	0.000	0.053
	Responder (N=37)	7.7±2.8	9.8±3.1	89.6±3.1						
DIET+150 (N=47)	Non-Responder (N=12)	8.1±3.9	8.7±3.6	9.1±3.7						
	Responder (N=35)	8.3±3.3	11.9±4.2	11.9±4.0						
DIET+250 (N=52)	Non-Responder (N=13)	8.9±3.1	9.1±3.7	7.7±2.8						
	Responder (N=39)	8.0±2.6	11.3±3.1	11.4±2.8						

4.3 Klotho Response

4.3.1 Specific Aim 1: Associations Between Klotho Concentration and Measures of Weight, Body Mass Index, Body Composition, and Cardiorespiratory Fitness

Analyses were conducted to examine the association between Klotho concentration and corresponding measures of weight, body mass index, body composition, and cardiorespiratory fitness. A Spearman rank-order correlation coefficient was performed for each variable (N=152; cardiorespiratory fitness: N=150). A non-parametric test was used due to skewness and slight kurtosis in Klotho concentration. Results are presented in Table 11.

At baseline, Klotho concentration was found to be inversely associated with lean body mass (p=0.021), with a lower lean mass associated with higher Klotho levels. Further, baseline Klotho concentration was inversely associated with absolute cardiorespiratory fitness (p=0.042), with a lower cardiorespiratory fitness level associated with a higher Klotho concentration. However, at baseline there were no significant associations between Klotho concentration and weight, BMI, fat mass, percent body fat, relative cardiorespiratory fitness, or cardiorespiratory fitness test time.

At 6 months, Klotho concentration was inversely associated with lean body mass (p=0.005), with a lower lean body mass being associated with a higher Klotho concentration. Moreover, Klotho concentration was significantly associated with percent body fat at 6 months (p=0.019), with a greater percent body fat being associated with a higher Klotho concentration.

However, at 6 months there were no significant associations between Klotho concentration and weight, BMI, fat mass, or cardiorespiratory fitness.

At 12 months, Klotho concentration was only found to be inversely associated to lean body mass (p=0.020), with less lean mass being associated with higher Klotho levels. There were no significant associations found at 12 months between Klotho concentration and measures of weight, BMI, fat mass, percent body fat, or cardiorespiratory fitness.

Table 11. Correlation between Klotho and corresponding measures of weight, body mass index, body composition, and cardiorespiratory fitness

Variable	**Assessment Period**	***Klotho (pg/mL)***		
		Baseline	6 Months	12 Months
Body Weight (kg)	Baseline	r= -0.121 (p=0.136)	-----	-----
	6 Months	-----	r= -0.137 (p=0.093)	-----
	12 Months	-----	-----	r= -0.147 (p=0.070)
Body Mass Index (kg/m^2)	Baseline	r= -0.103 (p=0.208)	-----	-----
	6 Months	-----	r= -0.012 (p=0.881)	-----
	12 Months	-----	-----	r= -0.112 (p=0.168)
Lean Mass (kg)	Baseline	r= -0.188 (p=0.021)	-----	-----
	6 Months	-----	r= -0.229 (p=0.005)	-----
	12 Months	-----	-----	r= -0.188 (p=0.020)
Fat Mass (kg)	Baseline	r=0.021 (p=0.797)	-----	-----
	6 Months	-----	r=0.072 (p=0.379)	-----
	12 Months	-----	-----	r= -0.014 (p=0.867)
Region Percent Body Fat	Baseline	r=0.129 (p=0.114)	-----	-----
	6 Months	-----	r=0.191 (p=0.019)	-----
	12 Months	-----	-----	r=0.088 (p=0.281)
Absolute Cardiorespiratory Fitness (L/min)	Baseline	r= -0.165 (p=0.042)	-----	-----
	6 Months	-----	r= -0.125 (p=0.129)	-----
	12 Months	-----	-----	r= -0.122 (p=0.140)
Relative Cardiorespiratory Fitness (ml/kg/min)	Baseline	r= -0.074 (p=0.367)	-----	-----
	6 Months	-----	r= -0.066 (p=0.425)	-----
	12 Months	-----	-----	r= --0.015 (p=0.856)
Cardiorespiratory Fitness (minutes)	Baseline	r= -0.042 (p=0.607)	-----	-----
	6 Months	-----	r= -0.117 (p=0.151)	-----
	12 Months	-----	-----	r= -0.050 (p=0.539)

4.3.2 Specific Aim 2: Examine the Change in Klotho Concentration by Intervention Group Across Time

A Repeated-measures ANOVA was conducted to examine the change in Klotho concentration by intervention group across the 12-month weight loss intervention (Table 12 and Figure7). A significant change in Klotho concentration across time (p=0.009) was observed, with Klotho concentration increasing at 6 months followed by a decrease in concentration at 12-months. However, there was no significant difference by intervention group (p=0.345), nor was there a significant treatment X time effect (p=0.329) across the 12-month intervention.

Table 12. Mean change in Klotho concentration by intervention condition and weight loss classification across the 12-month behavioral weight loss intervention

	Timepoint Mean ± SD			P-Value		
Condition	Baseline	6 Months	12 Months	Time	Treatment	Treatment x Time
DIET N=52	900.4±411.7	942.4±449.3	863.2±377.6	0.009	0.345	0.329
DIET+PA150 N=48	898.6±327.8	979.7±445.0	944.1±409.1			
DIET+PA250 N=52	998.7±392.7	1031.6±460.6	1014.0±471.7			

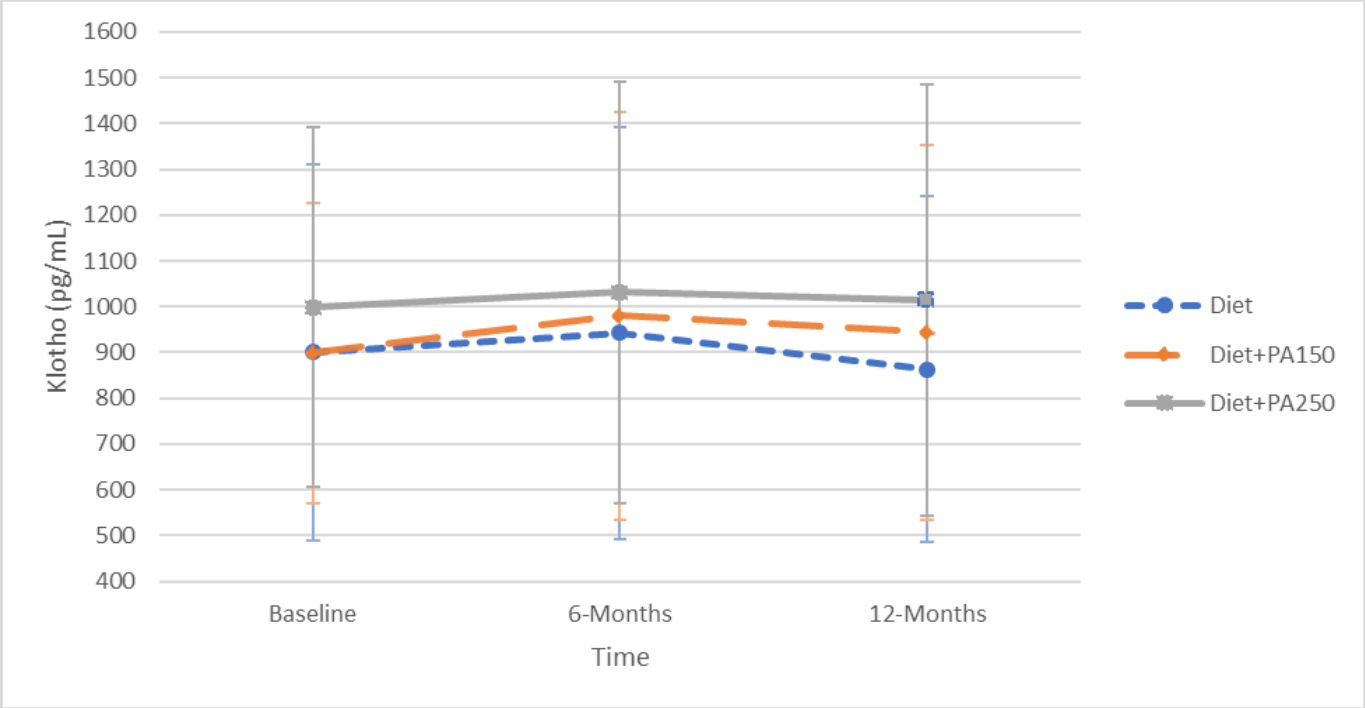

Figure 7. Change in mean Klotho concentration by intervention group across the 12-month behavioral weight loss intervention.

4.3.3 Specific Aim 3: Examine the Change in Klotho Concentration by Intervention Group and Weight Loss Classification Across Time

Analyses were conducted to examine the change in Klotho concentration across the 12-month behavioral weight loss intervention by intervention group and by weight loss response classification. Analyses performed was a three-factor repeated-measures ANOVA (treatment X weight loss response classification X time) to account for the randomized intervention group assignments, with results presented in Table 13. There were no significant differences by weight loss response ($p=0.477$). Moreover, the weight loss response X time ($p=0.284$), and treatment X classification X time effects ($p=0.748$) was not statistically significant.

Table 13. Change in Klotho (pg/mL) by treatment condition and weight loss response (responder vs. non-responder)

Treatment	Category	Klotho (pg/mL)			p-values					
		Baseline	6 Months	12 Months	Treatment	Category	Time	Treatment x Time	Category X Time	Category X Treatment X Time
DIET (N=52)	Non-Responder (N=15)	1051.1±562.1	1055.6±553.7	943.3±413.1	0.538	0.477	0.094	0.216	0.284	0.748
	Responder (N=37)	839.3±322.4	896.5±399.0	830.7±363.2						
DIET+150 (N=48)	Non-Responder (N=13)	750.0±257.1	784.7±277.6	805.8±276.1						
	Responder (N=35)	953.7±337.0	1052.2±476.0	995.5±440.9						
DIET+250 (N=52)	Non-Responder (N=13)	958.0±339.4	936.9±309.3	933.7±480.2						
	Responder (N=39)	1012.3±412.2	1063.2±500.4	1040.7±472.1						

4.3.4 (Exploratory) Baseline Klotho Concentration to Predict Change in Weight, Body Composition, and Cardiorespiratory Fitness by Intervention Group and Weight Loss Classification

Exploratory analyses were conducted to examine whether baseline Klotho concentration is associated with measures of change at either 6 months or 12 months for weight, body composition, or cardiorespiratory fitness. Spearman rank order coefficients were computed, with results presented in Table 14. No significant correlations were found between baseline Klotho concentration and change in weight, change in body composition, or change in cardiorespiratory fitness.

Table 14. Correlation between Baseline Klotho and change in weight, body composition, and cardiorespiratory fitness

Variable		Baseline Klotho
Body Weight (kg)	Change from Baseline to 6 Months*	r=0.064 (p=0.430)
	Change from Baseline to 12 Months**	r=0.013 (p=0.878)
Lean Mass (kg)	Change from Baseline to 6 Months*	r=0.188 (p=0.147)
	Change from Baseline to 12 Months**	r=0.125 (p=0.124)
Fat Mass (kg)	Change from Baseline to 6 Months*	r=0.031 (p=0.708)
	Change from Baseline to 12 Months**	r= -0.015 (p=0.856)
Percent Body Fat	Change from Baseline to 6 Months*	r=0.019 (p=0.817)
	Change from Baseline to 12 Months**	r= -0.013 (p=0.869)
Absolute Cardiorespiratory Fitness (L/min)	Change from Baseline to 6 Months*	r=0.126 (p=0.125)
	Change from Baseline to 12 Months**	r=0.079 (p=0.337)
Relative Cardiorespiratory Fitness (ml/kg/min)	Change from Baseline to 6 Months*	r=0.103 (p=0.211)
	Change from Baseline to 12 Months**	r=0.042 (p=0.614)
Cardiorespiratory Fitness (minutes)	Change from Baseline to 6 Months*	r= -0.034 (p=0.679)
	Change from Baseline to 12 Months**	r= -0.051 (p=0.536)
*Indicates change computed as Month 6 – Baseline **Indicates change computed as Month 12 – Baseline		

Analyses were also conducted to examine whether change in Klotho concentration at 6 months was associated with measures of change at 6 months for weight, body composition, or cardiorespiratory fitness. Spearman rank order correlation coefficients were computed, with results presented in Table 15. No significant correlations were observed between change in Klotho concentration at 6 months and change in weight, change in body composition, or change in cardiorespiratory fitness at 6 months.

Table 15. Correlation between change in Klotho at 6 months and change in weight, body composition, and cardiorespiratory fitness at 6 months

Variable		Baseline Klotho
Body Weight (kg)	Change from Baseline to 6 Months*	r= -0.106 (p=0.194)
Lean Mass (kg)	Change from Baseline to 6 Months*	r= -0.030 (p=0.718)
Fat Mass (kg)	Change from Baseline to 6 Months*	r= -0.113 (p=0.166)
Percent Body Fat	Change from Baseline to 6 Months*	r= -0.072 (p=0.381)
Absolute Cardiorespiratory Fitness (L/min)	Change from Baseline to 6 Months*	r= -0.095 (p=0.246)
Relative Cardiorespiratory Fitness (ml/kg/min)	Change from Baseline to 6 Months*	r= -0.003 (p=0.711)
Cardiorespiratory Fitness (minutes)	Change from Baseline to 6 Months*	r= -0.015 (p=0.856)
*Indicates change computed as Month 6 – Baseline		

Further, analyses were conducted to examine whether change in Klotho concentration at 12 months was associated with measures of change at 12 months for weight, body composition, or cardiorespiratory fitness. Spearman rank order correlation coefficients were computed, with results presented in Table 16. No significant correlations were observed between change in Klotho concentration at 12 months and change in weight, change in body composition, or change in cardiorespiratory fitness at 12 months.

Table 16. Correlation between change in Klotho at 12 months and change in weight, body composition, and cardiorespiratory fitness at 12 months

Variable		Baseline Klotho
Body Weight (kg)	Change from Baseline to 12 Months**	r= -0.023 (p=0.781)
Lean Mass (kg)	Change from Baseline to 12 Months**	r= -0.068 (p=0.404)
Fat Mass (kg)	Change from Baseline to 12 Months**	r= -0.020 (p=0.809)
Percent Body Fat	Change from Baseline to 12 Months**	r= -0.037 (p=0.652)
Absolute Cardiorespiratory Fitness (L/min)	Change from Baseline to 12 Months**	r=0.009 (p=0.918)
Relative Cardiorespiratory Fitness (ml/kg/min)	Change from Baseline to 12 Months**	r=0.064 (p=0.437)
Cardiorespiratory Fitness (minutes)	Change from Baseline to 12 Months**	r= -0.069 (p=0.399)
**Indicates change computed as Month 12 – Baseline		

Further exploratory analyses were performed to examine whether change in weight, change in body composition, and change in cardiorespiratory fitness differed by quartile of baseline Klotho. Quartiles were defined as Quartile 1: 0-638 pg/mL, Quartile 2: 639-877 pg/mL, Quartile 3: 878-1117 pg/mL, and Quartile 4: $\geq$1118 pg/mL. Three-factor repeated-measure ANOVA's (treatment X quartile X time) were performed for each variable (see Tables 17-24). These analyses were repeated for only "responders" and a similar pattern to the full sample was observed (see Appendix B: Tables 26-34).

Analysis revealed body weight significantly decreased across time (p=0.000), with no significant difference by treatment conditions (p=0.604) or quartile (p=0180) (Table 17). Similarly, body mass index significantly decreased across time (p=0.000), with no significant difference by treatment condition (p=0.402) or quartile (p=0.140). However, there was a significant treatment X quartile X time effect (p=0.044) across the 12-month intervention (Table 18).

Table 17. Change in Weight (kg) by treatment condition and Quartile of Baseline Klotho

Treatment	Baseline Klotho Quartile	Weight (kg)			p-values					
		Baseline	Change from Baseline to 6 Months	Change from Baseline to 12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=52)	Quartile 1 (N=38)	93.8±13.2	-12.1±7.0	-14.3±10.2	0.604	0.180	0.000	0.176	0.985	0.057
	Quartile 2 (N=38)	85.7±7.7	-8.5±6.8	-9.6±9.4						
	Quartile 3 (N=38)	90.8±16.9	-10.8±7.3	-11.8±10.8						
	Quartile 4 (N=38)	92.0±10.4	-6.7±6.5	-8.1±9.8						
DIET+150 (N=48)	Quartile 1 (N=38)	92.2±13.7	-10.3±8.0	-11.4±8.9						
	Quartile 2 (N=38)	95.0±11.1	-15.1±7.6	-16.4±7.9						
	Quartile 3 (N=38)	87.8±15.9	-8.8±6.9	-10.3±9.7						
	Quartile 4 (N=38)	88.7±8.1	-16.0±6.8	-18.6±8.0						
DIET+250 (N=52)	Quartile 1 (N=38)	93.7±16.6	-10.3±8.9	-12.7±12.5						
	Quartile 2 (N=38)	93.2±12.6	-11.0±6.8	-11.7±9.0						
	Quartile 3 (N=38)	80.2±9.8	-12.6±5.2	-13.1±5.3						
	Quartile 4 (N=38)	88.5±14.1	-9.7±5.5	-11.9±6.7						

Table 18. Change in Body Mass Index (kg/m2) by treatment condition and Quartile of Baseline Klotho

Treatment	**Baseline Klotho Quartile**	**Body Mass Index (kg/m^2)**			**p-values**					
		Baseline	Change from Baseline to 6 Months	Change from Baseline to 12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=52)	Quartile 1 (N=38)	33.1±3.5	-4.1±0.7	-4.9±1.0	0.402	0.140	0.000	0.117	0.975	0.044
	Quartile 2 (N=38)	31.5±3.0	-3.1±1.0	-3.5±1.7						
	Quartile 3 (N=38)	31.8±3.1	-3.7±0.8	-4.1±1.9						
	Quartile 4 (N=38)	32.9±3.0	-2.3±1.3	-2.9±2.0						
DIET+150 (N=48)	Quartile 1 (N=38)	32.2±3.6	-3.6±0.4	-4.0±0.8						
	Quartile 2 (N=38)	33.3±3.6	-5.4±0.2	-5.9±0.1						
	Quartile 3 (N=38)	31.7±4.5	-3.1±0.5	-3.7±0.2						
	Quartile 4 (N=38)	32.1±2.4	-5.7±0.4	-6.6±0.6						
DIET+250 (N=52)	Quartile 1 (N=38)	34.1±4.5	-3.7±1.1	-4.6±0.1						
	Quartile 2 (N=38)	32.2±3.8	-3.8±0.2	-4.1±0.3						
	Quartile 3 (N=38)	29.2±2.8	-4.6±0.3	-4.7±0.5						
	Quartile 4 (N=38)	31.4±4.4	-3.5±0.1	-4.3±0.2						

For measures of body composition, data revealed lean mass significantly decreased across the 12-month intervention (p=0.000), with no significant differences by treatment condition (p=0.567) or quartile (p=0.539) (Table 19). Similarly, fat mass significantly decreased over time (p=0.000), with no significant differences by treatment condition (p=0.237) or quartile (p=0.207) (Table 20). A similar pattern of response was observed with percent body fat, with a significant decrease across the 12-month intervention (p=0.000) (Table 21). However, there was a significant treatment X quartile X time effect (p=0.022) for percent body fat revealed.

Table 19. Change in Lean Mass (kg) by treatment condition and Quartile of Baseline Klotho

Treatment	Baseline Klotho Quartile	Lean Mass (kg)			p-values					
		Baseline	Change from Baseline to 6 Months	Change from Baseline to 12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=52)	Quartile 1 (N=38)	50.6±9.4	-2.2±1.9	-2.1±2.0	0.567	0.539	0.000	0.551	0.545	0.075
	Quartile 2 (N=38)	44.3±3.9	-1.5±1.6	-1.4±1.8						
	Quartile 3 (N=38)	48.7±11.5	-2.2±2.1	-2.0±2.4						
	Quartile 4 (N=38)	46.5±7.7	-0.8±1.7	-0.7±1.7						
DIET+150 (N=48)	Quartile 1 (N=38)	51.3±11.2	-1.4±2.2	-1.7±2.3						
	Quartile 2 (N=38)	51.4±9.1	-2.2±2.2	-2.7±2.6						
	Quartile 3 (N=38)	46.3±8.8	-0.9±1.5	-1.0±1.6						
	Quartile 4 (N=38)	46.5±8.5	-2.9±2.3	-2.6±2.6						
DIET+250 (N=52)	Quartile 1 (N=38)	45.6±5.5	-1.5±2.0	-1.7±2.3						
	Quartile 2 (N=38)	50.3±8.8	-2.3±1.6	-2.2±2.3						
	Quartile 3 (N=38)	44.4±4.7	-1.0±1.3	-0.8±0.8						
	Quartile 4 (N=38)	46.3±7.7	-0.8±1.3	-1.2±1.4						

Table 20. Change in Fat Mass (kg) by treatment condition and Quartile of Baseline Klotho

Treatment	Baseline Klotho Quartile	Fat Mass (kg)			p-values					
		Baseline	Change from Baseline to 6 Months	Change from Baseline to 12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=52)	Quartile 1 (N=38)	39.8±7.3	-9.7±5.3	-11.8±8.7	0.237	0.207	0.000	0.137	0.987	0.096
	Quartile 2 (N=38)	38.3±4.1	-6.8±5.2	-7.8±7.7						
	Quartile 3 (N=38)	39.0±6.4	-8.6±5.9	-9.7±9.0						
	Quartile 4 (N=38)	42.1±4.4	-5.4±4.7	-7.0±8.0						
DIET+150 (N=48)	Quartile 1 (N=38)	37.6±5.3	-8.7±6.3	-9.5±7.5						
	Quartile 2 (N=38)	40.2±5.9	-12.5±6.2	-13.1±7.4						
	Quartile 3 (N=38)	38.5±10.6	-7.7±5.8	-9.0±8.6						
	Quartile 4 (N=38)	39.1±4.9	-12.8±5.5	-15.4±6.4						
DIET+250 (N=52)	Quartile 1 (N=38)	44.8±12.3	-8.7±6.9	-10.7±10.2						
	Quartile 2 (N=38)	39.7±10.7	-8.4±5.2	-9.2±7.0						
	Quartile 3 (N=38)	32.8±6.6	-11.4±4.3	-11.9±5.2						
	Quartile 4 (N=38)	39.0±8.2	-8.5±5.0	-10.3±1.2						

Table 21. Change in Percent Fat Mass (%) by treatment condition and Quartile of Baseline Klotho

Treatment	Baseline Klotho Quartile	Percent Fat Mass (%)			p-values					
		Baseline	Change from Baseline to 6 Months	Change from Baseline to 12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=52)	Quartile 1 (N=38)	42.7±5.3	-5.9±2.5	-7.7±3.6	0.158	0.400	0.000	0.094	0.884	0.022
	Quartile 2 (N=38)	45.0±1.7	-4.4±2.1	-5.5±3.9						
	Quartile 3 (N=38)	43.5±3.8	-5.3±1.9	-7.0±4.2						
	Quartile 4 (N=38)	46.2±3.8	-3.1±0.9	-4.5±1.6						
DIET+150 (N=48)	Quartile 1 (N=38)	41.4±5.4	-5.9±2.9	-6.6±3.6						
	Quartile 2 (N=38)	42.7±5.8	-8.2±2.3	-8.9±2.8						
	Quartile 3 (N=38)	43.6±6.9	-4.9±0.9	-6.2±1.6						
	Quartile 4 (N=38)	44.6±6.0	-8.3±2.3	-10.9±2.2						
DIET+250 (N=52)	Quartile 1 (N=38)	47.5±5.4	-4.5±0.9	-6.2±3.5						
	Quartile 2 (N=38)	42.6±8.2	-4.9±0.9	-5.7±1.9						
	Quartile 3 (N=38)	41.0±4.2	-9.6±1.8	-10.0±1.4						
	Quartile 4 (N=38)	44.2±5.0	-5.9±2.4	-7.1±2.3						

Data for measures of cardiorespiratory fitness are shown in Tables 22 – 24. There was a significant difference in absolute cardiorespiratory fitness (L/min) by treatment condition across the 12-month intervention (p=0.036), with no significant difference by quartile (p=0.719) (Table 22). Moreover, relative cardiorespiratory fitness revealed a significant increase across time (p=0.000), with

significant differences by intervention condition (p=0.016) (Table 23). A similar pattern of response was observed when test termination time was used to represent cardiorespiratory fitness (Table 24).

Table 22. Change in Absolute Cardiorespiratory Fitness (L/min) by treatment condition and Quartile of Baseline Klotho

Treatment	**Baseline Klotho Quartile**	**Absolute Cardiorespiratory Fitness (L/min)**			**p-values**					
		Baseline	Change from Baseline to 6 Months	Change from Baseline to 12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=51)	Quartile 1 (N=35)	2.2±0.5	-0.2±0.3	-0.1±0.3	0.354	0.719	0.081	0.036	0.456	0.788
	Quartile 2 (N=36)	1.8±0.2	-0.1±0.2	-0.2±0.3						
	Quartile 3 (N=38)	2.2±0.8	-0.1±0.2	-0.2±0.2						
	Quartile 4 (N=38)	1.9±0.5	0.0±0.3	0.0±0.4						
DIET+150 (N=45)	Quartile 1 (N=35)	2.1±0.6	0.0±0.2	0.0±0.2						
	Quartile 2 (N=36)	2.2±0.6	0.0±0.3	0.0±0.2						
	Quartile 3 (N=38)	2.0±0.5	0.0±0.2	0.0±0.2						
	Quartile 4 (N=38)	2.0±0.4	0.0±0.3	0.0±0.2						
DIET+250 (N=51)	Quartile 1 (N=35)	1.9±0.3	-0.1±0.1	0.0±0.2						
	Quartile 2 (N=36)	2.2±0.6	0.1±0.2	0.0±0.2						
	Quartile 3 (N=38)	2.0±0.3	-0.1±0.2	0.0±0.2						
	Quartile 4 (N=38)	2.0±0.4	0.0±0.2	0.0±0.3						

Table 23. Change in Relative Cardiorespiratory Fitness (ml/kg/min) by treatment condition and Quartile of Baseline Klotho

Treatment	**Baseline Klotho Quartile**	**Relative Cardiorespiratory Fitness (ml/kg/min)**			**p-values**					
		Baseline	Change from Baseline to 6 Months	Change from Baseline to 12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=51)	Quartile 1 (N=35)	23.6±5.0	1.5±2.9	3.0±4.1	0.016	0.355	0.000	0.000	0.607	0.313
	Quartile 2 (N=36)	21.5±2.0	0.8±2.5	0.4±3.7						
	Quartile 3 (N=38)	23.6±4.4	2.8±3.3	1.6±4.6						
	Quartile 4 (N=38)	20.5±5.5	1.8±3.6	1.9±4.2						
DIET+150 (N=45)	Quartile 1 (N=35)	23.5±5.2	3.4±3.7	4.1±4.1						
	Quartile 2 (N=36)	23.9±4.8	4.7±5.5	6.3±3.4						
	Quartile 3 (N=38)	23.3±5.4	2.6±3.7	3.3±4.3						
	Quartile 4 (N=38)	22.2±3.9	5.6±4.6	5.9±3.6						
DIET+250 (N=51)	Quartile 1 (N=35)	20.1±2.4	1.8±2.7	2.9±3.8						
	Quartile 2 (N=36)	23.9±4.8	4.2±3.2	3.6±2.8						
	Quartile 3 (N=38)	24.5±3.4	3.8±2.8	4.4±3.3						
	Quartile 4 (N=38)	22.1±3.5	3.0±3.7	3.6±4.6						

Table 24. Change in Cardiorespiratory Fitness (min) by treatment condition and Quartile of Baseline Klotho

Treatment	Baseline Klotho Quartile	Cardiorespiratory Fitness (min)			p-values					
		Baseline	Change from Baseline to 6 Months	Change from Baseline to 12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=51)	Quartile 1 (N=35)	7.8±2.8	2.3±0.0	2.0±0.2	0.007	0.373	0.000	0.009	0.996	0.803
	Quartile 2 (N=37)	7.7±1.4	1.1±0.8	0.0±1.1						
	Quartile 3 (N=38)	8.3±2.5	1.7±0.9	1.1±1.0						
	Quartile 4 (N=38)	5.6±3.2	3.9±0.5	1.2±0.5						
DIET+150 (N=45)	Quartile 1 (N=35)	8.5±3.5	3.0±1.4	3.0±1.0						
	Quartile 2 (N=37)	8.4±3.5	3.1±0.8	3.9±1.5						
	Quartile 3 (N=38)	8.1±4.1	2.2±0.4	2.3±0.5						
	Quartile 4 (N=38)	8.0±2.4	3.4±0.8	2.8±1.1						
DIET+250 (N=51)	Quartile 1 (N=35)	6.4±2.9	1.4±0.0	1.5±0.2						
	Quartile 2 (N=37)	9.0±3.1	2.9±0.6	2.5±0.1						
	Quartile 3 (N=38)	8.3±1.2	2.5±0.9	2.5±0.3						
	Quartile 4 (N=38)	8.0±2.8	2.5±0.5	2.1±0.9						

5.0 Discussion

This study was conducted to examine changes in Klotho concentration in relation to a 12-month behavioral weight loss intervention in adults with overweight or obesity. This study is important for the following reasons. First, few analyses have been conducted to examine the chronic changes in Klotho levels over time in adults with obesity, participating in a behavioral weight loss program. Moreover, studies that have investigated change in Klotho concentration have focused on response to an acute bout of exercise.[11] The chronic changes that occur in Klotho concentration have not been examined. Moreover, whether weight loss has an effect on Klotho concentration is yet to be completely understood. Therefore, the current investigation examined how weight loss in conjunction with physical activity plays a role in Klotho concentration over a period of 12 months.

5.1 Behavioral Weight Loss Intervention

Data from the current study reveal a significant decrease in weight across the 12-month intervention. 29% (111 of 383) of the originally recruited participants lost $\geq$ 10% of their baseline weight at 6 months and maintained this weight loss for the end of the 12-month intervention (Table 3). Similar weight loss patterns have been shown in the literature, with the STEP-UP[135] randomized trial reporting ~20% of participants losing >10% of baseline weight and maintained this magnitude of weight loss throughout the duration of the trial.[135] However, for the

current study there were no differences in the amount of weight loss by intervention condition (DIET vs. DIET+PA150 vs. DIET+PA250).

Measures of body composition resulted in significant decreases in both volume of lean and fat mass across the 12-month intervention. Further, body composition was significantly different by weight loss response classification (Tables 5-7). This would be expected due to the stratification by weight loss of "responder" and "non-responder." With loss of lean and fat mass being greater in the "responders" vs. "non-responders."

Results show a significant increase in cardiorespiratory fitness across the 12-month intervention, with significant differences by intervention condition (Tables 8-10). The DIET+PA250 group had greater increases in relative cardiorespiratory fitness compared to the DIET+PA150 group, with both the DIET+PA150 and DIET+PA250 groups having greater increases in cardiorespiratory fitness compared to the DIET alone group. Results from the current study coincide with the literature on behavioral weight loss interventions that contain physical activity as a component, with the highest prescribed volume of activity having greater improvements in cardiorespiratory fitness.[43,135,136]

5.2 Klotho Concentration

This study aimed to examine change in Klotho concentration in participants who were overweight or obese undergoing a behavioral weight loss intervention, which also compared the effects of diet alone vs. diet combined with physical activity. For the current study, mean Klotho concentration was ~930 pg/mL. Amitani et al.,[17] estimates normal weight individuals to have a Klotho concentration of 1391.6±145.0 pg/mL and individuals with obesity to have a Klotho

concentration of 847.1±111.3 pg/mL.[17] The concentration of Klotho in the current study appears to be consistent with the level observed in other studies of adults with obesity.

The premise of this study was to examine the potential link between obesity and klotho concentration. Obesity has been linked to many chronic conditions and promotion of advanced aging processes.[127,129] Klotho has also been shown to be a marker of premature aging processes.[12,13] Therefore, it was hypothesized when individuals who are overweight or obese undergo a weight loss intervention, Klotho concentration would increase and remain increased. However, the current study did not support this hypothesis, with Klotho concentration increasing modestly from baseline to 6 months, but not sustaining this rise in concentration from 6- to 12 months. Though not statistically significant, there appeared to be no change in Klotho concentration among individuals who lost weight with diet alone. However, results revealed a greater increase in Klotho concentration among individuals prescribed a higher volume of physical activity (Table 12 Figure 7), suggesting it may not be obesity that is linked to Klotho concentration, but rather physical activity that may have an important impact on Klotho concentration.

5.2.1. Klotho Concentration, Obesity, and Weight Loss

Klotho concentration, to the best our of knowledge, has not been reported in the literature for obese, but otherwise healthy adults prior to, during, and following a behavioral weight loss intervention. This 12-month intervention was effective in improving measures of weight and BMI status, body composition, and relative cardiorespiratory fitness (Tables 3-10). Participants in the current study were stratified by weight loss response ("responder" and "non-responder"), however Klotho concentration did not differ by classification or intervention condition (Table

13). While the intervention was successful in decreasing level of obesity, the effect on Klotho concentration was not as hypothesized. The decline in obesity among participants, lead to a modest and significant increase in Klotho concentration from baseline to 6 months, followed by a decline in Klotho concentration from 6- to 12 months (Table 12 and Figure 7). Results suggest Klotho concentration may not be directly linked to obesity as originally hypothesized. Although weight loss did not appear to improve Klotho concentration among overweight and obese individuals, it also did not result in a Klotho concentration below baseline levels. Thus, weight loss does not appear to negatively influence Klotho concentration.

Patterns of the body retracting back to baseline levels, similar to what was observe with Klotho in this study, has also been shown in patients who have undergone bariatric surgery. A randomized clinical trial from Courcoulas et al., observed significant weight loss among individuals who underwent bariatric surgery, which was maintained up to three years following the intervention.[137] Moreover, 60% individuals who underwent Roux-en-Y Gastric Bypass (RYGB) surgery and 45% of individuals who underwent Lapriscopic adjustable gastric band (LAGB) surgery had complete or partial remission of type 2 diabetes at year one following surgery. However, three years following surgery only 29% of the RYGB and 40% of the LAGB participants remained in remission,[137] potentially suggesting that the return to baseline levels in Klotho concentration in the current study may not be a unique phenomenon, but rather the body's potential desire to return to baseline levels despite weight loss maintenance. This warrants further investigation.

While not significant, an intriguing observation from this study revealed that a greater amount of physical activity prescribed was associated with a greater increase in Klotho concentration in the first 6 months of weight loss, as well as, a somewhat blunted decline in

Klotho concentration during months 6 to 12, with weight continually being maintained or lost (Table 12 Figure 7). This may indicate reducing sedentary time and increasing muscle activity through exercise may provide improvements in Klotho concentration, with a higher volume of physical activity being more beneficial than lower volumes to produce a change within the context of obesity and weight loss treatment.

5.2.2. Klotho Concentration and Body Composition

It is known that individuals with overweight and obesity tend to have greater functional impairment and decrements in musculoskeletal health. Klotho concentration has also been linked to declines in functional status in older adults.[104,107] Thus, it would be hypothesized that higher levels of obesity would be associated with lower levels of Klotho concentration as measured by weight, BMI, lean mass, fat mass, and percent body fat. However, this relationship was not observed in this particular study. These results could be due to a few reasons. The participants were an average of 45 years of age, which is younger than individuals examined in the literature that have shown relationships between Klotho and age-related health outcomes. Moreover, the participants in this current study did not have physical impairments which may have influenced the outcomes and their association with Klotho.

Results from the current study reveal higher levels of Klotho concentration in individuals with a lower volume of lean mass (Table 17), potentially meaning volume of lean mass may not be completely linked to Klotho concentration. It is unknown if Klotho concentration may be affected by the quality of the skeletal muscle or lean body mass, rather than the total volume of lean body mass. This could be hypothesized because of obesity's link to many metabolic alterations in muscle including changes in cellular location of fatty acid transporter proteins,

decreased mitochondrial enzyme activity, and defects in mitochondrial morphology.[138-140] With these metabolic alterations, obesity is typically associated with an insulin resistant state, potentially where Klotho concentration may be impacted. The link between insulin/IGF-1 and Klotho concentration may be affected in obese individuals due to the increased volume of lean body mass and intramuscular adipose tissue that accumulates. Within this pathway, obesity may decrease oxidative capacity and mitochondrial function, and therefore increasing oxidative stress on the body. Therefore, a potential hypothesis that may be drawn from these results is Klotho concentration is not influenced by the volume of lean body mass, but by the quality of the muscular tissue. This hypothesis warrants further investigation.

This study used DXA to assess volume of lean mass. However, DXA does not provide a measure of muscle quality. Thus, measures to assess lean tissue and other components of body composition (e.g. MRI, CT, tissue biopsy, etc.) may be necessary to further investigate these relationships with Klotho in humans.

Exercise has been shown throughout the literature to somewhat blunt lean body mass loss when individuals with obesity undergo weight loss.[39,51] In an exploratory analysis, although not significant, results show individuals with a low baseline Klotho concentration do not preserve their lean body mass, even with high levels of physical activity being performed, compared to individuals with higher baseline levels of Klotho (Table 17). The prescribed mode of exercise in this study was primarily aerobic activity (e.g. walking). However, it is unknown is if other modes of physical activity (e.g. resistance exercise) would have resulted in different changes in lean mass loss and whether this would be associated with Klotho concentration. This may warrant further investigation.

5.2.3. Klotho Concentration as a Predictor to Key Variables

Klotho concentration was analyzed as a predictor to changes in weight, body composition, and cardiorespiratory fitness. The literature has shown lower levels of Klotho concentration to be predictive of a decline in musculoskeletal health, cardiovascular disease, cognitive health, among other outcomes.[104-107] However, this current study did not include measures of chronic disease and musculoskeletal health. Results did not consistently reveal baseline quartile of Klotho concentration to predict change in measures of weight, body composition, or cardiorespiratory fitness. Thus, these variables included in weight loss treatment, may not be appropriate when assessing to Klotho concentration as a predictor of intervention response.

5.3. Limitations and Future Directions

While this study adds to the literature regarding Klotho and its association to body composition, weight loss, and physical activity, it is not without limitations that could impact the interpretation of the observed results. These limitations should also be considered as an opportunity for improving future research in this area of study. These include:

1. This sample contained individuals who were overweight or obese but otherwise relatively healthy individuals. Participants were required to have the ability to walk on a treadmill at 3 miles per hour to measure cardiorespiratory fitness. Therefore, individuals in this study may not have functional impairments needed to observe associations with Klotho concentration that was originally hypothesized. This

outcome can potentially be due to the possibility that the level of obesity for participants in this study had not yet led to a decline in functional ability. Individuals with a broader range in functional ability should be examined to better understand the effect of weight and functional status on Klotho concentration.

2. This study contains a subsample of the original 383 participants recruited for this standard behavioral weight loss intervention. The 152 participants who were included in this subsample were stratified into level of weight response classification to the weight loss intervention. Though weight loss of $\geq$10% is clinically meaningful, whether Klotho concentration significantly increases with weight loss at even higher levels may warrant further research.
3. The current study involved moderate to high volumes of prescribed physical activity. As stated earlier, a higher volume of physical activity may produce significant changes in Klotho concentration, with diet alone producing no changes in Klotho concentration. Results from this study suggest future studies should asses the relationship between varying doses of prescribed physical activity and Klotho response.
4. The prescribed physical activity in this study was primarily aerobic activity (e.g. walking). Moreover, volume of lean mass though non-significant, appeared to decrease further among individuals with lower baseline Klotho levels, even when a high volume of aerobic physical activity was prescribed. It is unclear how prescription of physical activity based on intensity or other modes of physical activity would have provided different results. Measuring other modes of physical activity, such as resistance training, may result in different effects on Klotho concentration and

lean mass. Further, it may be that only those who perform high intensities of physical activity, produce significant and sustainable changes in Klotho concentration, however this is unknown. Although this analysis is outside of scope for this particular study, in the future studies could analyze how physical activity status may impact Klotho concentration within and outside of the context of a weight loss intervention.

5. Individuals in this study were an average of 45 years of age, representing a younger population than typically reported in Klotho literature. The potential impact this intervention has on Klotho concentration in older individuals should be considered in future studies with obesity and measures of weight loss treatment.
6. Lean mass has been used as a measure of musculoskeletal health (functional ability), and has been found to be associated with Klotho concentration, however there were no measures of this in the current study. As a measure of musculoskeletal health, functional ability and the effect it has on Klotho concentration, via measures such as the Senior Performance Physical Battery, Senior Fitness Test, 6-Minute Walk, and 400 Meter Walk, may be important to include in future research studies.[141,142]
7. Results from the current study show an inverse relationship between lean mass and Klotho concentration. These findings suggest that total volume of lean mass may not be a link to Klotho concentration as was hypothesized. What is not known is whether the quality of lean mass would provide additional understanding of this relationship. The measures of lean mass in this study did not account for an assessment of muscle quality. Therefore, future research studies should consider exploring both quality and volume of lean mass to better understand this relationship.

5.4. Summary

In summary, this study quantified Klotho concentration in subjects who underwent a standard behavioral weight loss intervention for 12 months for the following Specific Aims.

1. (Specific Aim 1) Examine Klotho concentration and its association to measures of body composition and fitness level: The current study revealed a significant an inverse relationship between lean body mass and Klotho concentration. However, no other measures were found to be statistically correlated with Klotho.
2. (Specific Aim 2) Examine the change in Klotho concentration by intervention group across the 12-month weight loss intervention: Results revealed significant changes in Klotho concentration across 12 months, however these changes did not differ by intervention group. Although non-significant, changes in Klotho concentration appeared greater among those individuals who were prescribed higher volumes of physical activity.
3. (Specific Aim 3) Examine the change in Klotho concentration by both intervention group and weight loss classification ("responder" and "non-responder") across the 12-month intervention: The analysis revealed no significant difference by intervention condition or by response to achieve a weight loss of $\geq$ 10% ("responders" vs. "non-responders").

This study provides evidence within the context of a behavioral weight loss intervention, that Klotho concentration significantly but modestly increases when individuals undergo a weight loss intervention. However, this change may not be sustained overtime, despite sustained weight loss. Although the premise of this study to link obesity with Klotho concentration

because of a mutual relationship to advanced aging, the results from this study do not support there to be a relationship between these outcomes. However, results from this study showed that high volumes of prescribed physical activity were modestly and non-significantly associated with greater increases in Klotho concentrations. This may suggest stimulating muscle activity via physical activity may be an important contributor to increase Klotho concentration within the context of obesity that may require additional investigation.

Appendix A

Human Klotho Elisa Kit Analysis

Klotho was analyzed using ELISA kit steps developed by Immuno-Biological Laboratories, Takasaki, Japan.[134]

Preparation steps were performed as follows:

1. Wash buffer concentration was diluted 40-fold with deionized water. The dilution was used for the assay as a wash buffer. (975 mL deionized water/25 mL wash buffer concentration)
2. Labeled antibody concentration was diluted 30-fold with the solution for labeled antibody in a prepared collecting container. (11.6 mL solution for antibody/400 µL labeled antibody concentration)
3. 0.5 mL of deionized water was added into the vial of Standard (recombinant human soluble α-Klotho) and was completely dissolved. Making the concentration of the standard to be 12,000 pg/mL. Seven test tubes for dilution of the standard were prepared, along with adding 300 µL of the EIA buffer into each tube. Next, 300 µL of the 12,000 pg/mL standard were placed into the tube 6,000 pg/mL (tube-1) and gently mixed. Afterword, 300 µL of the mixed liquid from tube-1 was placed into the tube 3,000 pg/mL (tube-2) and gently mixed. Following, a dilution two-fold of the

standard solution was done in a series to set up 8 points of diluted standard between 6,000 pg/mL and 93.75 pg/mL as follows:

Tube-1	6,000	pg/mL
Tube-2	3,000	pg/mL
Tube-3	1,500	pg/mL
Tube-4	750	pg/mL
Tube-5	375	pg/mL
Tube-6	187.5	pg/mL
Tube-7	93.75	pg/mL
Tube-8	46.875	pg/mL

4. Test samples were diluted with EIA buffer 2-fold: 140 µL EIA buffer/140 µL test sample

The measurement procedure was performed as follows:[134]

1. Test sample blank wells were be determined, and 100 µL of EIA buffer was placed into the wells.
2. 100 µL of prepared test samples and 100 µL of prepared standard were placed into appropriate wells.
3. An incubation period of 60 minutes with the plate lid was performed at room temperature.
4. The plate was washed with the prepared wash buffer four times, with all the liquid completely removed following the fourth wash.
5. 100 µL of the prepared labeled antibody was added to the wells.

6. An incubation period of 30 minutes with the plate lid was performed at room temperature.
7. The plate was washed with the prepared wash buffer five times, with all the liquid completely removed following the fifth wash.
8. 100 µL of Chormogen - TMB solution was added into the wells.
9. An incubation period of 30 minutes in the dark was performed at room temperature.
10. 100 µL of the Stop solution was added to the wells.
11. Removal of dirt and drops of water on the bottom of the plate was done, as well as, confirmation that no bubbles are on the surface of the liquid. Then the optical density of the standard and the test samples was measured against a test sample blank, with the measurement wavelength at 450 nm. The minimum level of detectability of the assay was 6.15 pg/mL.

Table 25. Breakdown of human soluble α-Klotho assay kit measurement procedures

Reagents	**Test Sample**	**Standard**	**Test Sample Blank**	**Reagent Blank**
	Test Sample 100 µL	Diluted standard (Tube-1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at room temperature with plate lid				
4 times (wash buffer more than 350 µL)				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at room temperature with plate lid				
5 times (wash buffer more than 350 µL)				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL

Calculation of the test result were performed as follows:

1. Plot the concentration of the standard on the x-axis and its O.D. on the y-axis. Draw a standard curve by applying appropriate regression curve on each plot (i.e. linear curve)
2. Read the concentration by applying the absorbance of the test samples on a standard curve.
3. Calculate the concentration of the test samples by multiplying dilution ratio of the test samples on the value.

Appendix B

Appendix B.1 Association Between Klotho Concentration and Measures of Weight, Body Composition, and Cardiorespiratory Fitness in "Responders"

Table 26. Correlation between Baseline Klotho and change in weight, body composition, and cardiorespiratory fitness in "Responders"

Variable		Baseline Klotho
Body Weight (kg)	Change from Baseline to 6 Months*	r=0.175 (p=0.066)
	Change from Baseline to 12 Months**	r=0.072 (p=0.450)
Lean Mass (kg)	Change from Baseline to 6 Months*	r=0.190 (p=0.046)
	Change from Baseline to 12 Months**	r=0.159 (p=0.095)
Fat Mass (kg)	Change from Baseline to 6 Months*	r=0.098 (p=0.309)
	Change from Baseline to 12 Months**	r=0.014 (p=0.887)
Percent Body Fat	Change from Baseline to 6 Months*	r=0.012 (p=0.901)
	Change from Baseline to 12 Months**	r= -0.046 (p=0.634)
Absolute Cardiorespiratory Fitness (L/min)	Change from Baseline to 6 Months*	r=0.215 (p=0.023)
	Change from Baseline to 12 Months**	r=0.119 (p=0.214)
Relative Cardiorespiratory Fitness (ml/kg/min)	Change from Baseline to 6 Months*	r=0.153 (p=0.110)
	Change from Baseline to 12 Months**	r=0.033 (p=0.731)
Cardiorespiratory Fitness (minutes)	Change from Baseline to 6 Months*	r=0.027 (p=0.777)
	Change from Baseline to 12 Months**	r= -0.007 (p=0.943)
*Indicates change computed as Month 6 – Baseline **Indicates change computed as Month 12 – Baseline N=111		

Table 27. Correlation between change in Klotho at 6 months and change in weight, body composition, and cardiorespiratory fitness at 6 months in "Responders"

Variable		Baseline Klotho
Body Weight (kg)	Change from Baseline to 6 Months*	r= -0.052 (p=0.585)
Lean Mass (kg)	Change from Baseline to 6 Months*	r= 0.053 (p=0.581)
Fat Mass (kg)	Change from Baseline to 6 Months*	r= -0.052 (p=0.590)
Percent Body Fat	Change from Baseline to 6 Months*	r= 0.030 (p=0.754)
Absolute Cardiorespiratory Fitness (L/min)	Change from Baseline to 6 Months*	r= -0.089 (p=0.352)
Relative Cardiorespiratory Fitness (ml/kg/min)	Change from Baseline to 6 Months*	r= -0.112 (p=0.244)
Cardiorespiratory Fitness (minutes)	Change from Baseline to 6 Months*	r= -0.066 (p=0.493)
*Indicates change computed as Month 6 – Baseline N=111		

Table 28. Correlation between change in Klotho at 12 months and change in weight, body composition, and cardiorespiratory fitness at 12 months in "Responders"

Variable		Baseline Klotho
Body Weight (kg)	Change from Baseline to 12 Months**	r= 0.075 (p=0.436)
Lean Mass (kg)	Change from Baseline to 12 Months**	r= 0.044 (p=0.648)
Fat Mass (kg)	Change from Baseline to 12 Months**	r= 0.070 (p=0.468)
Percent Body Fat	Change from Baseline to 12 Months**	r= 0.012 (p=0.901)
Absolute Cardiorespiratory Fitness (L/min)	Change from Baseline to 12 Months**	r=0.038 (p=0.691)
Relative Cardiorespiratory Fitness (ml/kg/min)	Change from Baseline to 12 Months**	r=0.057 (p=0.553)
Cardiorespiratory Fitness (minutes)	Change from Baseline to 12 Months**	r= 0.012 (p=0.902)
**Indicates change computed as Month 12 – Baseline N=111		

Appendix B.2 Baseline Quartile Klotho Concentration to Predict Change in Measures of Weight, Body Composition, and Cardiorespiratory Fitness in "Responders"

Table 29. Change in Weight (kg) by treatment condition and Quartile of Baseline Klotho in "Responders"

Treatment	Baseline Klotho Quartile	Weight (kg)			p-values					
		Baseline	6 Months	12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=37)	Quartile 1 (N=27)	94.3±14.0	80.0±10.8	77.1±10.0	0.988	0.448	0.000	0.340	0.624	0.861
	Quartile 2 (N=28)	83.4±9.4	69.8±7.8	67.0±7.4						
	Quartile 3 (N=27)	91.5±18.5	77.0±14.9	73.9±15.1						
	Quartile 4 (N=29)	91.6±11.0	80.4±8.6	76.5±12.4						
DIET+150 (N=35)	Quartile 1 (N=27)	92.6±12.9	76.4±9.6	74.4±11.7						
	Quartile 2 (N=28)	93.5±10.5	76.7±8.8	75.1±10.4						
	Quartile 3 (N=27)	92.7±13.4	79.1±12.6	75.9±12.9						
	Quartile 4 (N=29)	88.7±8.1	72.6±4.9	70.0±6.1						
DIET+250 (N=39)	Quartile 1 (N=27)	98.5±18.7	82.9±15.6	78.7±17.8						
	Quartile 2 (N=28)	94.6±13.7	80.0±13.0	78.3±12.8						
	Quartile 3 (N=27)	81.2±10.0	67.0±9.3	66.8±8.4						
	Quartile 4 (N=29)	88.1±15.0	75.5±13.4	72.7±13.0						

Table 30. Change in Body Mass Index (kg/m2) by treatment condition and Quartile of Baseline Klotho in "Responders"

Treatment	Baseline Klotho Quartile	Body Mass Index (kg/m^2)			p-values					
		Baseline	6 Months	12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=37)	Quartile 1 (N=27)	32.8±3.6	27.9±3.6	26.9±3.3	0.917	0.337	0.000	0.218	0.564	0.731
	Quartile 2 (N=28)	31.2±3.5	26.1±3.0	25.1±3.0						
	Quartile 3 (N=27)	31.6±2.4	26.7±2.1	25.5±2.0						
	Quartile 4 (N=29)	32.0±2.9	28.1±2.6	26.6±2.0						
DIET+150 (N=35)	Quartile 1 (N=27)	32.2±2.9	26.7±2.6	26.0±2.8						
	Quartile 2 (N=28)	33.4±3.8	27.4±3.1	26.8±3.3						
	Quartile 3 (N=27)	33.0±4.9	28.1±4.4	27.0±4.4						
	Quartile 4 (N=29)	32.1±2.4	26.4±2.3	25.5±2.9						
DIET+250 (N=39)	Quartile 1 (N=27)	35.5±4.6	30.0±4.1	28.4±5.0						
	Quartile 2 (N=28)	32.5±3.9	27.5±3.9	26.9±3.9						
	Quartile 3 (N=27)	29.6±2.8	24.4±2.6	24.3±2.4						
	Quartile 4 (N=29)	31.4±4.3	27.0±4.0	25.9±3.5						

Table 31. Change in Lean Mass (kg) by treatment condition and Quartile of Baseline Klotho in "Responders"

Treatment	Baseline Klotho Quartile	Lean Mass (kg)			p-values					
		Baseline	6 Months	12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=37)	Quartile 1 (N=27)	51.5±9.9	48.8±8.9	48.8±9.6	0.688	0.505	0.000	0.517	0.260	0.354
	Quartile 2 (N=28)	43.0±4.4	40.6±4.1	40.6±4.2						
	Quartile 3 (N=27)	50.0±12.9	47.1±11.2	47.2±10.7						
	Quartile 4 (N=29)	46.0±9.7	44.3±9.1	44.1±10.3						
DIET+150 (N=35)	Quartile 1 (N=27)	52.4±9.8	49.7±9.1	49.8±9.3						
	Quartile 2 (N=28)	50.2±8.8	47.7±8.1	47.4±8.2						
	Quartile 3 (N=27)	47.2±6.3	45.6±6.5	45.8±6.2						
	Quartile 4 (N=29)	46.5±8.5	43.6±6.5	43.9±6.1						
DIET+250 (N=39)	Quartile 1 (N=27)	47.2±6.2	45.1±5.6	44.9±6.0						
	Quartile 2 (N=28)	49.9±7.4	47.0±6.6	46.9±6.5						
	Quartile 3 (N=27)	45.1±4.5	43.8±4.8	44.3±4.8						
	Quartile 4 (N=29)	46.5±8.9	45.5±9.4	45.1±9.3						

Table 32. Change in Fat Mass (kg) by treatment condition and Quartile of Baseline Klotho in "Responders"

Treatment	Baseline Klotho Quartile	Fat Mass (kg)			p-values					
		Baseline	6 Months	12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=37)	Quartile 1 (N=27)	39.4±7.7	28.0±7.4	27.7±8.2	0.749	0.572	0.000	0.227	0.726	0.928
	Quartile 2 (N=28)	37.3±5.0	26.5±4.0	28.0±8.5						
	Quartile 3 (N=27)	38.6±6.6	26.9±4.2	26.2±8.1						
	Quartile 4 (N=29)	42.0±3.2	33.2±3.6	28.2±6.9						
DIET+150 (N=35)	Quartile 1 (N=27)	36.8±5.6	23.6±5.8	25.2±7.1						
	Quartile 2 (N=28)	39.8±6.1	26.0±6.9	23.7±3.5						
	Quartile 3 (N=27)	42.2±10.9	30.5±10.5	24.0±6.1						
	Quartile 4 (N=29)	39.1±4.9	26.3±6.5	29.3±4.3						
DIET+250 (N=39)	Quartile 1 (N=27)	47.6±14.5	34.8±11.7	21.6±5.2						
	Quartile 2 (N=28)	41.2±10.7	30.1±10.7	24.8±7.6						
	Quartile 3 (N=27)	33.1±7.0	20.5±5.8	27.4±10.8						
	Quartile 4 (N=29)	38.4±8.1	27.3±7.6	23.7±6.7						

Table 33. Change in Percent Fat Mass (%) by treatment condition and Quartile of Baseline Klotho in "Responders"

Treatment	Baseline Klotho Quartile	Percent Fat Mass (%)			p-values					
		Baseline	6 Months	12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=37)	Quartile 1 (N=27)	42.0±5.5	35.2±7.4	32.9±8.1	0.462	0.412	0.000	0.147	0.699	0.517
	Quartile 2 (N=28)	45.0±1.6	38.0±1.9	35.5±2.4						
	Quartile 3 (N=27)	42.7±4.2	35.4±3.9	32.5±5.2						
	Quartile 4 (N=29)	46.6±4.8	41.8±5.7	38.8±5.0						
DIET+150 (N=35)	Quartile 1 (N=27)	40.2±4.8	31.1±7.1	29.2±5.7						
	Quartile 2 (N=28)	43.0±6.0	34.0±8.4	32.8±8.5						
	Quartile 3 (N=27)	45.3±7.0	38.0±8.5	35.2±9.3						
	Quartile 4 (N=29)	44.6±6.0	36.3±8.3	33.7±8.2						
DIET+250 (N=39)	Quartile 1 (N=27)	48.0±6.8	41.3±7.1	38.1±9.2						
	Quartile 2 (N=28)	43.5±7.0	37.0±8.4	35.6±9.6						
	Quartile 3 (N=27)	40.7±4.4	30.2±5.3	29.6±4.2						
	Quartile 4 (N=29)	43.8±5.3	36.1±7.2	34.4±6.2						

Table 34. Change in Absolute Cardiorespiratory Fitness (L/min) by treatment condition and Quartile of Baseline Klotho in "Responders"

Treatment	Baseline Klotho Quartile	Absolute Cardiorespiratory Fitness (L/min)			p-values					
		Baseline	6 Months	12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=37)	Quartile 1 (N=27)	2.3±0.5	2.1±0.4	2.1±0.4	0.201	0.579	0.230	0.030	0.335	0.933
	Quartile 2 (N=27)	1.9±0.2	1.7±0.2	1.6±0.2						
	Quartile 3 (N=27)	2.1±0.8	2.1±0.7	2.0±0.8						
	Quartile 4 (N=29)	1.9±0.5	1.8±0.5	1.8±0.6						
DIET+150 (N=34)	Quartile 1 (N=27)	2.4±0.5	2.3±0.6	2.4±0.6						
	Quartile 2 (N=27)	2.2±0.6	2.3±0.7	2.2±0.5						
	Quartile 3 (N=27)	2.0±0.4	2.0±0.4	2.0±0.5						
	Quartile 4 (N=29)	2.0±0.4	2.0±0.4	2.0±0.3						
DIET+250 (N=39)	Quartile 1 (N=27)	1.9±0.3	1.8±0.3	1.9±0.3						
	Quartile 2 (N=27)	2.2±0.5	2.3±0.5	2.2±0.5						
	Quartile 3 (N=27)	2.0±0.3	1.9±0.3	2.0±0.3						
	Quartile 4 (N=29)	2.0±0.5	2.0±0.6	2.0±0.6						

Table 35. Change in Relative Cardiorespiratory Fitness (ml/kg/min) by treatment condition and Quartile of Baseline Klotho in "Responders"

Treatment	Baseline Klotho Quartile	Relative Cardiorespiratory Fitness (ml/kg/min)			p-values					
		Baseline	6 Months	12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=37)	Quartile 1 (N=27)	24.0±4.9	25.8±4.0	27.7±3.8	0.032	0.532	0.000	0.001	0.495	0.937
	Quartile 2 (N=27)	22.4±1.8	24.1±4.0	24.0±4.3						
	Quartile 3 (N=27)	22.8±4.6	26.9±5.1	26.6±5.9						
	Quartile 4 (N=29)	20.2±4.1	22.7±4.3	23.2±4.0						
DIET+150 (N=34)	Quartile 1 (N=27)	25.8±5.0	30.7±6.2	32.1±6.1						
	Quartile 2 (N=27)	23.9±4.8	30.1±9.1	30.3±6.8						
	Quartile 3 (N=27)	21.7±4.9	26.2±6.1	27.3±6.5						
	Quartile 4 (N=29)	22.2±3.9	27.8±5.9	28.1±4.9						
DIET+250 (N=39)	Quartile 1 (N=27)	19.2±1.3	22.1±1.3	24.5±1.9						
	Quartile 2 (N=27)	23.7±4.2	29.1±4.6	28.3±5.0						
	Quartile 3 (N=27)	24.6±3.6	28.9±4.0	29.8±3.3						
	Quartile 4 (N=29)	22.2±3.8	26.6±5.0	27.6±5.5						

Table 36. Change in Cardiorespiratory Fitness (min) by treatment condition and Quartile of Baseline Klotho

Treatment	Baseline Klotho Quartile	Cardiorespiratory Fitness (min)			p-values					
		Baseline	6 Months	12 Months	Treatment	Baseline Klotho Quartile	Time	Treatment X Time	Baseline Klotho Quartile X Time	Baseline Klotho Quartile X Treatment X Time
DIET (N=37)	Quartile 1 (N=27)	8.2±278	10.7±2.5	10.4±2.5	0.018	0.462	0.000	0.006	0.892	0.790
	Quartile 2 (N=27)	8.3±1.4	8.8±2.8	8.7±2.6						
	Quartile 3 (N=27)	8.2±2.8	10.6±0.9	10.3±3.7						
	Quartile 4 (N=29)	5.0±3.5	7.6±2.8	7.2±2.6						
DIET+150 (N=34)	Quartile 1 (N=27)	9.5±3.1	13.7±4.7	13.8±3.4						
	Quartile 2 (N=27)	8.4±3.5	11.6±4.3	12.3±5.0						
	Quartile 3 (N=27)	7.5±4.2	11.2±4.8	10.9±3.9						
	Quartile 4 (N=29)	8.0±2.4	11.4±3.2	10.8±3.5						
DIET+250 (N=39)	Quartile 1 (N=27)	5.4±3.1	8.7±3.1	9.3±2.9						
	Quartile 2 (N=27)	8.6±2.6	12.5±3.2	12.1±2.8						
	Quartile 3 (N=27)	8.3±1.3	10.9±2.2	11.2±0.9						
	Quartile 4 (N=29)	8.0±2.9	11.0±3.1	11.4±3.3						

Bibliography

1. Hales CM, Carroll MD, Fryar CD, Ogden CL. *Prevalence of obesity among adults and youth: United States, 2015-2016.* US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 2017.
2. Van Gaal LF, Mertens IL, Christophe E. Mechanisms linking obesity with cardiovascular disease. *Nature.* 2006;444(7121):875.
3. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *Journal of the American Medical Association.* 2012;307(5):491-497.
4. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *Journal of the American Medical Association.* 2014;311(8):806-814.
5. Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *Journal of the American Medical Association.* 2003;289(1):76-79.
6. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation.* 2006;113(6):898-918.
7. Field AE, Coakley EH, Must A, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. *Archives of Internal Medicine.* 2001;161(13):1581-1586.
8. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. *Journal of the American Medical Association.* 1999;282(16):1523-1529.
9. Vgontzas AN, Papanicolaou DA, Bixler EO, et al. Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *The Journal of Clinical Endocrinology & Metabolism.* 2000;85(3):1151-1158.
10. Wolk R, Shamsuzzaman AS, Somers VK. Obesity, sleep apnea, and hypertension. *Hypertension.* 2003;42(6):1067-1074.
11. Avin KG, Coen PM, Huang W, et al. Skeletal muscle as a regulator of the longevity protein, Klotho. *Frontiers in Physiology.* 2014;5:189.
12. Kuro-o M, Matsumura Y, Aizawa H, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature.* 1997;390(6655):45.
13. Xu Y, Sun Z. Molecular basis of Klotho: from gene to function in aging. *Endocrine Reviews.* 2015;36(2):174-193.
14. Drüeke TB, Massy ZA. Circulating Klotho levels: clinical relevance and relationship with tissue Klotho expression. *Kidney International.* 2013;83(1):13-15.
15. Martin A, David V, Quarles LD. Regulation and function of the FGF23/klotho endocrine pathways. *Physiological Reviews.* 2012;92(1):131-155.

16. Matsubara T, Miyaki A, Akazawa N, et al. Aerobic exercise training increases plasma Klotho levels and reduces arterial stiffness in postmenopausal women. *American Journal of Physiology-Heart and Circulatory Physiology.* 2013;306(3):H348-H355.
17. Amitani M, Asakawa A, Amitani H, et al. Plasma klotho levels decrease in both anorexia nervosa and obesity. *Nutrition.* 2013;29(9):1106-1109.
18. Razzaque MS. The role of Klotho in energy metabolism. *Nature Reviews Endocrinology.* 2012;8(10):579.
19. Ohnishi M, Kato S, Akiyoshi J, Atfi A, Razzaque MS. Dietary and genetic evidence for enhancing glucose metabolism and reducing obesity by inhibiting klotho functions. *The Federation of American Societies for Experimental Biology Journal.* 2011;25(6):2031-2039.
20. Physical Activity Guidelines Advisory Committee. 2018 Physical Activity Guidelines Advisory Committee Scientific Report. In: Services USDoHaH, ed. Washington, D.D.2018.
21. Dalise S, Cavalli L, Ghuman H, et al. Biological effects of dosing aerobic exercise and neuromuscular electrical stimulation in rats. *Scientific Reports.* 2017;7(1):10830.
22. Flegal KM, Graubard BI, Williamson DF, Gail MH. Cause-specific excess deaths associated with underweight, overweight, and obesity. *Journal of the American Medical Association.* 2007;298(17):2028-2037.
23. Finkelstein EA, Trogdon JG, Cohen JW, Dietz W. Annual medical spending attributable to obesity: payer-and service-specific estimates. *Health Affairs.* 2009;28(5):w822-w831.
24. Serra-Majem L, Bautista-Castaño I. Etiology of obesity: two “key issues” and other emerging factors. *Nutricion Hospitalaria.* 2013;28(5).
25. Weinsier RL, Hunter GR, Heini AF, Goran MI, Sell SM. The etiology of obesity: relative contribution of metabolic factors, diet, and physical activity. *The American Journal of Medicine.* 1998;105(2):145-150.
26. Ravussin E. Energy metabolism in obesity: studies in the Pima Indians. *Diabetes Care.* 1993;16(1):232-238.
27. Ravussin E, Lillioja S, Knowler WC, et al. Reduced rate of energy expenditure as a risk factor for body-weight gain. *New England Journal of Medicine.* 1988;318(8):467-472.
28. Neel JV. Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress”? *American Journal of Human Genetics.* 1962;14(4):353.
29. Galgani J, Ravussin E. Energy metabolism, fuel selection and body weight regulation. *International Journal of Obesity.* 2009;32(S7):S109.
30. Speakman JR. Thrifty genes for obesity, an attractive but flawed idea, and an alternative perspective: the ‘drifty gene’hypothesis. *International Journal of Obesity.* 2008;32(11):1611.
31. Donahoo WT, Levine JA, Melanson EL. Variability in energy expenditure and its components. *Current Opinion in Clinical Nutrition & Metabolic Care.* 2004;7(6):599-605.
32. Seidell J, Muller D, Sorkin J, Andres R. Fasting respiratory exchange ratio and resting metabolic rate as predictors of weight gain: the Baltimore Longitudinal Study on Aging. *International Journall of Obesity and Related Metabolic Disorders.* 1992;16(9):667-674.
33. Filozof C, Gonzalez C. Predictors of weight gain: the biological-behavioural debate. *Obesity Reviews.* 2000;1(1):21-26.

34. Lang A, Froelicher ES. Management of overweight and obesity in adults: behavioral intervention for long-term weight loss and maintenance. *European journal of cardiovascular nursing : journal of the Working Group on Cardiovascular Nursing of the European Society of Cardiology.* 2006;5(2):102-114.
35. Wadden TA, Stunkard AJ. *Handbook of Obesity Treatment.* Guilford Press; 2002.
36. Wing RR, Venditti E, Jakicic JM, Polley BA, Lang W. Lifestyle intervention in overweight individuals with a family history of diabetes. *Diabetes Care.* 1998;21(3):350-359.
37. Ross R, Janssen I, Tremblay A. Obesity reduction through lifestyle modification. *Canadian Journal of Applied Physiology.* 2000;25(1):1-18.
38. Hagan RD, Upton SJ, Wong L, Whittam J. The effects of aerobic conditioning and/or caloric restriction in overweight men and women. *Medicine & Science in Sports & Exercise.* 1986;18(1):87-94.
39. Jakicic JM, Otto AD. Treatment and prevention of obesity: what is the role of exercise? *Nutrition Reviews.* 2006;64(suppl_1):S57-S61.
40. Executive Summary. *Obesity Research.* 1998;6(S2):51S-179S.
41. Wu T, Gao X, Chen M, Van Dam R. Long-term effectiveness of diet-plus-exercise interventions vs. diet-only interventions for weight loss: a meta-analysis. *Obesity Reviews.* 2009;10(3):313-323.
42. Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Medicine & Science in Sports & Exercise.* 2009;41(2):459-471.
43. Jakicic JM, Clark K, Coleman E, et al. Appropriate intervention strategies for weight loss and prevention of weight regain for adults. *Medicine & Science in Sports & Exercise.* 2001.
44. Physical Activity Guidelines Advisory Committee. Physical activity guidelines advisory committee report, 2008. *Washington, DC: US Department of Health and Human Services.* 2008;2008:A1-H14.
45. Initiative NOE. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. *National Institutes of Health.* 1998:98-4083.
46. Wing RR, Goldstein MG, Acton KJ, et al. Behavioral science research in diabetes: lifestyle changes related to obesity, eating behavior, and physical activity. *Diabetes Care.* 2001;24(1):117-123.
47. Wing RR. Behavioral weight control. *Handbook of Obesity Treatment.* 2002;2:301-317.
48. Klem ML, Wing RR, McGuire M, T.Seagle, M. H, Hill JO. A descriptive study of individuals successful at long-term maintenance of substantial weight loss. *The American Journal of Clinical Nutrition* 1997:239-246.
49. Phelan S, Wing RR. Long-term weight loss maintenance. *The American Journal of Clinical Nutrition.* 2005;82(1):222S-225S.
50. Pronk NP, Wing RR. Physical activity and long-term maintenance of weight loss. *Obesity Research.* 1994;2(6):587-599.
51. Jakicic JM, Marcus BH, Gallagher KI, Napolitano M, Lang W. Effect of exercise duration and intensity on weight loss in overweight, sedentary women: a randomized trial. *Journal of the American Medical Association.* 2003;290(10):1323-1330.

52. Jakicic JM, Winters C, Lang W, Wing RR. Effects of intermittent exercise and use of home exercise equipment on adherence, weight loss, and fitness in overweight women: a randomized trial. *Journal of the American Medical Association.* 1999;282(16):1554-1560.
53. Jakicic JM, Marcus BH, Lang W, Janney C. Effect of exercise on 24-month weight loss maintenance in overweight women. *Archives of Internal Medicine.* 2008;168(14):1550-1559.
54. Jeffery RW, Wing RR, Sherwood NE, Tate DF. Physical activity and weight loss: does prescribing higher physical activity goals improve outcome? *The American Journal of Clinical Nutrition.* 2003;78(4):684-689.
55. Pi-Sunyer FX, Becker DM, Bouchard C, et al. Clinical guidelines for the identification, evaluation, and treatment of overweight and obesity in adults-the evidence report. In: Health NIo, ed. Vol 61998:51S-209S.
56. Blackburn G. Effect of degree of weight loss on health benefits. *Obesity Research.* 1995;3(S2):211s-216s.
57. Jensen MD, Ryan DH, Apovian CM, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation.* 2014;129(25 Suppl 2):S102-138.
58. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Reports.* 1985;100(2):126.
59. Powers SK, Howley ET. *Exercise Physiology: Theory and Applications to Fitness and Performance.* McGraw-Hill New York; 2004.
60. Warburton DE, Nicol CW, Bredin SS. Health benefits of physical activity: the evidence. *Canadian Medical Association Journal.* 2006;174(6):801-809.
61. Warburton DE, Gledhill N, Quinney A. The effects of changes in musculoskeletal fitness on health. *Canadian Journal of Applied Physiology.* 2001;26(2):161-216.
62. Seidell JC, Cigolini M, Deslypere J-P, Charzewska J, Ellsinger B-M, Cruz A. Body fat distribution in relation to physical activity and smoking habits in 38-year-old European men: the European Fat Distribution Study. *American Journal of Epidemiology.* 1991;133(3):257-265.
63. Tremblay A, Després J-P, Leblanc C, et al. Effect of intensity of physical activity on body fatness and fat distribution. *The American Journal of Clinical Nutrition.* 1990;51(2):153-157.
64. Slattery ML, McDonald A, Bild DE, et al. Associations of body fat and its distribution with dietary intake, physical activity, alcohol, and smoking in blacks and whites. *The American Journal of Clinical Nutrition.* 1992;55(5):943-949.
65. Maiorana A, O'driscoll G, Taylor R, Green D. Exercise and the nitric oxide vasodilator system. *Sports Medicine.* 2003;33(14):1013-1035.
66. Warburton D, Haykowsky MJ, Quinney HA, et al. Blood volume expansion and cardiorespiratory function: effects of training modality. *Medicine & Science in Sports & Exercise.* 2004;36(6):991-1000.
67. Berg A, Halle M, Franz I, Keul J. Physical activity and lipoprotein metabolism: epidemiological evidence and clinical trials. *European Journal of Medical Research.* 1997;2(6):259-264.

68. Halle M, Berg A, Baumstark M, König D, Keul J. Lipoprotein (a) in endurance athletes, power athletes, and sedentary controls. *Medicine & Science in Sports & Exercise.* 1996;28(8):962-966.
69. O'Connor GT, Hennekens CH, Willett WC, et al. Physical exercise and reduced risk of nonfatal myocardial infarction. *American Journal of Epidemiology.* 1995;142(11):1147-1156.
70. DuRant RH, Baranowski T, Rhodes T, et al. Association among serum lipid and lipoprotein concentrations and physical activity, physical fitness, and body composition in young children. *The Journal of Pediatrics.* 1993;123(2):185-192.
71. Tell GS, Vellar OD. Physical fitness, physical activity, and cardiovascular disease risk factors in adolescents: the Oslo Youth Study. *Preventive Medicine.* 1988;17(1):12-24.
72. Taimela S, Viikari J, Porkka K, Dahlen G. Lipoprotein (a) levels in children and young adults: the influence of physical activity. The Cardiovascular Risk in Young Finns Study. *Acta Paediatrica.* 1994;83(12):1258-1263.
73. Brenes G, Dearwater S, Shapera R, LaPorte RE, Collins E. High density lipoprotein cholesterol concentrations in physically active and sedentary spinal cord injured patients. *Archives of Physical Medicine and Rehabilitation.* 1986;67(7):445-450.
74. American College of Sports Medicine. Appropriate intervention strategies for weight loss and prevention of weight regain for adults. *Medicine & Science in Sports & Exercise.* 2001;33(12):2145-2156.
75. Wallberg-Henriksson H, Rincon J, Zierath JR. Exercise in the Management of Non—Insulin-Dependent Diabetes Mellitus. *Sports Medicine.* 1998;25(1):25-35.
76. Young JC. Exercise prescription for individuals with metabolic disorders. *Sports Medicine.* 1995;19(1):43-54.
77. Kelley DE, Goodpaster BH. Effects of physical activity on insulin action and glucose tolerance in obesity. In:1999.
78. Paffenbarger RS, Jung DL, Leung RW, Hyde RT. Physical activity and hypertension: an epidemiological view. *Annals of Medicine.* 1991;23(3):319-327.
79. Blair SN, Goodyear NN, Gibbons LW, Cooper KH. Physical fitness and incidence of hypertension in healthy normotensive men and women. *Journal of the American Medical Association.* 1984;252(4):487-490.
80. Adamopoulos S, Piepoli M, McCance A, et al. Comparison of different methods for assessing sympathovagal balance in chronic congestive heart failure secondary to coronary artery disease. *American Journal of Cardiology.* 1992;70(20):1576-1582.
81. Tiukinhoy S, Beohar N, Hsie M. Improvement in heart rate recovery after cardiac rehabilitation. *Journal of Cardiopulmonary Rehabilitation and Prevention.* 2003;23(2):84-87.
82. Gokce N, Vita JA, Bader DS, et al. Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. *The American Journal of Cardiology.* 2002;90(2):124-127.
83. Kobayashi N, Tsuruya Y, Iwasawa T, et al. Exercise training in patients with chronic heart failure improves endothelial function predominantly in the trained extremities. *Circulation Journal.* 2003;67(6):505-510.
84. Hambrecht R, Wolf A, Gielen S, et al. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *New England Journal of Medicine.* 2000;342(7):454-460.

85. McGavock J, Mandic S, Lewanczuk R, et al. Cardiovascular adaptations to exercise training in postmenopausal women with type 2 diabetes mellitus. *Cardiovascular Diabetology.* 2004;3(1):3.
86. Wessel TR, Arant CB, Olson MB, et al. Relationship of physical fitness vs body mass index with coronary artery disease and cardiovascular events in women. *Journal of the American Medical Association.* 2004;292(10):1179-1187.
87. Katzmarzyk PT, Church TS, Janssen I, Ross R, Blair SN. Metabolic syndrome, obesity, and mortality: impact of cardiorespiratory fitness. *Diabetes Care.* 2005;28(2):391-397.
88. Penedo FJ, Dahn JR. Exercise and well-being: a review of mental and physical health benefits associated with physical activity. *Current Opinion In Psychiatry.* 2005;18(2):189-193.
89. Helmrich SP, Ragland DR, Leung RW, Paffenbarger Jr RS. Physical activity and reduced occurrence of non-insulin-dependent diabetes mellitus. *New England Journal of Medicine.* 1991;325(3):147-152.
90. Wei M, Gibbons LW, Kampert JB, Nichaman MZ, Blair SN. Low cardiorespiratory fitness and physical inactivity as predictors of mortality in men with type 2 diabetes. *Annals of Internal Medicine.* 2000;132(8):605-611.
91. Holmes MD, Chen WY, Feskanich D, Kroenke CH, Colditz GA. Physical activity and survival after breast cancer diagnosis. *Journal of the American Medical Association.* 2005;293(20):2479-2486.
92. Barry VW, Baruth M, Beets MW, Durstine JL, Liu J, Blair SN. Fitness vs. fatness on all-cause mortality: a meta-analysis. *Progress in Cardiovascular Diseases.* 2014;56(4):382-390.
93. Hainer V, Toplak H, Stich V. Fat or Fit: What Is More Important? *Diabetes Care.* 2009;32(suppl 2):S392-S397.
94. Fogelholm M, van Marken LW. Comparison of body composition methods: a literature analysis. *European Journal of Clinical Nutrition.* 1997;51(8):495-503.
95. Fogelholm M. Physical activity, fitness and fatness: relations to mortality, morbidity and disease risk factors. A systematic review. *Obesity Reviews.* 2010;11(3):202-221.
96. Eisenmann JC, Wickel EE, Welk GJ, Blair SN. Relationship between adolescent fitness and fatness and cardiovascular disease risk factors in adulthood: the Aerobics Center Longitudinal Study (ACLS). *American Heart Journal.* 2005;149(1):46-53.
97. Matsumura Y, Aizawa H, Shiraki-Iida T, Nagai R, Kuro-o M, Nabeshima Y-i. Identification of the Human Klotho Gene and Its Two Transcripts Encoding Membrane and Secreted Klotho Protein. *Biochemical and Biophysical Research Communications.* 1998;242(3):626-630.
98. Wang Y, Sun Z. Current understanding of klotho. *Ageing Research Reviews.* 2009;8(1):43-51.
99. Chen C-D, Podvin S, Gillespie E, Leeman SE, Abraham CR. Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. *Proceedings of the National Academy of Sciences.* 2007;104(50):19796-19801.
100. Imura A. alpha-Klotho; a central regulator of Ca homeostasis. Paper presented at: Proceedings of Annual Meeting of the Physiological Society of Japan Proceedings of Annual Meeting of the Physiological Society of Japan2007.
101. Huang C-L. Regulation of ion channels by secreted Klotho: mechanisms and implications. *Kidney International.* 2010;77(10):855-860.

102. Pedersen L, Pedersen SM, Brasen CL, Rasmussen LM. Soluble serum Klotho levels in healthy subjects. Comparison of two different immunoassays. *Clinical Biochemistry.* 2013;46(12):1079-1083.
103. Begega A, Alvarez-Suarez P, Sampedro-Piquero P, Cuesta M. Effects of Physical Activity on the Cerebral Networks. In: *Physical Activity and the Aging Brain.* Elsevier; 2017:3-11.
104. Semba RD, Cappola AR, Sun K, et al. Relationship of low plasma klotho with poor grip strength in older community-dwelling adults: the InCHIANTI study. *European Journal of Applied Physiology.* 2012;112(4):1215-1220.
105. Shardell M, Semba RD, Rosano C, et al. Plasma klotho and cognitive decline in older adults: findings from the InCHIANTI study. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences.* 2015;71(5):677-682.
106. Semba RD, Cappola AR, Sun K, et al. Plasma klotho and cardiovascular disease in adults. *Journal of the American Geriatrics Society.* 2011;59(9):1596-1601.
107. Semba RD, Ferrucci L, Sun K, et al. Low plasma klotho concentrations and decline of knee strength in older adults. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences.* 2015;71(1):103-108.
108. Shimoyama Y, Nishio K, Hamajima N, Niwa T. KLOTHO gene polymorphisms G-395A and C1818T are associated with lipid and glucose metabolism, bone mineral density and systolic blood pressure in Japanese healthy subjects. *Clinica Chimica Acta.* 2009;406(1-2):134-138.
109. Cheikhi A, Barchowsky A, Sahu A, et al. Klotho: An elephant in aging research. *Journal of Gerontology: Aging, Biological Science, Medical Science.* 2019.
110. Kawano KI, Ogata N, Chiano M, et al. Klotho gene polymorphisms associated with bone density of aged postmenopausal women. *Journal of Bone and Mineral Research.* 2002;17(10):1744-1751.
111. Yamada Y, Ando F, Niino N, Shimokata H. Association of polymorphisms of the androgen receptor and klotho genes with bone mineral density in Japanese women. *Journal of Molecular Medicine.* 2005;83(1):50-57.
112. Tsezou A, Furuichi T, Satra M, Makrythanasis P, Ikegawa S, Malizos KN. Association of KLOTHO gene polymorphisms with knee osteoarthritis in Greek population. *Journal of Orthopaedic Research.* 2008;26(11):1466-1470.
113. Rhee E, Oh K, Yun E, et al. Relationship between polymorphisms G395A in promoter and C1818T in exon 4 of the KLOTHO gene with glucose metabolism and cardiovascular risk factors in Korean women. *Journal of Endocrinological Investigation.* 2006;29(7):613-618.
114. Arking DE, Becker DM, Yanek LR, et al. KLOTHO allele status and the risk of early-onset occult coronary artery disease. *The American Journal of Human Genetics.* 2003;72(5):1154-1161.
115. Deary IJ, Harris SE, Fox HC, et al. KLOTHO genotype and cognitive ability in childhood and old age in the same individuals. *Neuroscience Letters.* 2005;378(1):22-27.
116. Houlihan L, Harris S, Luciano M, et al. Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936. *Genes, Brain and Behavior.* 2009;8(2):238-247.

117. Imamura A, Okumura K, Ogawa Y, et al. Klotho gene polymorphism may be a genetic risk factor for atherosclerotic coronary artery disease but not for vasospastic angina in Japanese. *Clinica Chimica Acta.* 2006;371(1-2):66-70.
118. Brownstein CA, Adler F, Nelson-Williams C, et al. A translocation causing increased α-Klotho level results in hypophosphatemic rickets and hyperparathyroidism. *Proceedings of the National Academy of Sciences.* 2008;105(9):3455-3460.
119. Ichikawa S, Imel EA, Kreiter ML, et al. A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis. *The Journal of Clinical Investigation.* 2007;117(9):2684-2691.
120. Arking DE, Krebsova A, Macek M, et al. Association of human aging with a functional variant of klotho. *Proceedings of the National Academy of Sciences.* 2002;99(2):856-861.
121. Di Bona D, Accardi G, Virruso C, Candore G, Caruso C. Association of Klotho polymorphisms with healthy aging: a systematic review and meta-analysis. *Rejuvenation Research.* 2014;17(2):212-216.
122. Stöhr R, Schuh A, Heine G, Brandenburg V. FGF23 in cardiovascular disease: innocent bystander or active mediator? *Frontiers in Endocrinology.* 2018;9:351.
123. Kim HJ, Kang E, Oh YK, et al. The association between soluble klotho and cardiovascular parameters in chronic kidney disease: results from the KNOW-CKD study. *BMC Nephrology.* 2018;19(1):51.
124. Maekawa Y, Yasuda O, Oguro R, et al. Klotho suppresses TNF-alpha-induced expression of adhesion molecules in the endothelium and attenuates NF-kB activation. *Endocrine.* 2009;35(3).
125. Huang C-L, Moe OW. Klotho: a novel regulator of calcium and phosphorus homeostasis. *European Journal of Physiology.* 2011;462(2):185-193.
126. Hui H, Zhai Y, Ao L, et al. Klotho suppresses the inflammatory responses and ameliorates cardiac dysfunction in aging endotoxemic mice. *Oncotarget.* 2017;8(9):15663.
127. Barton M. Obesity and aging: determinants of endothelial cell dysfunction and atherosclerosis. *Pflügers Archiv-European Journal of Physiology.* 2010;460(5):825-837.
128. Barton M. Aging and biomedicine 2005: Where should we go from here? In: Elsevier Science; 2005.
129. Ahima RS. Connecting obesity, aging and diabetes. *Nature Medicine.* 2009;15(9):996.
130. Barton M, Haudenschild CC. Endothelium and atherogenesis: endothelial therapy revisited. *Journal of Cardiovascular Pharmacology.* 2001;38:S23-S25.
131. Woo K, Chook P, Yu C, et al. Overweight in children is associated with arterial endothelial dysfunction and intima-media thickening. *International Journal of Obesity.* 2004;28(7):852.
132. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *The Journal of Clinical Investigation.* 1996;97(11):2601-2610.
133. American College of Sports Medicine. *ACSM's guidelines for exercise testing and prescription.* Lippincott Williams & Wilkins; 2013.
134. Yamazaki Y, Imura A, Urakawa I, et al. Establishment of sandwich ELISA for soluble alpha-Klotho measurement: Age-dependent change of soluble alpha-Klotho levels in healthy subjects. *Biochemical and Biophysical Research Communications.* 2010;398(3):513-518.

135. Jakicic JM, Tate DF, Lang W, et al. Objective physical activity and weight loss in adults: The step-up randomized clinical trial. *Obesity.* 2014;22(11):2284-2292.
136. Jakicic JM, Rickman AD, Lang W, et al. Time-based physical activity interventions for weight loss: a randomized trial. *Medicine & Science in Sports & Exercise.* 2015;47(5):1061.
137. Courcoulas AP, Belle SH, Neiberg RH, et al. Three-year outcomes of bariatric surgery vs lifestyle intervention for type 2 diabetes mellitus treatment: a randomized clinical trial. *Journal of the American Medical Association: Surgery.* 2015;150(10):931-940.
138. Consitt LA, Bell JA, Houmard JA. Intramuscular lipid metabolism, insulin action, and obesity. *IUBMB Life.* 2009;61(1):47-55.
139. Goodpaster BH, Wolf D. Skeletal muscle lipid accumulation in obesity, insulin resistance, and type 2 diabetes. *Pediatric Diabetes.* 2004;5(4):219-226.
140. He J, Watkins S, Kelley DE. Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. *Diabetes.* 2001;50(4):817-823.
141. Oh B, Cho B, Choi H-C, et al. The influence of lower-extremity function in elderly individuals' quality of life (QOL): an analysis of the correlation between SPPB and EQ-5D. *Archives of Gerontology and Geriatrics.* 2014;58(2):278-282.
142. Langhammer B, Stanghelle JK. Functional fitness in elderly Norwegians measured with the Senior Fitness Test. *Advances in Physiotherapy.* 2011;13(4):137-144.