# A nonrandomized trial of a pre-operative physical activity program on bariatric surgery candidates as evaluated by pre- and post-operative physical activity- and obesity-related biomarkers

by

**Alex Edye-Mazowita**

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of Master of Science

Faculty of Kinesiology & Recreation Management

University of Manitoba

Winnipeg

## Abstract

Physical activity, obesity, and bariatric surgery independently exert effects on cytokines related to inflammation. Less is known of the effect of a physical activity intervention on the cytokine profile of obese patients prior to undergoing bariatric surgery, particularly since the standard cytokine profile of a bariatric surgery candidate is not established. Indices contained within the cytokine profile may predict suitability for bariatric surgery and prognosticate long-term outcomes. This thesis will examine the cytokine profile of 20 patients of the Centre for Metabolic and Bariatric Surgery in Winnipeg, Manitoba in addition to the effects of a 16 week pre-operative physical activity intervention for 7 of those patients.

The study recruited patients (n=26, BMI = 47.1 ± 6.2) from the Centre for Metabolic and Bariatric Surgery (CMBS) in Winnipeg, Manitoba between September 2017 and May 2018. A time-series quasi-experimental design was used to evaluate the effect of the intervention, which took place at the University of Manitoba's Active Living Centre. Outcomes measured included physical fitness, cardiovascular disease risk, mental health, and self-compassion measurements. The focus of this thesis will be to examine the effect of the intervention on several biomarkers found in tissue samples drawn at the baseline and 16-week time points. These measurements will include cytokines as measured via magnetic bead multiplex assay.

Significance was reached in several pre-post improvements of cardiovascular endurance and muscular strength as well as mental health, including the primary outcome of 6 minute walk test. Baseline cytokine profiles of 20 participants were comparable to demographically similar cohorts. Pre-post changes in cytokine profiles did not reach significance. Significant correlations were found between baseline and 16-week cytokine data and leg press and/or BMI.

The intervention effectively improved several measures of health and is generalizable to other pre-operative bariatric patients. The data can be used to inform the development of future physical activity trials for patients waiting for bariatric surgery.

## Acknowledgement

**Academic**

- Dr. Todd Duhamel (Thesis Advisor)
- Dr. Krista Hardy (Advisory Committee Member)
- Dr. Stephen Cornish (Advisory Committee Member)

**Other**

- Participants of the ENCOURAGEing Start program, who demonstrated inspirational outlook and energy.
- Coworkers and students at the University of Manitoba who assisted with the program, data collection, or general assistance including the Duhamel lab group (Nicole Carswell, Nicole Johnston, Sophia Mbabaali, Josie Papadopoulos, Teri Moffat, David Kent, Kevin Boreskie, Nicole Sigurdson, Alexandra Rose), Denise Cornish, Jody Bohonos, Jay Gamey and Lexi Margolis.
- Centre for Metabolic and Bariatric Surgery and Winnipeg Regional Health Authority staff, including Kathleen Clouston, Janet Antoshko, and Andrea Rodrigue.
- Jana Goodmanson for cooking and bringing me food while I was writing.

# Table of Contents


Abstract ii

Acknowledgement iv

List of tables vii

List of figures viii

Chapter 1: Literature Review 1

1.1. What is Obesity? 1

1.2. Effects of obesity 2

1.3. Causes of obesity 2

1.3.a. Lifestyle and Physical Activity 3

1.4. Treatment of obesity 5

1.4.a. Publicly available interventions 5

1.4.b. Bariatric surgery 7

1.5.a. Adipose tissue and adipokines 8

1.5.b.Rationale for adipokines and other molecules measured in this study 10

1.6 Manitoba's bariatric surgery program and rationale for thesis 17

Chapter 2: Methods 19

2.1. Overview 19

2.2. Existing CMBS framework 19

2.3. Development of the ENCOURAGEing Start program 22

2.4. Content of intervention 24

2.5. Integration of ES referrals into CMBS pre-surgical program 29

2.6. Sample size and participant assignment methods 30

2.7. Data collection 34

2.8. My specific thesis 44

2.9 Statistics 44

Chapter 3: Results 47

3.1. Data for participants completing all baseline appointments 47

3.2. Data for participants completing all baseline and 16-week appointments 52

Chapter 4: Discussion 61

4.1. Outcomes from the ENCOURAGEing Start study 61

4.2a Leptin 63

4.2b IL-1β 66

4.2c MCP-1 68

4.2d IL-6 71

4.2e TNF 74

4.2f Osteonectin 76

4.2g Osteocalcin 78

4.2h Summary of analyte panel 80

4.3. Directions for future research 83

4.3a. Recruitment and sample size 83

4.3b. Analytes 86

4.3c. Method of analysis 90

4.3d. Appointment and intervention timing 90

4.3e. Intervention structure and content 92

4.4. Conclusion 94

Appendix A: Informed Consent Form 96

Appendix B: Map for 6 minute walk test in Active Living Centre 104

Appendix C: Information sheet given to CMBS patients at seminar 107

Appendix D: Magnetic bead panel manufacturer's guide (sample excerpt) 109

Appendix E: Scatterplot graphs of bivariate correlations at baseline and 16-week time points 121

Appendix F: Bivariate correlations between delta values of cytokines and assessment measures 130

Appendix G: ENCOURAGEing Start safety and emergency response document 132

References 138

## List of Tables


Table 1: Weight classification by BMI 1

Table 2: Biomarkers analyzed in thesis 14

Table 3: Overview of intervention structure 25

Table 4: Sample Walk & Talk questions 27

Table 5: Number of participants in each ENCOURAGEing Start cohort 31

Table 6: Landmarks for anthropometric measurements of obese patients 35

Table 7: Data collection throughout ENCOURAGEing Start 42

Table 8: Characteristics and scores of participants completing baseline data collection and baseline blood draw 47

Table 9: Baseline comparisons of Canadian pre-surgical bariatric cohorts 49

Table 10: Cytokine profile of participants completing baseline data collection and baseline blood draw 51

Table 11: Characteristics and pre-post changes of participants completing all baseline and 16-week appointments 53

Table 12: Pre-post Paffenbarger questionnaire 56

Table 13: Pre-post cytokine data 57

Table 14: Bivariate correlations using baseline and 16-week data 59

Table 15: Δ (post - baseline) cytokine profiles for each of the 7 participants 60

Table 16: Interstudy serum leptin comparisons 64

Table 17: Interstudy serum IL-1β comparisons 67

Table 18: Interstudy serum MCP-1 comparisons 69

Table 19: Interstudy serum IL-6 comparisons 72

Table 20: Interstudy serum TNF comparisons 76

Table 21: Interstudy serum osteonectin comparisons 78

Table 22: Interstudy serum osteocalcin comparisons 80

Table 23: Other commonly used analytes in post-bariatric surgery panels 83

Table 24: Biomarkers of future interest 89

## List of Figures

Figure 1: Pathway into the CMBS program 21

Figure 2: Structure of CMBS pre-surgical appointments 22

Figure 3: ENCOURAGEing Start development team 23

Figure 4: Overlapping timelines of CMBS and ENCOURAGEing Start programs 32

Figure 5. Participant allocation information using CONSORT guidelines 33

Figure 6: Facebook post from a physical activity intervention 93

Figure 7: Relationships between leptin and other outcomes at baseline and post 123

Figure 8: Relationships between IL-1β and other outcomes at baseline and post 124

Figure 9: Relationships between MCP-1 and other outcomes at baseline and post 125

Figure 10: Relationships between IL-6 and other outcomes at baseline and post 126

Figure 11: Relationships between TNFα and other outcomes at baseline and post 127

Figure 12: Relationships between osteonectin and other outcomes at baseline and post 128

Figure 13: Relationships between osteocalcin and other outcomes at baseline and post 129

## List of Equations

Equation 1: Calculation of baseline inflammatory composite 46

Equation 2: Calculation of d' in a two-tailed t-test without correlation 83

# Chapter 1: Literature Review

## 1.1 What is Obesity?

Obesity is a chronic disease which may be defined as abnormal or excessive fat accumulation that may impair health, and/or a body mass index equal (BMI) greater than 29.9[1]. This condition affects approximately 1 in 4 Canadian adults[2], and self-reported data indicates that rates of obesity in Manitoba (30.4%) are higher than that national average (23.1%)[3]. This prevalence contributes to the rising economic burden of overweight and obesity in Canada, which has been estimated as high as 11 billion dollars[4]. Along with the significant financial cost, it carries an even more significant individualized cost - obesity is the second leading cause of death in Western countries[5].

**Table 1. Weight Classification by BMI**[6]

| Weight class | BMI ($kg/m^2$) |
|---|---|
| Underweight | <18.5 |
| **Normal weight** | 18.5-24.9 |
| **Overweight** | 25.0-29.9 |
| **Obese** | |
| **Class I** | 30.0-34.9 |
| **Class II** | 35.0-39.9 |
| **Class III** | 40.0-49.9 |

Though questions have been raised about the validity of using BMI as a tool to define obesity (as it cannot distinguish between fat and fat-free mass), population-level research indicates that a very high BMI is actually a more accurate predictor of cardiovascular mortality than criterion measurements such as percent body fat or fat mass[7].

## 1.2 Effects of Obesity

As defined by the World Health Organization, obesity delineates a risk of harm in addition to its categorization of weight[8] although a growing body of research has identified a phenotype known as metabolically healthy obesity (MHO). However, there is a multitude of definitions for MHO ranging from an absence of dyslipedemia, hyperglycemia, and hypertension to those who have less than 2 metabolic syndrome components. As such, the proportion of MHO could be estimated between 6 and 60%[9]. Regardless of the initial estimate, 30-50% of MHO individuals will convert longitudinally to the unhealthy phenotype[9] . The popular term "healthy at every size" may also be misleading, as there is a paucity of data concerning MHO in obesity classes II and above[10,11]. Consequently, the majority of obese individuals are indeed at risk for type 2 diabetes[12], hypertension[12], impaired bone metabolism[13] and osteoarthritis[14], stroke[15], coronary artery disease[16], left ventricular hypertrophy[17], depression[18], premature death[19], and other chronic diseases. This collection of associated chronic conditions causes an incremental association between BMI over 30.0 and pharmaceutical costs, work absenteeism, short-term disability claim incidence, and Workers' Compensation claim incidence[20]. Most evidence indicates that these relationships are J-shaped curves, with the economic burden rapidly increasing with a higher BMI[20].

## 1.3 Causes of Obesity

Obesity is an excess of stored energy in the form of adipose tissue, which results in increased body weight. This is not to say that net caloric balance, or the adage of "energy in versus energy out" (while technically correct) is the sole determinant of obesity. Several risk factors for obesity have been identified, including hereditary factors and gut microbe profile[21].

However, physiologic and genetic factors may not fully explain the burgeoning obesity rates - consideration must also be given to the context in which these profiles manifest. Contextual correlates for obesity include socioeconomic status, built environmental factors, lifestyle, social network, gender, age, culture, and food landscape[21]. It may be worthwhile to note that obesity in Canada is not as associated with low socioeconomic status as it may be in other countries[22], and obesity is more prevalent in rural areas than urban. For example, obesity reaches a national low in urban British Columbia (5.3%) to a high in rural Northern Saskatchewan (35.9%)[23].

### 1.3.a Lifestyle and Physical Activity

Lower total energy requirements in activities of daily living are linked with higher rates of obesity[24]. This has become increasingly evident in Canada with the advent of technological advances resulting in an increasing number of sedentary desk jobs, while leisure-time physical activity remains constant[25]. With occupation-related and home-related physical activity's observed precipitous decline over recent decades, researchers have surmised that the weekly energy expenditure has declined with them[26], with one estimation being that an average woman may be expending 868 fewer calories per week while an average man may be expending 980 fewer calories per week. Lending further credence to this societal factor is that objective body weights measured by the National Health and Nutrition Examination Survey over a 30 year period were predicted by occupation[26], and the prevalence of moderate-intensity physical activity occupations decreased from 48% in 1960 to 20% in 2008[26].

These stagnating physical activity levels in the face of rising obesity rates are cause for concern, as physical activity has been shown to attenuate the association between sitting time and mortality in a general population[27]. Similarly, reduced exercise capacity is a strong predictor

of mortality[28]. Whether measured by body mass index, waist circumference, or percent body fat, obese individuals who are physically fit have strikingly better outcomes than their unfit peers. For example, in the most physically fit obese populations, all-cause mortality and cardiovascular disease risk is lowered to a level less than that of unfit lean subjects[29]. This protective effect may dissipate in severe cases of obesity. Large cohort studies thus far tend to include a limited range of body mass index, generally excluding populations in Class II obesity and above (>35). It is noted that more data is needed to draw conclusions about the effects of physical activity on individuals above this range (>35)[30], or whether achieving good cardiorespiratory as measured by age-specific normative data is possible above a certain body mass index. Individuals with higher stages of obesity will face significant barriers to physical activity, which may include mechanical ventilatory constraints[31], decreased mental health and quality of life[32], and pain and progression in knee osteoarthritis[33] (resulting in surgery to rectify the pain).

Most research examining the relationship between physical activity and health outcomes in obese populations focuses on cardiovascular fitness, but resistance training has a demonstrated role in maintaining or gaining lean muscle mass, which reduces risk of sarcopenia and its subsequent reduction in resting metabolic rate[34]. These health benefits are gained despite total body weight remaining unchanged or even increased by resistance training alone[35]. Resistance training combined with aerobic exercise may offer small incremental benefits to certain markers such as hemoglobin $A_{1C}$[35].

## 1.4 Treatment of Obesity

### 1.4.a Publically Available Interventions

The two interventions which likely come to mind when considering weight loss are reduction in caloric intake and/or increase in physical activity. However, research dating back several decades[36,37] shows that these interventions may result in an initial success in weight loss, followed by a gradual return to baseline - this holds true for individuals who are overweight or obese, with or without type 2 diabetes. Unfortunately, these unsuccessful attempts to lose weight may cause physiological change which further reduces the odds of losing weight[38]. Enter the weight loss industry, which has produced countless programs and tools purported to assist in the weight loss process, and is worth USD $72 billion in the United States alone[39]. These range from multi-level marketing (also known as a pyramid scheme) companies selling stomach wraps to accredited medical fitness facilities offering staff-led group interventions. Though these products present an overwhelming variety of methodologies, none has been shown to achieve clinically significant long-term weight loss. Definitions of clinically significant weight loss for obese individuals have evolved over recent decades, from a 1998 guideline recommending achieving a BMI under 30[40] to the more recent 2013 recommendation by the National Institutes of Health to achieve 5% loss of body mass[41]. However, it should be noted that health benefits, including glycemic measures, may be possible with as little as 3% weight loss, and that more benefits will accompany increased weight loss[41].

Commercial programs may include in-person group meetings, online support, and meal plans. Weight Watchers is the most widely-used program in this category. A 2015 review of randomized controlled trials examining Weight Watchers outcomes found a statistically significant but clinically insignificant weight loss at 6 months[42], which is not a period of follow-up lengthy enough to tout permanent weight loss success (12 months is a recommended minimum[41]). Jenny Craig, Atkins Diet and Nutrisystem programs have been found to have

similar results[42]. However, it should be noted that adherence is not objectively tracked in the majority of these, and other, similar weight loss intervention studies.

Meal replacements such Medifast, Optifast and HMR show stronger effects in the short-term but their effects are attenuated within 12 months and are then comparable to the interventions listed above[42].

Intermittent fasting is a weight loss technique whereby individuals deliberately restrict caloric based on a predetermined schedule, such as eating nothing for 24 hours, and then eating ad libitum for 24 hours. One randomized controlled trial reports this technique to evoke a 50-100% improvement in the total cholesterol of individuals who are obese[43]. As with most novel weight loss interventions, there is a large amount of anecdotal evidence supplied in support of this method yet there is a paucity of randomized controlled trials studying the effects of intermittent fasting on humans, let alone any greater than 12 months in length[44,45].

Ketogenic diets, which severely restrict dietary carbohydrate intake but allow free consumption of protein and fat, have a beneficial effect on weight loss when properly integrated into a long-term lifestyle rather than used as a short intervention. This method can reduce caloric intake while suppressing feelings of hunger[46]. Several reviews focusing on people who are obese found that a ketogenic diet was sustainable, lead to improved mood[47], and also had therapeutic effects including reduced LDL and triglycerides, increased HDL and other reduced cardiovascular risk factors[48,49,50]. It also has beneficial effects for individuals with type 2 diabetes[48]. Individuals may have several barriers to adherence in this diet (as with all other diets), including socioeconomic status, availability of ingredients, motivation, and perceived unpalatability.

The Biggest Loser is one of several television shows documenting idealized, intense weight loss journeys in which participants are aided by prepared low-calorie meals, motivational competition, and highly demanding exercise programs. A 6 year longitudinal study[51] followed subjects who previously participated in The Biggest Loser, and found that while 9 of 14 subjects had kept some weight off (while the other 5 were near or above their pre-competition weight), the resting metabolic rate of all participants had decreased by 704 ± 427 calories. This study illustrated the potential for persistent metabolic adaptation with contemporaneous unhealthy and rapid weight loss.

Several prescription drugs such as orlistat, lorcaserin and liraglutide are approved for short- or long-term treatment of obesity. Mechanisms of action for such drugs may include uptake inhibition of catecholamines, serotonin, or more than one neurotransmitter[52]. Orlistat is unique in its contribution to weight loss, and blocks approximately one-third of fat absorption. Although these drugs may result in modestly effective weight loss, common side effects including gastrointestinal disturbances, insomnia, cognitive problems, and elevated heart rate contribute to their limited long-term desirability[21].

### 1.4.b Bariatric Surgery

Bariatrics is the branch of medicine which studies and treats obesity, while bariatric surgery refers to surgical modification of the stomach or intestine and is indicated by the National Institutes of Health (NIH) for patients who have a BMI over 40 (or over 35 with comorbid conditions) and for whom other treatments have failed[52]. Unlike commercial products, or physical activity and nutrition interventions performed in isolation, bariatric surgery elicits clinically significant weight loss results. Patients in Manitoba undergo a type of bariatric surgery

known as Roux-en-Y Gastric Bypass (RYGB), a restrictive and malabsorptive procedure in which a small pouch (15-30 ml) is created from the stomach, and then attached to the small intestine (length 100-150 cm) after bypassing the majority of the stomach and duodenum[53]. This connection, or anastomosis, between the stomach pouch and duodenum is beneficial both because of the reduced stomach space, and reduced fat absorption in the bypassed duodenum segment. Though its popularity has been overtaken in the United States by sleeve gastrectomy (a procedure which results in comparatively less weight loss at the two year time point[54]) as of 2013, RYGB remains the standard offering in Canada[55]. RYGB assists subjects in successfully losing and keeping off 20-30% of their body weight in the short-term[53] and a reduced but still clinically significant amount after 10 years[56]. Therefore, it stands as the most, and perhaps only, method of eliciting clinically significant weight loss which can be maintained in the long-term.

### 1.5.a Adipose Tissue and Adipocytes

With similar total body fat percentages, an individual with increased visceral fat mass will be at higher cardiometabolic risk than an individual with increased subcutaneous fat mass[57]. However, removal of this visceral fat via liposuction does not necessarily improve an inflammatory profile[58], thus suggesting that factors other than the simple presence of fat itself account for the associated effects of obesity.

This presence of adipose tissue (whether measured as weight, waist circumference, or skinfolds) was historically the main focus in obesity research exploring the relationship to cardiovascular and metabolic outcomes. However, contemporary research delves deeper into *why* adiposity has these effects. Is there a quantifiable explanation why caloric intake is deregulated? Does excess weight in itself lead to type 2 diabetes, dyslipidemia and hypertension? Much of the

discussion in the area now focuses on the adipose tissue itself, and the bioactive molecules known as adipokines it produces. Adipokines are a specific type of cytokine, which is a broad category of peptides involved in cell signaling during both healthy and pathological states. These molecules, cytokine and adipokine alike, affect metabolic processes including satiety, insulin sensitivity, and inflammation[59,60]. The origin of a cytokine does not necessarily predict its function. For example, adipocytes produce both pro-inflammatory (IL-6) and anti-inflammatory (adiponectin) adipokines[61]. Cytokines may also be pleiotropic. For example, when IL-6 is expressed as a myokine (a cytokine released from muscle) it has an anti-inflammatory effect, as opposed to its pro-inflammatory effect when expressed as an adipocyte[62].

Adipose tissue can be differentiated by type and by location. White adipose tissue is the main form of energy storage in humans. When available carbohydrates are insufficient to supply the body's needs, lipids are mobilized through the process of lipolysis and these stores are depleted[63]. Conversely, white adipose tissue cells will increase in size and number from excess energy intake. White adipose tissue is metabolically active, influenced by the sympathetic nervous system and capable of releasing hormones which may subsequently affect further metabolic and inflammatory processes[64]. Brown adipose tissue assists thermoregulation, and is most abundantly present in infants. Its quantity decreases with age although some conditions may cause further production of brown adipose tissue[65]

Fat location is traditionally described in terms of either visceral adipose tissue or subcutaneous adipose tissue. Visceral adipose tissue may also be referred to as abdominal or central obesity. Accumulation of visceral fat is associated with pathological conditions including insulin resistance, several cancers, and prolonged hospital stays[66]. Though visceral adiposity tends to increase with age, the amount of visceral adipose tissue relates to the magnitude of

health risk[67]. Subcutaneous adipose tissue poses relatively lower health risk, and may be more metabolically healthy. For example, subcutaneous adipose tissue may produce a more favorable ratio of interleukin-6 and tumor necrosis factor α[68]. Recent research also suggests that visceral and subcutaneous adipose tissues do not express the same patterns of adipokines[69].

Adipose tissue transplants improve adipokine profiles and effectively treat type 2 diabetes in mice[70]; however, no similar treatment has been tested in humans. Known methods of ameliorating adipokine levels in humans includes an improvement in diet, weight loss, therapeutic administration, or physical activity - the latter of which will be the focus of the intervention central to this thesis.

### 1.5.b Rationale for adipokines and other molecules measured in this study

This section comprises a brief discussion on the analytes selected for measurement in this thesis, followed by a summary of their characteristics in **Table 2.** Eight of the 9 analytes are cytokines, which as described above are a broad category of substances which are secreted by certain cells to act on other cells. Adipokines (secreted by fat cells) and myokines (secreted by muscle cells) are types of cytokines of interest in the area of bariatric medicine and physical activity, two fields which intersect in this intervention.

The study of adipokines was birthed by the discovery of leptin in 1994, which linked a hormone produced by adipose tissue (known as an adipocyte) to food intake and energy expenditure[71]. Leptin remains a target of research in obesity, as its properties include increase of anorexigenic (appetite inhibiting) and decrease of orexigenic (appetite stimulating) peptides in the hypothalamus[72]. In what might seem a paradox, obesity is associated with high leptin levels[73]. This may be due to obesity increasing the body's leptin resistance[74]. More recently, it has been suggested that leptin's roles include signaling energy deficiency in the brain, and so

while leptin therapy appears to have no use to combat obesity[75] it may have a beneficial role for individuals who have already begun losing weight. Leptin therapy to treat the weight-loss induced condition of relative leptin insufficiency[76], including after roux-en-y gastric bypass surgery, has been shown to result in reduction of sensitivity to food cues and maintenance of energy expenditure[77].

With the discovery that adipose tissue is a metabolically active endocrine organ and capable of crosstalk with several other systems[78], research sought to determine the role of its other secretions, of which there are more than 600[79]. Many of these 600 adipokines exert effects on insulin resistance (e.g. adiponectin, leptin, retinol binding protein-4), inflammation (tumor necrosis factor alpha, monocyte chemoattractant protein-1, resistin) and cardiovascular risk factors (e.g. visfatin, serum amyloid A-3, pentraxin-3)[80]. Adiponectin, discovered in 1995, is an example of an anti-inflammatory, insulin-sensitizing adipokine, notable for its status as the most abundant protein secreted by white adipose tissue[81].

Interleukin-6 and tumor necrosis factor alpha are additional pro-inflammatory cytokines included in this study. The effects of bariatric surgery on these 2 molecules are unclear[82], though their increase in the presence of both aerobic exercise and obesity is established[83,84]. Apelin is an adipokine whose plasma levels increase with obesity and decrease following roux-en-y gastric bypass surgery[85,86]. Though not detailed in Table 2, it is notable that a meta-analysis has found evidence that depression may also elevate proinflammatory cytokines[87].

Inclusion of myokines in the analysis was indicated as an additional lens through which to examine the relationships of obesity and physical activity, as the thesis intervention was to include measurements of muscular strength and endurance. As noted earlier, myokines are a bioactive molecule that are released when myocytes contract. The characteristics of an individual

who is obese as they relate to skeletal muscle are unique: they may have a significant quantity of absolute lean body mass, particularly in the lower body, but a relatively lower percentage of lean body mass due to obesity. Furthermore, the high rates of sedentary behavior in individuals who are obese[24] may cause altered myokine response[88]. As such, two myokines will be measured in this thesis. Secreted Protein Acidic and Rich in Cysteine (SPARC, also known as osteonectin) and Irisin are the 2 myokines included in the analysis.

SPARC may be a somewhat novel measurement in the context of this study. SPARC is associated with insulin resistance and obesity, and its secretion is increased by leptin[89]. It is established that SPARC will increase with both acute and chronic aerobic exercise, but may decrease significantly with an 8 week weight loss regime[90]. The effects of physical activity on SPARC in individuals who are obese may yield further insight into the benefits, and potential drawbacks, of different types of physical activity in this population.

Irisin is a particularly relevant myokine, as its interactions with obesity, and especially bariatric surgery, are poorly understood. Maintaining or increasing irisin levels pre-operatively through physical activity may ameliorate long-term weight maintenance due to irisin's role in energy expenditure. This is salient for bariatric surgery patients, as most significant weight loss journeys will inevitably involve loss of lean body mass (though significantly less so if the patient is physically active)[91].

The majority of targeted cytokines may be described as adipokines because they are secreted by adipocytes. However, none of the targeted cytokines are exclusively secreted by adipocytes. Even leptin is secreted by other tissues, including hepatocytes and enterocytes[92]. Conversely, IL-6 and irisin, frequently examined as exercise-induced myokines, are also secreted

to a lesser degree by adipocytes[62,93]. It may therefore by disingenuous to refer to any of the analytes exclusively as either an adipokine or myokine.

As measured by the analytes described above, the effect of a pre-operative physical activity program for bariatric surgery candidates may provide insight into the health benefits of improvement of physical fitness in the absence of significant weight loss. Their relationships, which are broadly established in the contexts of obesity, inflammation, physical activity, and remission of type 2 diabetes, are a clinically relevant tool with which long-term health outcomes may be prognosticated with or without bariatric surgery. Such data may also elucidate the relationship between these measurements pre- and post-bariatric surgery, of which there is a paucity of longitudinal data. Understanding the long-term effects of these markers may assist in the development of more extensive pre- and post-bariatric surgery quality indicators.

| **Table 2: Biomarkers analyzed in thesis** ↑ Increases ↓ Decreases ↔ Unchanged | | | | |
|---|---|---|---|---|
| **Molecule Name and *type*** | **Description** | **Effects from exercise** | **Relation to obesity** | **Effects from roux-en-y gastric bypass surgery** |
| Leptin<br><br>*Adipokine* | Hormone produced by adipose tissue (and other organs). Acts on central nervous system to suppress food intake and increase energy expenditure through cardiovascular system and brown adipose tissue [92,94] | ↓by aerobic[95]<br>↔ by strength training[96] | Positively correlated with body fat mass and body mass index[92], exercise-related decreases more significant with lower body fat[95] | Very strong evidence showing ↓ [97] |
| Interleukin 1 beta (IL1β)<br><br>*Adipokine* | Cytokine which has an overall pro-inflammatory effect, and which may also have a role in neurophysiological process of anxiety[98] | ↑ by aerobic[99]<br>↑ long-term up-regulation[100] | ↑ levels in obese individuals [83] | ↓ by RxY[101] |
| Apelin<br><br>*Adipokine* | Peptide secreted by adipose tissue, considered an adipokine[102]. Expressed in several systems including digestive, osseous, muscular. Has effects on blood pressure[103], food intake, muscle hypertrophy[104]. | ↓ by aerobic program[105], though it ↑ acutely in healthy subjects doing maximal exercise[106]. | ↑ levels in obese individuals[102]. | ↓ by RxY[85] |

| **Molecule Name and *type*** | **Description** | **Effects from exercise** | **Relation to obesity** | **Effects from roux-en-y gastric bypass surgery** |
|---|---|---|---|---|
| Monocyte chemoattractant protein-1 (MCP-1/CCL2)<br><br>*Cytokine* | Chemotactic cytokine produced by many cell types. Regulates migration of monocytes and memory T cells, may have use in treatment for atherosclerosis, insulin-resistant diabetes[107] | ↑ by aerobic[108]<br>↓ by strength training[109] | ↑ levels in obese individuals[110] | ↓ as soon as 3 months post-op[111,112] |
| Interleukin 6 (IL-6)<br><br>*Cytokine* | Pro-inflammatory cytokine and anti-inflammatory myokine. Influences immune response, energy metabolism in muscle and fat and osteoclast formation | ↑by aerobic (up to 100 fold). Produced by muscle fibers during exercise[113].<br>↑ by strength training | Positively correlated with, percentage body fat[84], waist circumference and BMI in children as young as 3[114]. | Effect is unclear[82] |
| Tumor necrosis factor alpha (TNF-α)<br><br>*Cytokine* | Regulates activation of macrophages and mediates inflammatory response. Pro-inflammatory and promotes insulin resistance[100] | ↑ by aerobic<br>↔ by strength training (changes depending whether sample is taken from plasma or muscle)[100] | ↑ levels in obese individuals [83] | Effect is unclear[82] |
| Osteonectin/Secreted protein acidic and rich in cysteine<br><br>*Myokine* | Myokine which reduces fat accumulation, inhibits adipogenesis and aids glucose metabolism[90]. May induce skeletal muscle atrophy[115]. | ↑ acutely by aerobic<br>↑ chronically by 4 weeks of aerobic training[116] | ↑ levels in obese individuals, can decrease significantly within 8 weeks[89]. | There appears to be no data. |

| **Molecule Name and *type*** | **Description** | **Effects from exercise** | **Relation to obesity** | **Effects from roux-en-y gastric bypass surgery** |
|---|---|---|---|---|
| Osteocalcin<br><br>*Protein* | Produced solely by bone. Involved in regulation of energy homeostasis and has some demonstrated association with inflammation[117]. Inversely related to IL-6[118] | ↑ acutely by aerobic exercise<br>↔ by strength training[119] | ↓ levels in obese individuals[118] | ↑ by RxY[120] |
| Irisin<br><br>*Myokine* | Exercise-induced adipomyokine that may stimulate browning of white adipose tissue, glucose uptake in skeletal and cardiac muscle[121]. | Unclear which type of exercise produces most ↑[122,123]. Neither has been shown to ↓. May be ↑ with habitual physical activity[124]. | Unclear[124]. | Few studies exist. One n=12 found that irisin↑ for 8 and ↓ for 4[125]. |

## 1.6 Manitoba's bariatric surgery program and Statement of the Problem

A detailed description of the patient flow through the Centre for Metabolic and Bariatric Surgery (CMBS) is provided in the Methods section. Briefly, patients in Manitoba are eligible for publicly funded bariatric surgery if they meet the NIH criteria described in 1.4.b. After entry into the program, patients will attend an information seminar, followed by a multidisciplinary evaluation by a nurse, kinesiologist, dietitian, and psychologist where a combination of standardized and individualized goals are set. Patients receive ongoing care prior to surgery wherein they will meet with these specialists two to four times over a six month period.

As discussed in 1.3.a., pre-operative physical activity levels are an important predictor of both short- and long-term success following surgery. Physically active patients will see greater likelihood of metabolic benefit from the retention of lean body mass during weight loss, fewer perioperative complications, resolution of pre-existing chronic disease, increased function, and long-term weight loss and maintenance[126]. Although pre- and post-operative physical and lifestyle management programs are recommended by the American Society of Metabolic and Bariatric surgeons[127], there is no standardized model of providing either type of physical activity support to patients in Manitoba. Due to a standard 18 month wait time for surgery and the substantial interim healthcare costs of obesity, the development of an upstream program to build patients' physical activity skills and self-efficacy is worthwhile. Based on this rationale, a pilot project for a physical activity program (ENCOURAGEing Start) was developed from 2016-2018 in partnership between the University of Manitoba and the CMBS. The goal was to calculate the sample size needed to demonstrate a statistically significant effect size for outcomes including the six minute walk test in a larger project.

This thesis, using data collected prior to and after the physical activity intervention that was conducted, will analyze the cytokine profile of patients at two time points. The baseline data of all patients who completed both physical function and blood sample baseline appointments will be used to measure a baseline cytokine profile of patients in the pre-surgical program. The 16-week data of all patients who completed both physical function and blood sample baseline and appointments and both physical function and blood sample 16-week appointments will be used to discuss the effect of the physical activity intervention.

# Chapter 2: Methods

## 2.1 Overview

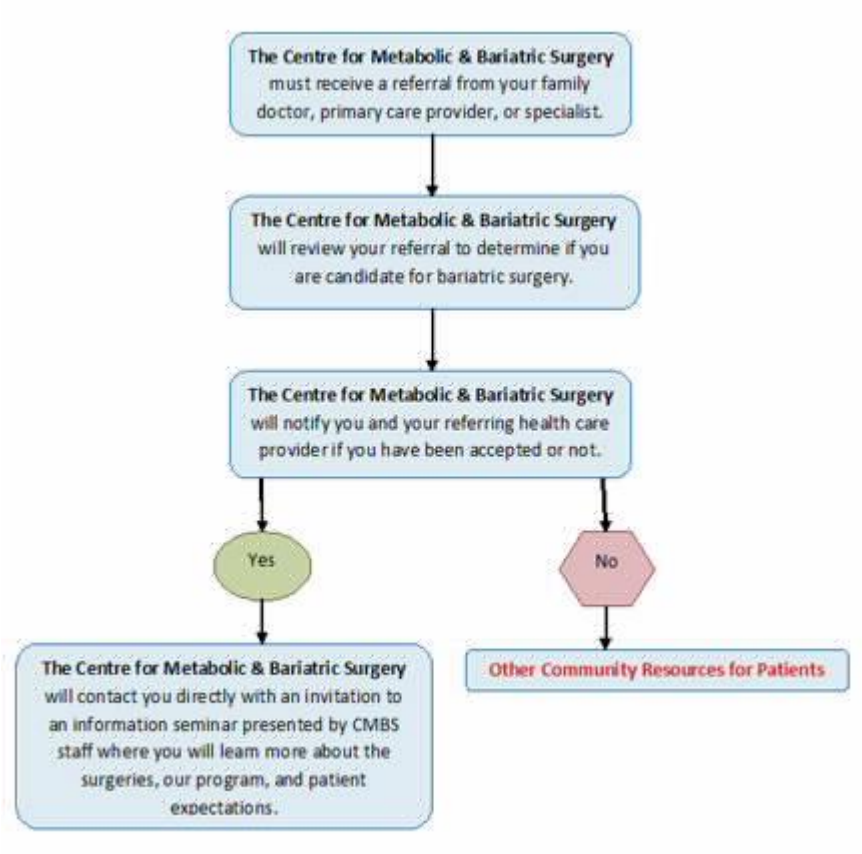
This thesis will focus on data collected during the ENCOURAGEing Start (ES) intervention, which was a time series quasi-experimental design used to determine the effects of a 16 week pre-operative physical activity program on musculoskeletal fitness, cardiovascular health, mental health, and quality of life of bariatric surgery candidates. The overall study's primary outcome was the 6 minute walk test. This chapter will begin by describing the care map of the CMBS, its involvement with ENCOURAGEing Start, and the intervention structure. Following this overview is a description of the unique components of this thesis, which analyzes the adipokines, myokines and cytokines of the study participants using data collected at baseline and the 16 week time point (**Table 7**). Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) guidelines were followed in the reporting of baseline and 16-week data. The study protocol was approved by the University of Manitoba's Health Research Ethics Board.

## 2.2 Existing CMBS framework

To better delineate the recruitment process and methodology for this study, it is appropriate to first discuss the existing framework of the program from which the participants were recruited - the Winnipeg Regional Health Authority's Metabolic and Bariatric Surgery Program, established in April 2012 at Victoria General Hospital (VGH) in Winnipeg, Manitoba. The structure of the existing CMBS pre-operative care pathway begins with a referral from a medical doctor into the program (**Figure 1**). The wait to enter the program may be as long as 18 months. After entering the program, patients first attended a group information seminar led by the CMBS team where the requirements and expectations of the program were described. Patients were given a client information book which contains information on bariatric surgery,

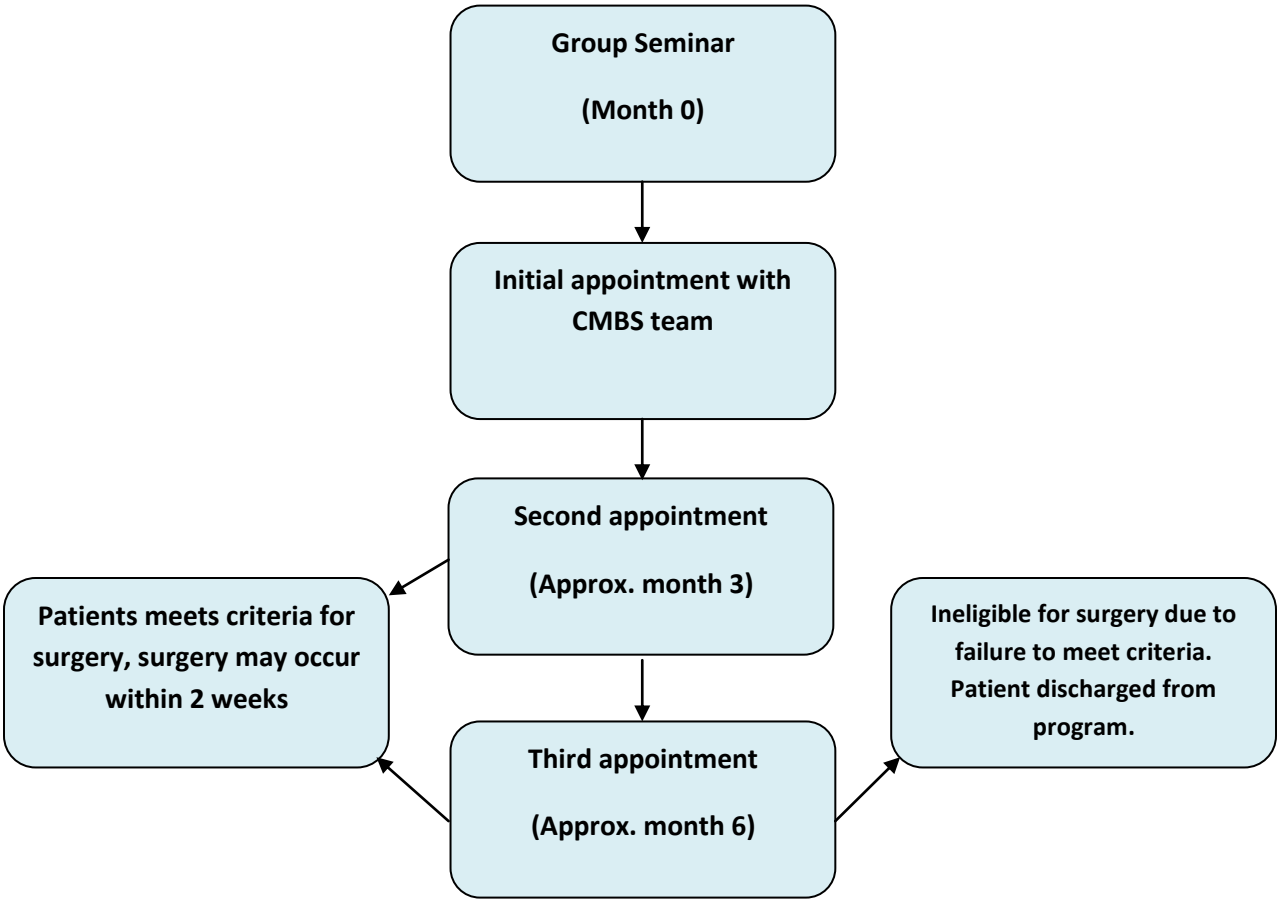
potential complications, the hospital stay, and diet changes before and after the surgery. These seminars typically occurred monthly with a new group of incoming patients.

**Figure 1. Pathway into the CMBS program**




Patients then began a series of personalized follow-up appointments with the CMBS team at intervals of approximately 3 months (**Figure 2**). Practitioners on the CMBS team assisted patients in their efforts to match their pre-surgical requirements such as increased physical activity or participation in the Craving Change© program. If participants were deemed to have met these requirements at the time of the second appointment, they were booked into surgery as soon as 4 weeks later after a surgeon consultation. Otherwise, patients attended a third appointment wherein a failure to meet pre-surgical requirements resulted in discharge from the program.

**Figure 2. Structure of CMBS pre-surgical appointments**

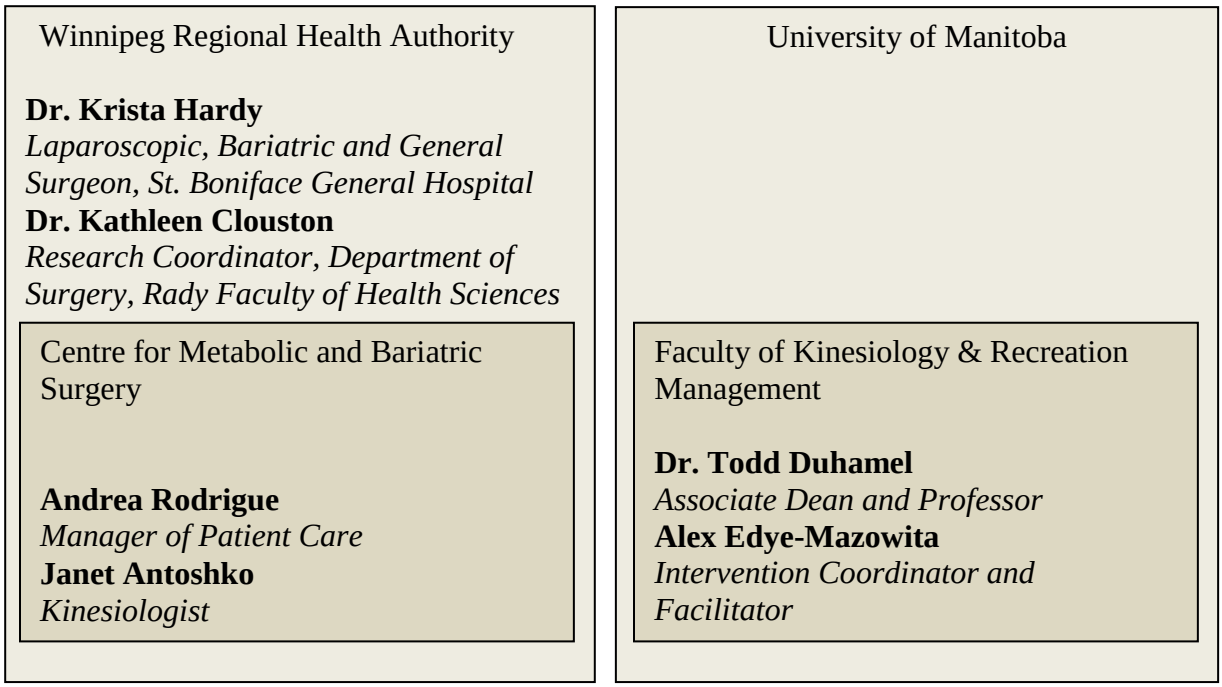



## 2.3 Development of the ENCOURAGEing Start program

As described in the literature review, an enhanced physical activity component may provide benefit to the pre- and post-surgical patient outcomes of the CMBS program. As such, development of ENCOURAGEing Start began in Summer 2016 with initial consultation between CMBS and University of Manitoba's Faculty of Kinesiology and Recreation Management to establish an intervention to support physical activity in patients waiting for roux-en-y gastric bypass surgery. Site visits were made to Victoria General Hospital beginning in September 2016 to observe the group seminar, discuss the existing program, and meet in a round-table format several times with the CMBS team to discuss what type of content might be most needed for the

ES program. Finally, drafts of the program content were shared and discussed in a series of meetings taking place both at VGH and FKRM. Individuals involved in the process are detailed in figure 3.

**Figure 3.ENCOURAGEing Start development team**




This study was informed by a previous randomized controlled trial by Kwok which took place between July 2014 and October 2015 with a similar cohort of pre-operative patients from CMBS[128]. The study had participants meet at another local gym three times per week for two weeks with a kinesiologist trainer, followed by ten weeks where the participants were responsible for achieving the physical activity prescriptions set by the trainer. Kwok's experimental group showed no difference in most measures, including objective weekly physical activity as assessed by waist-worn accelerometer. Inherent weaknesses to the design may have been the lack of structured social interaction, brevity of the introduction phase, lack of variety,

and a rigidity to the strength training prescription that may have held participants back from greater rates of progression back during this introductory phase of resistance training (as prescriptions for the entirety of the 12 week intervention were derived from baseline 1 repetition maximum).

## 2.4 Content of intervention

The ENCOURAGEing Start intervention was created by a Canadian Society for Exercise Physiology Certified Exercise Physiologist (CSEP-CEP) in consultation with CMBS staff. A brief overview of the program is described in Table 3. Safety and emergency guidelines developed for this intervention can be found in Appendix F.

**Table 3. Overview of intervention structure**

| | Structured components | Unstructured components |
|---|---|---|
| Weeks 1-8 | **Tuesday**: one session of walking program integrating discussion and skill development<br>**Thursday**: one session of instructor-led structured exercise. Type of exercise changes weekly to provide participants a cross-section of what programs are available in community (i.e. cardio machines, yoga, recreational sports)<br>**Weekly**: Homework assignments<br>**Every two weeks**: educational seminar | Membership to University gym which includes access to equipment, track and pool as well as group fitness classes |
| Week 9-16 | **Tuesday:** window of time where instructor is available to assist participants or answer questions in person (optional)<br>**Every two weeks:** contact in-person or by phone/email between instructor and participant to maintain motivation and assist as needed | Membership to University gym which includes access to equipment, track and pool as well as group fitness classes |

***Weeks 1-8 - Active Living Center membership***

Patients were given an access card to the Active Living Center that offered the same benefits as a full membership for the duration of the program. The instructor educated patients on several free features that could be used with this card including a pool and fitness classes (including aquacize and stretch).

***Weeks 1-8 - Tuesday "Walk and Talk" program***

Walk & Talk is designed to promote social support and behavior modification while gaining the added health benefits of moderate-intensity exercise with short bouts of vigorous intensity exercise. In short, patients walked in pairs on a track while discussing relevant health- and physical activity-related topics (**Table 4**), engaged in "checkstops" wherein they interact with the instructor, learned or demonstrated a skill, did an exercise, or discussed a question in more depth. An example of a checkstop might be learning how to measure exercise heart rate, and then seeing if the speed of a regular lap puts the patient in a moderate-intensity heart rate zone. A later example might be selecting descriptors (e.g. done alone, learn a skill, free) from a series of cards and identifying a physical activity that matches these descriptors. Each exercise session contained two checkstops. Patients were provided a list of themed questions for each session, although patients were informed that they could organically create their own health-related topics.

**Table 4. Sample Walk & Talk questions**

| Week 1: Walk & Talk question prompts | |
|---|---|
| Briefly describe yourself to your partner – perhaps a bit about your job and family situation – and then share an interesting fact! | Are there people in your life who would benefit from you adopting a healthier lifestyle, or who already benefit from your current healthy lifestyle? In what way? |
| When it comes to your overall health, what are some aspects you are satisfied with? What are some aspects you wish were better? | To what factors do you attribute your current health? |
| How does managing your health connect to what you consider to be important in life? | Share an example of a personal success. This can be anything from learning to do home renovations to overcoming shyness and taking a dance lesson! |
| What compelled you to attend this program? | In what way would improving your health benefit the aspects of your life you value? |
| In your day-to-day life, in what ways do you feel strong and capable? In what ways do you feel challenged? | What is a source or sources of motivation for you to become more active? |

Patients were encouraged to do a "hot lap" after both partners had fully answered each question. This was described as a higher-intensity, non-conversational lap where the patient would feel that they were working hard. It was identified in the first week of the intervention that some patients may be uncomfortable walking on the track for an hour, as there were no benches or chairs to rest nearby. Consequently, patients were encouraged to use stationary bikes or elliptical machines if they felt more confident. In this case, they were asked to visit the instructor 15 minutes and 45 minutes into the session for their checkstops.

***Weeks 1-8 - education seminar***

In weeks 1, 3, 5 and 7 of the program, patients participated in a larger group discussion in a non-active setting. This included sharing stores, an educational component from the instructor, and guided discussion. One example is the topic of connecting to local physical activity resources that was done in week 7.

***Weeks 1-8 - homework***

Homework was given to patients in most weeks, and was presented to them on Thursdays. These assignments always tied into questions for the next week's Walk and Talk session. Examples of homework include one week of tracking physical activity, setting a SMART goal, coming to the Active Living Center on their own, or identifying different types of social support in their life.

***Weeks 1-8 -Thursday mixed activity sessions***

Participants engaged in a variety of physical activity on the Thursday sessions, which was designed to offer an approximation of what programs they could find in the community and lower future barriers of intimidation and knowledge. Scheduling of external instructors and space availability dictated slight differences between the three cohorts, but examples included a Zumba class, a yoga class, resistance training circuit, a spin class, and recreational sports such as badminton and floor hockey.

***Weeks 9-16 - open time with instructor, and option to participate in other cohorts' programming***

The instructor was accessible on Tuesday for a one hour period prior to the scheduled Walk and Talk time for weeks 1-8 of the following cohort. No structure was given to this meeting, but patients were informed that they could use the time for whatever they wished. Examples of what patients discussed with the instructor included a refresher on exercise machines, and a discussion about barriers to healthy living stemming from the patient's employment. Patients were informed that they were able to join in the next cohort's Walk and Talk sessions, education seminars, and Thursday activities.

***Weeks 9-16 - membership to Active Living Center***

Membership benefits, including gym membership and fitness classes, continued until the end of the program.

***Weeks 9-16 - phone call or email follow-ups***

If a patient had not met with the instructor in the open time for more than 2 weeks, the instructor reached out to the patient to inquire whether any assistance was needed.

## 2.5 Integration of ES referrals into CMBS pre-surgical program

A one-page information sheet and consent-to-contact form for ES (Appendix C) were added to the package received by the patients at the CMBS group information seminar. Patients were able to read this one-page summary of the study and return the signed consent-to-contact form to CMBS alongside the standard paperwork if they were interested in participation in ES.

The information sheet also contained a contact number for the ES Intervention Coordinator, which patients were told they could call for any study-related questions. The CMBS Kinesiologist was familiar with ES and was available to answer questions at patient appointments as well. Patients were provided the incentive of a 16 week membership to a fitness facility (Active Living Center) and access to an exercise specialist (the ES Intervention Coordinator). No direct financial incentive was provided.

The CMBS Clinic Coordinator passed any signed consent-to-contact forms to a Research Coordinator, who then passed the information to the Intervention Coordinator monthly via in-person delivery at the University of Manitoba Active Living Centre. As such, this was a self-selected cohort of patients whose eligibility was established by their presence in the CMBS program.

### 2.6 Sample size and participant assignment methods

ENCOURAGEing Start targeted recruitment of 48 patients, as it was determined that this would provide sufficient power for the primary outcome (six minute walk test) for the overall study. This was based on a conservative sample size calculation (two-tailed, 90% power) and a dropout rate of 40%. Three cohorts were planned to support recruitment feasibility (e.g.: number of patients attending CMBS seminar, and Faculty of Kinesiology & Recreation Management resources). Patients were assigned into the next available cohort (**Table 5**) once the baseline appointment was completed.

My thesis will examine biomarker data from the ENCOURAGEing Start intervention participants, therefore it is limited to the sample recruited for the original purpose.

**Table 5.Number of participants in each ENCOURAGEing Start cohort**

| | Cohort start date | Participants who completed baseline appointment and entered cohort |
|---|---|---|
| Cohort 1 | Nov. 21 2017 | 6 |
| Cohort 2 | Feb. 13 2018 | 9 |
| Cohort 3 | May 1 2018 | 11 |

Participants were entered into the next available ENCOURAGEing Start cohort due to the structure of the CMBS program. As the participants spent more time in the CMBS program, they moved closer towards a potential surgical date, which would necessitate withdrawal from ENCOURAGEing Start and eliminate the opportunity for 8-week and/or 16-week data collection **(Figure 4)**. Even so, several participants dropped out of the study due to receiving a surgery date (**Figure 5**), which itself may reflect positively on the effect of the intervention. Participants who underwent surgery while participating in ENCOURAGEing Start were permitted to attend ENCOURAGEing Start and use their gym membership post-recovery during the remaining portion of the 16 weeks. Neither participants nor research and intervention staff were blinded, and all participants were aware that they were participating in a novel intervention beyond the standard of care.

**Figure 4.Overlapping timelines of CMBS and ENCOURAGEing Start programs**

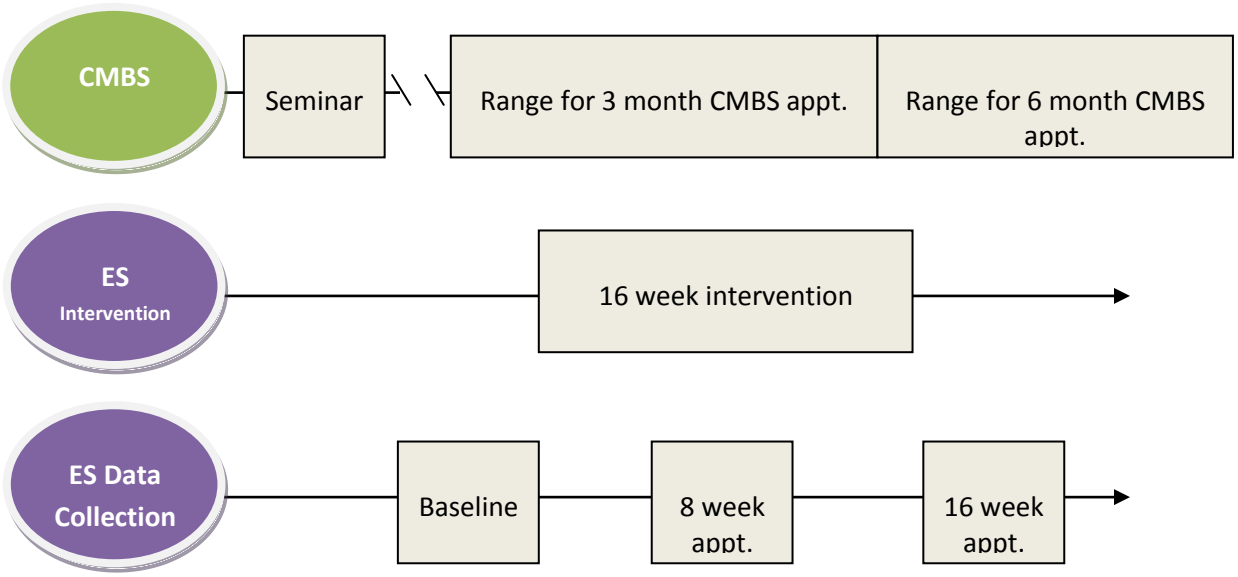
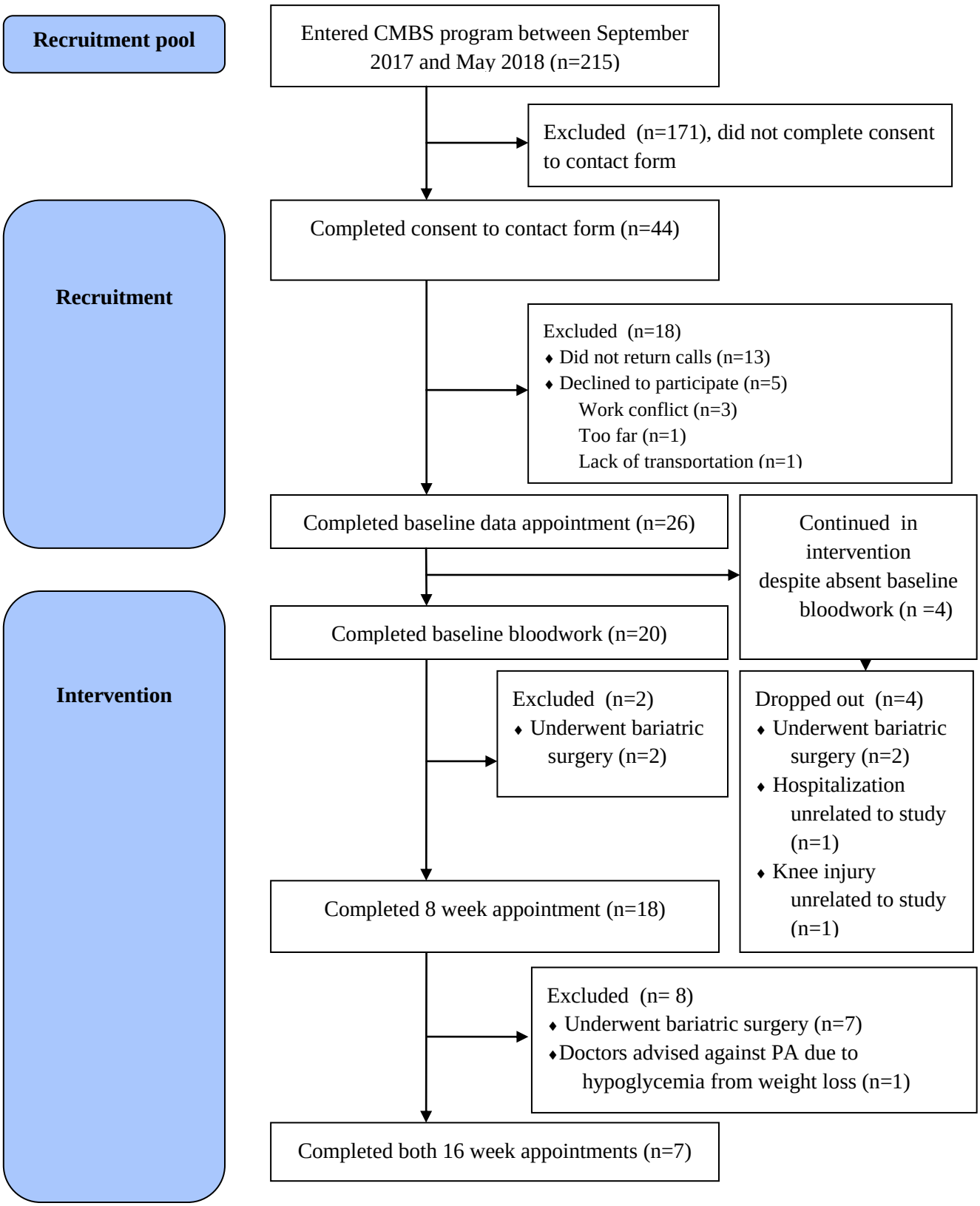


**Figure 5.Participant allocation information using CONSORT guidelines**[129]



## 2.7 Data Collection

Both the data collection appointments and intervention itself occurred in the University of Manitoba Active Living Center. Though the majority of the building is occupied by a gym space, the Applied Research Center subsection is comprised of several quiet and private rooms suitable for the nature of testing and discussion inherent to the study. The building also contains a blood lab that was used for the collection of samples. The sum of data collection methods listed below constitute the ENCOURAGEing Start protocol.

### *Blood draws*

One day before blood was drawn, patients were given a reminder call by a team member. During the call, patients were reminded to fast beginning at midnight, take medications as normal, and avoid smoking, tobacco, and exercise that morning. Patients reported to the ALC in the morning between 6:30 AM and 10:00 AM for blood draws. Samples were drawn by a trained Phlebotomist into 2 x 8mL lithium heparin vacutainers. Samples were centrifuged at 1,600g for 15 minutes at $4^{o}$ C less than half an hour after collection. Supernatant was pipetted into labeled plastic serum sample tubes and stored at $-80^{o}$ C prior to analysis.

### *Anthropometric measurements*

Height and weight were taken at the start of each appointment. This was followed by circumference measurements of the neck, waist, and hips. The landmarks below were used to standardize the anthropometric measurements.

**Table 6.Landmarks for anthropometric measurements of obese patients, CMBS**

| | **Neck** | **Waist** | **Hip** |
|---|---|---|---|
| Location | Midway of the neck **Men: Below Adams Apple | Midway between the lowest rib and upper iliac crest ***Usually at level with belly button* | At the femoral (greater) trochanter ***Largest part of the buttocks* |
| Measure | Start at the front of the neck, wrap around back to the start, take measurement | Wrap the tape measure horizontally around the waist ***Measure at the end of a gentle expiration* | Wrap the tape measure horizontally around the largest part of the buttocks, take measurement |

*Adapted from Ben-Noun, Sohar & Laor (2012).Neck circumference as a simple screening measure for identifying overweight and obese patients. Obesity Research, 9 (8), pp: 470-477*

Weight, percent body fat and skeletal muscle mass were measured using an InBody 720 bioelectrical impedance analysis device. Before the test, individuals were instructed to use the bathroom if possible, and had been standing for several minutes during the circumference measurements described above.

***Cardiovascular measurements***

Measurements of blood pressure were taken throughout the appointment. Large artery elasticity assessment and small artery elasticity assessment were taken once during the appointment. The measurement device used was the HD/PulseWave$^{TM}$ CR-2000 Research

CardioVascular Profiling System (Profiler) from Hypertension Diagnostics. This device is indicated for use in research-grade measurement of both blood pressure and arterial elasticity in human subjects. Measurements captured by this device are reproducible, with one study finding only 3% variability in intra-visit measures 5 minutes apart[130]. Research staff were trained in the use of the Profiler and performed all measurements. Blood pressure was also measured using a mercury sphygmomanometer and stethoscope.

1. **Resting, sitting blood pressure**

Blood pressure (via Profiler then sphygmomanometer) was captured after the patient sat for 5 minutes completing study questionnaires. Patients' feet were flat on the ground during this time, with their hands being supported on a table while measurements were being recorded.

**2. Resting, supine blood pressure and arterial assessments**

Blood pressure, large arterial assessment and small arterial assessment by the Profiler were performed as the patient lay supine on a powered treatment table. Blood pressure was then measured by sphygmomanometer. Patients who stated that they found the position uncomfortable were given pillows to position beneath their knees and/or head.

**3. Immediate post-moderate-intensity exercise**

Blood pressure (via Profiler then sphygmomanometer) was captured after the patient engaged in 3 minutes of moderate-intensity exercise at a workload of 5 METs. Patients chose to walk on a treadmill at either 2.5 mph/6 grade or 2.9 mph/4 grade. An equivalent metabolic demand was calculated using ACSM equations for a Monark cycle ergometer in case any patient

could not walk on the treadmill. All patients successfully completed the exercise on the treadmill and the Monark cycle ergometer was not used. After exercise, patients transferred immediately back into a supine position on the same treatment table, approximately 3 feet away from the treadmill.

***Musculoskeletal fitness tests***

A battery of musculoskeletal fitness tests were performed at each time point. Tests were performed in the order listed.

*Grip strength*

Staff demonstrated how to hold the JAMAR dynamometer to the participant by testing it on themselves, and explained how the dynamometer works by squeezing it as tightly as possible. After the participant was sitting on a chair, the staff instructed the participant to bend their arm at a 90 degree angle and to position their thumb around one side and their fingers around the other side of the handle to determine whether the size was adjusted appropriately. Staff ensured the red indicator needle was in the "0" position by turning the dial.

Measurements began with the participant's right hand and then proceed to the left hand before measuring both sides again. Participants were encouraged to squeeze as long and as tightly as possible for the best result until the needle stopped rising, and results were recorded to the nearest 2 kg. These instructions were in accordance with Canadian Society for Exercise Physiology testing guidelines[131].

*30 second chair stand*

Participants were instructed to sit in the middle of the chair, which was placed against a wall. They were then instructed to cross arms across their chest with hands resting on the opposite shoulder and keep feet flat on the ground while in a sitting position. With a verbal signal from staff, participants rose to a full standing position and back to a sitting position as many times as possible within 30 seconds. If the participant was over halfway to a standing position when 30 seconds had elapsed, it was counted as a stand. If the participant used their arms to stand, the test would be stopped and 0 would be the recorded score (this scenario did not occur). These instructions were in accordance with the Centre for Disease Control guidelines[132].

*5 meter walk*

The 5 meter walk test was integrated as a secondary measure of gait speed alongside the 6 minute walk test, as it may be more immediately responsive to improvement in fitness in certain populations. Gait speed itself has been identified as an indicator of a patient's function and performance of activities of daily living[133]. Prior to the testing session, research staff used a measuring wheel to determine and mark a 5 meter distance in a large room. Endpoints were marked using tape, and Layfette Instruments timing mats were positioned at the near edge of this tape. To start the test, participants stood with their lead foot one to two feet behind the first timing mat. With a verbal signal from staff, participants walked at their normal walking speed one to two feet past the second timing mat. Three trials were done. If a participant failed to step on one or both of the timing mats, an additional trial was performed.

*Indirect one repetition maximum leg press*

This measurement was included to capture muscular strength, which may have a protective effect against the typical significant loss of fat-free mass and skeletal muscle mass[134] seen after surgical weight loss. Leg press has been used as a marker of strength in studies focusing on post-roux-en-y gastric bypass patient[35]. The leg press used in this protocol was a Hammer Strength plate-loaded unilateral model.

Research staff had the participant warm up by performing 5 to 10 repetitions at what they perceived to be 40-60% of their one repetition maximum (1RM). If the participant could not estimate their appropriate 1RM weight, the warm up was started at 1/3 of their body weight. The participant rested for 1 minute while staff added or removed an appropriate amount of weight based on the participant's success during the warm up. The participant then had the participant perform another trial of as many repetitions as possible (up to 10 repetitions), followed by a 3 minute rest period. The 1RM trials then began as staff increased the weight progressively by 2.5-20.0 kg and had the participant complete the next attempt - up to 4 sets, with a maximum of 10 repetitions per set. Research staff used a table from the American College of Sports Medicine[135] to predict the participant's 1RM.

*6 minute walk test*

This measurement was the primary outcome of ENCOURAGEing Start. The 6 minute walk test is not only a validated measure of functional capacity, but is one of the most-used markers of function in pre- and post-bariatric surgery studies[136]. This test was performed on a flat 200 meter track. Participants used their walking aids (i.e. cane, walker) during the test, and a

warm-up was not performed. Research staff instructed the participant before the test, stating "The object of this test is to walk as far as possible for 6 minutes. You will be walking around the track. Six minutes is a long time to walk, so you will be exerting yourself. You may get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able."

During the test, participants were instructed to walk in the 5th lane of the track, which had been previously measured and marked in its location relative to fixed structural landmarks (**Appendix B**). Staff walked approximately 2 meters behind the participant during the test and informed participants at the conclusion of every minute, and every 15 seconds during the last minute.

***Data Collection - Questionnaires***

Patients completed different questionnaires at certain time points, as described on the following page. Participants were asked to begin the questionnaires during their data collection appointment while they were sitting for 5 minutes before measurements of blood pressure, and again when the research assistant was programming the accelerometer. Participants then returned to the questionnaires to complete them after conclusion of all other tests in that appointment. Due to the length of the self-compassion series of questionnaires, participants were asked to complete this section of the package at home and return it in the same envelope with their accelerometer. Packages were marked with participant numbers to reconcile the responses.

### *Baseline Comorbidities*

Participants' baseline comorbidities were self-reported via the PAR-Q+ questionnaire. These statements were not confirmed with CMBS records, physical exam, laboratory testing nor radiographic imaging. Several questions in the PAR-Q+ are open ended, therefore patients endorsed a wide range of comorbidities. As such, mental illnesses (e.g. "mental illness", "OCD", "anxiety") were combined into a category of "self-reported mental illness" for the purpose of reporting the data.

### *Accelerometry*

Participants were given an Actical accelerometer at the conclusion of each data collection appointment. This device was used to capture physical activity for a 7 day period. Initialization was programmed to occur at 6 AM the day after the appointment. Patients were instructed to wear the device for one week before returning it to the Active Living Center. Participants received an information sheet illustrating proper placement of the accelerometer, considerations, and instructions to contact the Intervention Coordinator if any problems were encountered. Data capture was 90 Hz, and was analyzed Actical Actigraph software.

**Table 7. Data collection throughout ENCOURAGEing Start**

| | Time span of data collected for ES | | | | |
|---|---|---|---|---|---|
| | Time span of data used in this thesis | | | | |
| | **Baseline** | **8 wks** | **16 wks** | **1 y** | **5 y** |
| Anthropometry | x | x | x | x | x |
| Cardiovascular profiler | x | x | x | x | x |
| Grip strength | x | x | x | x | x |
| 30 second chair stand | x | x | x | x | x |
| 5 meter walk | x | x | x | x | x |
| Indirect 1RM leg press | x | x | x | x | x |
| 6 minute walk | x | x | x | x | x |
| Accelerometer | x | x | x | x | x |
| Blood draw | x | | x | x | x |
| **Questionnaires completed as part of data collection appointment** | | | | | |
| Laval Health Related Quality of Life | x | x | x | x | x |
| Patient Satisfaction Questionnaire (PSQ-18) | x | x | x | x | x |
| Patient Health Questionnaire (PHQ-9) | x | x | x | x | x |
| Paffenbarger Physical Activity Scale | x | x | x | x | x |
| Exhaustion (based on CES-D) | x | x | x | x | x |
| Nutrition | x | x | x | x | x |
| Hospital Anxiety and Depression Scale (HADS) | x | x | x | x | x |

| **Self-Compassion and Physical Activity Questionnaire (option of taking home package to complete)** | | | | | |
|---|---|---|---|---|---|
| 1- Self-Compassion Scale | x | - | x | x | x |
| 2- (MHQ)-Health Anxiety Subscale | x | - | x | x | x |
| 3- Self-Esteem Scale | x | - | x | x | x |
| 4- Affective Responses to a Real Health Problem | x | - | - | - | - |
| 5- Response to Illness Questionnaire (completed if #4 is completed) | x | - | - | - | - |
| 6- Physical Activity Intentions (Part A) | x | - | - | - | - |
| Physical Activity Intentions (Part B) | - | - | x | - | - |
| Physical Activity Intentions (Part C) | - | - | - | x | x |
| 7- Social Physique Anxiety Scale | x | - | x | x | x |
| 8- Body Appreciation Scale-2 | x | - | x | x | x |
| 9- Exercise Task Self-Efficacy-Over the Duration of the Intervention (next 16 weeks) | x | - | - | - | - |
| 10- Exercise Task Self-Efficacy- Now until Surgery | - | - | x | - | - |
| 11- Exercise Task Self-Efficacy-Over the Next Year | - | - | - | x | x |
| 12- Sedentary Behavior Task Self-Efficacy: Over the course of the Intervention (16 weeks) | x | - | - | - | - |
| 13- Sedentary Behavior Task Self-Efficacy: Now until Surgery | - | - | x | - | - |
| 14- Sedentary Behavior Task Self-Efficacy: Over the Next Year | - | - | x | x | x |
| 15- Exercise Regulations Questionnaire (BREQ-3) | x | | x | x | x |

## 2.8 My specific thesis

Blood samples were collected and stored using the procedure described in section 2.7, allowing for analysis of the cytokines via magnetic bead-based multiplex assay (MILLIPLEX Custom Bio-Plex Assay) using the MAGPIX instrument and MILLIPLEX Analyst Software. During this procedure, the samples were added to a mixture of color-coded beads (one color for every analyte), pre-coated with analyte-specific capture antibodies. The MAGPIX uses a magnet to hold the beads in a layer before one light-emitting diode (LED) to determine the analyte being detected, and the second LED quantifies the bound analyte by measuring signal strength. The assay kit is manufactured by Millipore and contains all reagents, serum matrix, bead diluent, assay buffer, cytokine detection antibodies, and the plate itself. Reagent preparation and measurements were performed according to instructions from the manufacturer. A sample of these instructions for a similar Millipore myokine analysis kit is available in **Appendix D.** This thesis will include analysis of baseline and 16 week time points for the biomarkers leptin, interleukin 1 beta (IL1β), apelin, monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), osteonectin, irisin and osteocalcin.

The primary outcome for my thesis is not the same as the primary outcome for the overall study (i.e. 6 minute walk test), but is instead pre-post changes in the biomarkers in Table 2, which the literature suggests would change with the intervention. A sample size calculation for an example biomarker will be provided in the statistics section.

## 2.9 Statistics

As described earlier, the sample size for this thesis is bound to that of the overall study, for which the targeted recruitment total of 48 patients was calculated with a conservative sample size (two-tailed, 90% power) for the primary outcome variable (6 minute walk test) using a

standard deviation of 50 meters, a minimum detectable difference (MDD) of 30.5 meters, and a dropout rate of 40%. The MDD used in this sample size calculation is in contrast to the previous study measuring the same test in CMBS patients, which used 70 meters[128]. The MDD of 30.5 meters has been identified as a conservative estimate of a clinically important distance in adults with pathology[137]. Using 80% power, the estimated sample size is 30 patients. However, this recruitment target was not achieved - although 44 potential participants completed the consent to contact form, only 20 completed baseline testing.

Although ES used a time-series design with 3 time points (i.e. baseline/pre, 8-weeks and 16-weeks/post) the biomarker data to be analyzed in this thesis as a primary outcome was captured in a pre-post structure. A Kolmogorov-Smirnov test was used to determine the distribution of the samples of patients with baseline and 16-week data, followed by a Student's T-test (parametric). The profile of baseline-only measurements, such as participant characteristics, will be built using group means and standard deviation for each biomarker. Analysis will be done using SPSS software v26.

An inflammatory composite and overall composite were created. The inflammatory composite included IL-6, IL-1β, TNFα and MCP-1 while the cytokine composite included all cytokines. Both were calculated by combining baseline and 16-week data into one column per cytokine ($n = 7$, $N = 14$) and calculating 14 Z scores per cytokine. Baseline and 16-week Z scores were then re-sorted into separate columns. Baseline and 16-week composite scores were computed using the appropriate time point for each cytokine. An example of the inflammatory composite is shown in Equation 1. A T-test was performed on the pre-post composite scores.

**Equation 1**: **Calculation of baseline inflammatory composite**

Baseline Inflammatory Composite =

(zBaselineIL6 + zBaselineIL1β + zBaselineTNFα + zBaselineMCP1) / 4

The cytokine composite was calculated using the same general approach seen in Equation 1, but using all cytokines.

# Chapter 3: Results

## 3.1 Data for participants completing all baseline appointments

As noted in Figure 5, 26 participants completed the baseline data collection appointments. Of those 26, 20 completed the baseline blood draw and 6 withdrew from the study. A summary of characteristics for individuals completing both baseline appointments is on Table 8.

**Table 8: Characteristics and scores of participants completing baseline data collection and baseline blood draw (n = 20)**

| | Cohort (n = 20) | Range |
|---|---|---|
| **Characteristic** | | |
| Age (years) | 47.9 ± 8.9 | 23 - 61 |
| Females (%) | 85.0 | - |
| BMI | 47.3 ± 5.6 | 31.4 - 54.4 |
| Body fat (%) | 48.7 ± 7.2 | 26.9 - 55.2 |
| Resting systolic BP (mmHg) | 145 ± 17 | 104 - 164 |
| Resting diastolic BP (mmHg) | 78 ± 12 | 61 - 107 |
| 4 test RDS score | 2.13 ± 1.26 | 0 - 4 |
| **Self-reported conditions and medications** | | |
| Type 2 Diabetes | 9 (45.0) | - |
| Hypertension | 17 (85.0) | - |

| | | |
|---|---|---|
| Osteoarthritis | 7 (35.0) | - |
| Self-reported mental illness | 7 (35.0) | - |
| Asthma | 4 (20.0) | - |
| Diabetes medication(s) | 10 (50.0) | - |
| Lipid medication(s) | 3 (15.0) | - |
| Blood pressure medication(s) | 12 (60.0) | |
| Thyroid medication(s) | 4 (20.0) | |
| Antidepressant medication(s) | 6 (30.0) | |
| **Assessment data** | | |
| 30 second chair stand (# reps) | 12.6 ± 3.6 | 6 - 18 |
| 5 meter gait (s) | 5.36 ± 1.48 | 4.45 - 11.45 |
| Indirect 1RM leg press (lb) | 277 ± 79 | 137 - 458 |
| 6 minute walk (m) | 445 ± 81 | 277 - 558 |
| PHQ 9 | 8.9 ± 7.1 | 0 - 27 |
| Laval QOL | 183 ± 50 | 86 - 258 |
| HADS Anxiety composite score | 7.3 ± 4.8 | 0 - 15 |
| HADS depression composite score | 5.6 ± 3.8 | 0 - 15 |

*Continuous variables expressed as mean ± standard deviation. Categorical variables expressed as N (%)*

Participant characteristics at baseline in this thesis were similar to the baseline experimental group in Kwok's study which recruited CMBS patients[128]. Comparisons between baseline participant data in this thesis, Kwok's study and other Canadian pre-surgical physical

activity interventions are shown in Table 9. Rasmussen Disease Score (RDS), a four-test screening tool to measure structural and functional changes in arteries in response to exercise[138], appears not to be previously reported in a bariatric population. Thirty five percent of participants scored in the abnormal range (RDS score > 2), which may suggest early-stage cardiovascular disease in asymptomatic patients[138].

**Table 9: Baseline comparisons of Canadian pre-surgical bariatric cohorts**

| | ES (Mean ± SD) | Kwok[128] (Mean ± SD) | Baillot[30] (Median) | Baillot[139] (Median) | Cassin[140] (Mean ± SD) |
|---|---|---|---|---|---|
| **Age** | 49.9 ± 13.7 | 47.5 ± 8.3 | 40.8 | 44.8 | 43.8 ± 10.7 |
| **BMI** | 47.3 ± 5.6 | 46.9 ± 5.4 | 51.4 | 46.6 | 49.6 ± 8.5 |
| **6 minute walk test** | 445 ± 81 | 460 ± 51 | 464 | 495 | - |
| **30 second chair stand (# reps)** | 12.6 ± 3.6 | 13.1 ± 2.5 | 13 | 17 | - |
| **PHQ9** | 8.9 ± 7.1 | - | - | - | 10.9 ± 6.9 |

All studies reported six minute walk tests lower than those of healthy individuals, to such an extent that they are lower than test scores of healthy 70-80 year old subjects (514 ± 71 m)[141]. According to PHQ9 cutoff scores[142], 8 of 20 of ES participants (40%) reported depressive symptoms in the moderate range or higher (PHQ-9 score ≥10). Cassin's study of 244 bariatric surgery candidates reported 52.5% subjects in this range. Ten percent of participants in ENCOURAGEing Start and 10.4% of participants in Cassin's study reported symptoms in the severe range (PHQ-9 score ≥20). Based on the demographic, fitness-related and psychological

factors at baseline, the ENCOURAGEing Start cohort is comparable to other Canadian studies of bariatric surgery candidates.

The dropout rate in ENCOURAGEing Start (61.5%) was greater than that of Kwok's study (40.0% in the control group). The most common reason for dropout in Kwok's study was time commitment, whereas none of the ENCOURAGEing Start dropouts described time commitment as a barrier. Instead, 68.6% received their surgery date, which necessitated withdrawal from the study before the completion of the 16 week physical activity intervention. The remaining dropouts possessed unique reasons for doing so, such as sustaining a significant musculoskeletal injury unrelated to ENCOURAGEing Start (e.g. slipped on ice).

In addition to the measurements recorded at the baseline data collection appointments, the participants profiled in this thesis section also attended their baseline blood draw appointment. The cytokine profile of these 20 participants are in Table 10.

**Table 10: Cytokine profile of participants completing baseline data collection and baseline blood draw (n=20)**

| | Mean (Mean ± SD) | Range |
|---|---|---|
| **Leptin (pg/mL)** | 19195 ± 9635 | 1618 - 38699 |
| **IL-1β (pg/mL)** | 1.84 ± 2.23 | 0.15 - 7.99 |
| **Apelin (pg/mL)** | Unreported | - |
| **MCP-1 (pg/mL)** | 688 ± 198 | 361 - 1155 |
| **IL-6 (pg/mL)** | 31.4 ± 59.6 | 0.46 - 217.3 |
| **TNFα (pg/mL)** | 18.9 ± 7.3 | 5.45 - 30.8 |
| **Osteonectin (ng/mL)** | 207 ± 131 | 70 - 732 |
| **Irisin (pg/mL)** | Unreported | - |
| **Osteocalcin (pg/mL)** | 3529 ± 2663 | 747 - 10454 |
| **Cytokine composite (SD)** | 0.015 | -0.64 - 0.97 |
| **Inflammatory composite (SD)** | 0.019 | -0.90 - 1.29 |

Leptin, IL-1β , MCP-1, TNFα, osteonectin and osteocalcin were within detectable ranges of the assay kit. Apelin is unreported, as its concentrations in the majority of samples (11 of 20) were below detectable limits. IL-6 was included in the analysis. However, it must be disclosed

that 5 of 20 measurements were below the detectable range of 3.2 pg/mL. The analysis was completed by imputing 3.2 pg/mL for those five values and should be interpreted with caution, as this substitution method will cause a left-censoring bias. Among the possible techniques to assign these IL-6 values (substitution with 0 or 0.5 detectable limit, probability plot methods, Kaplan-Meier estimates, etc) there is no objective solution[143]. Irisin was unreported because its concentration in the majority of samples (14 of 20) were below detectable limits.

### 3.2 Data for participants completing all baseline and 16-week appointments

Of the 20 participants who completed the baseline data collection appointment and baseline blood draw, 11 dropped out due to receiving a surgery date. Seven completed both the 16-week data collection appointment and 16-week blood draw appointments (Figure 5). The following part of this results section will now focus entirely on the descriptive data and results of these 7 participants. Their descriptive data and results are reported in Table 11.

**Table 11: Characteristics and pre-post changes of participants completing all baseline and 16-week appointments (n = 7)**

| | Baseline (Mean ± SD) | 16 week (Mean ± SD) | Mean Δ (16 weeks - baseline) ± SD | Sig. (2-tailed) |
|---|---|---|---|---|
| **Characteristic** | | | | |
| BMI | 44.5 ± 6.6 | 44.5 ± 7.0 | -0.04 ± 0.75 | 0.885 |
| Female (%) | 85.7% | - | - | - |
| Body fat (%) | 45.3 ± 8.8 | 44.4 ± 9.1 | -0.89 ± 0.83 | **0.030*** |
| Resting systolic BP (mmHg) | 130 ± 16 | 136 ± 17 | 5.4 ± 15 | 0.361 |
| Resting diastolic BP (mmHg) | 76 ± 12 | 79 ± 7 | 3 ± 12 | 0.475 |
| 4 test RDS score | 3.0 ± 0.7 | 1.8 ± 2.1 | -1.2 ± 1.6 | 0.178 |
| **Self-reported conditions and medications** | | | | |
| Type 2 Diabetes | 1 (14.3) | - | - | - |
| Hypertension | 6 (85.7) | - | - | - |
| Osteoarthritis | 1 (14.3) | - | - | - |
| Self-reported mental illness | 1 (14.3) | - | - | - |
| Asthma | 1 (14.3) | - | - | - |
| Diabetes medication(s) | 4 (57.1) | - | - | - |
| Lipid medication(s) | 3 (42.8) | - | - | - |

| | | | | |
|---|---|---|---|---|
| Blood pressure medication(s) | 4 (57.1) | - | - | - |
| Thyroid medication(s) | 1 (14.2) | - | - | - |
| Antidepressant medications(s) | 2 (28.4) | - | - | - |
| **Assessment data** | | | | |
| 30 second chair stand (# reps) | 12.6 ± 3.2 | 16.3 ± 3.0 | 3.7 ± 1.9 | **0.002*** |
| 5 meter gait (m) - | 5.03 ± 0.37 | 4.72 ± 3.1 | -0.3 ± 0.3 | 0.077 |
| Indirect 1RM leg press (lb) | 308 ± 82 | 405 ± 74 | 98 ± 72 | **0.012*** |
| 6 minute walk (m) | 501 ± 37 | 555 ± 54 | 54 ± 34 | **0.006**** |
| PHQ9 | 12.4 ± 7.7 | 8.3 ± 5.9 | -4.4 ± 4.2 | **0.039*** |
| Laval QOL | 189 ± 46 | 225 ± 38 | 35 ± 37 | **0.045*** |
| HADS Anxiety | 7.6 ± 5.3 | 5.4 ± 5.4 | -2.1 ± 3.6 | .169 |
| HADS Depression | 6.1 ± 2.8 | 3.9 ± 3.2 | -2.3 ± 3.8 | .160 |

*Continuous variables expressed as mean ± standard deviation. Categorical variables expressed as N (%)*

The unchanged weight from pre-post is to be expected, as increased physical activity of bariatric patients in the absence of dietary change is estimated to cause loss of only 0.1-5.2 kg[144]. However, participants did decrease body fat percentage. This magnitude of body fat percentage decrease has been reported in other physical activity intervention studies[145,85] which found pre-post cytokine relationships similar to those in Table 2**.** As participants maintained weight with this decrease in body fat percentage, it can be deduced that lean body mass was not negatively

affected. This retention or increase of lean body mass is associated with improved post-surgical outcomes[146].

Scores in the 30 second chair stand increased by 3.7 ± 1.9. This test is used primarily to measure a patient's own progress, and there are no norms for age groups less than 60 years of age. If we utilize norms for older populations, the ENCOURAGEing Start participants did move from the "below average" category (<14) to the "average" category (14-19)[132].

Indirect 1RM leg press scores increased by 98 ± 72 pounds. Like the 30 second chair stand, there are no established general population norms. However, the percentage of improvement seen in the mean values (32%) is greater than several studies reporting the effect of resistance training in both pre- and post-operative bariatric patients[147,148,149]. This percentage of improvement may be greater than that seen in recreationally trained adults[150].

The PHQ9 mean scores decreased from a moderate (10-14) to mild (5-9) range, indicating a reduction in depressive symptoms.

Compared to Kwok's pre-operative physical activity intervention offered to CMBS patients, the ENCOURAGEing Start intervention showed a stronger effect size in their shared primary outcome of the 6 minute walk test (10.7% vs. 5.9% improvement over baseline)[128]. ENCOURAGEing Start's post-value for 6 minute walk score (555 ± 54 m) is similar to one year post-operative standard-care subjects from another Canadian study (550 ± 55 m) [146]. It is unknown whether a minimal clinically important difference score exists for the 6 minute walk test in a pre-surgical bariatric population, but it has been suggested that a difference of at least 30.5-80 meters is required to be certain of a statistically significant improvement in an obese

population[137,151] (although the study suggesting the upper range reported younger subjects with a lower BMI than in my thesis).

Changes in weekly physical activity were measured by accelerometer and Paffenbarger Questionnaire at all time points. Data from the Paffenbarger is reported in Table 12. Analysis of accelerometer data has not yet been completed but will be reported in the future within a separate manuscript.

**Table 12: Pre-post Paffenbarger questionnaire (n=7)**

| | **Baseline (Mean ± SD)** | **16 week (Mean ± SD)** | **Mean Δ (16 weeks - baseline) ± SD** | **Sig. (2-tailed)** |
|---|---|---|---|---|
| **Total number of blocks walked in the last 7 days (1 mile = 12 blocks = 1.6 kilometers)** | 42 ± 46 | 43 ± 20 | 0 ± 38 | .985 |
| **Total flights of stairs climbed UP in the past 7 days** | 108 ± 176 | 152 ± 191 | 44 ± 77 | .181 |
| **Total minutes of LIGHT sports/recreation in the past 7 days** | 198 ± 469 | 821 ± 1852 | 623 ± 1987 | .439 |
| **Total minutes of MODERATE sports/recreation in the past 7 days** | 61 ± 92 | 397 ± 754 | 335 ± 779 | .298 |
| **Total minutes of HEAVY sports/recreation in the past 7 days** | 2 ± 6 | 49 ± 89 | 46 ± 91 | .225 |
| **Total calories expended from activity (calculated from answers above)** | 2810 ± 2938 | 9422 ± 14491 | 6611 ± 15993 | .316 |

These data confirm that the ENCOURAGEing Start participants received a sufficient dose of physical activity to enact detectable change in fitness outcomes, which supports the usage of the "effects from exercise" column reported in Table 2 as an indicator of expected biomarker values. It is on this basis that the cytokine profiles of these 7 participants were analyzed. Their baseline and post data can be seen in Table 13.

**Table 13: Pre-post cytokine data (n=7)**

| | Baseline (Mean ± SD) | 16 weeks (Mean ± SD) | Mean Δ (16 weeks - baseline) ± SD | Sig (2-tailed) |
|---|---|---|---|---|
| **Leptin (pg/mL)** | 15526 ± 10011 | 18112 ± 10439 | 2586 ± 4517 | 0.181 |
| **IL-1β (pg/mL)** | 1.56 ± 2.84 | 1.43 ± 2.71 | -0.13 ± 0.25 | 0.233 |
| **Apelin (pg/mL)** | Unreported | Unreported | - | - |
| **MCP-1 (pg/mL)** | 714 ± 230 | 760 ± 339 | 46 ± 241 | 0.627 |
| **IL-6 (pg/mL)** | 33.5 ± 81.1 | 31.9 ± 74.3 | -1.64 ± 7.17 | 0.567 |
| **TNFα (pg/mL)** | 18.6 ± 4.3 | 17.1 ± 4.1 | -1.48 ± 2.61 | 0.185 |
| **Osteonectin (ng/mL)** | 180 ± 58 | 175 ± 46 | -5.63 ± 18.4 | 0.45 |
| **Irisin (pg/mL)** | Unreported | Unreported | - | - |
| **Osteocalcin (pg/mL)** | 4018 ± 2973 | 3536 ± 2681 | -483 ± 576 | 0.068 |
| **Cytokine composite (SD)** | 0.021 | -0.021 | -0.042 | 0.672 |
| **Inflammatory composite (SD)** | 0.033 | -0.033 | -0.066 | 0.669 |

Leptin, IL-1β , MCP-1, TNFα, osteonectin and osteocalcin were within detectable ranges of the assay kit . Apelin was unreported as its concentrations in the majority of samples (4 of 7) were below detectable limits at both time points. IL-6 was included in the analysis, though 2 measurements at both the baseline and 16-week time points were below the detectable range of 3.2 pg/mL. The analysis was completed imputing 3.2 pg/mL for those four values, with the rationale previously described[143]. Irisin was not reported as its concentrations in the majority of samples (5 of 7) were below detectable limits at both time points. No significance was found in the pre-post cytokine data when performing a paired samples T-test. A $\log_{10}$ transformation of the cytokine data (not shown) did not alter the outcome of the analysis.

Baseline and post data for each participant (n = 7 ; N = 14) was utilized to correlate and graph cytokine profiles by 3 outcome measures (6 minute walk test, leg press, and BMI). This data is reported in Table 14 and graphed in Appendix E. The majority of correlations (4 of 7) between cytokines and BMI reached significance, followed by 2 of 7 between cytokines and leg press, and none between cytokines and the 6 minute walk test.

**Table 14: Bivariate correlations using baseline and 16-week data (n=7 ; N=14)**

| | 6 minute walk | Leg press | BMI |
|---|---|---|---|
| Leptin | r = 0.057<br>p = 0.847 | r = 0.454<br>p = 0.103 | r = 0.603<br>**p = 0.022*** |
| IL-1β | r = 0.234<br>p = 0.420 | r = -0.467<br>p = 0.092 | r = -0.882<br>**p < 0.001**** |
| MCP-1 | r = -0.138<br>p = 0.637 | r = 0.673<br>**p = 0.008*** | r = 0.449<br>p = 0.107 |
| IL-6 | r = 0.228<br>p = 0.433 | r = -0.476<br>p = 0.085 | r = -0.878<br>**p < 0.001**** |
| TNFα | r = -0.273<br>p = 0.344 | r = 0.048<br>p = 0.871 | r = 0.247<br>p = 0.394 |
| Osteonectin | r = -0.381<br>p = 0.178 | r = 0.545<br>**p = 0.044*** | r = 0.944<br>**p < 0.001**** |
| Osteocalcin | r = 0.349<br>p = 0.222 | r = -0.284<br>p = 0.326 | r = -0.190<br>p = 0.516 |

**correlation is significant at the 0.05 level (two-tailed)*
**** correlation is significant at the <0.01 level (two-tailed)*

Finally, the change (Δ) observed in each individual's participant is reported in Table 15. The Δ is reported as (post - baseline), meaning that a positive value indicates an increased concentration of that cytokine at the 16-week time point. No participant's overall cytokine profile encompassed a majority of the mean values reported in Table 13, which is to say that each participant displayed a unique cytokine response to the intervention. Interparticipant Δ values for 4 of the 7 analytes were at least tenfold different, and 6 of the 7 analytes were not unanimous in the direction of their change. Also included in the table is the change in composite scores derived from the baseline and 16-week Z scores of inflammatory cytokines (IL-6, IL-1β, TNFα, MCP-1).

**Table 15: Δ (post - baseline) cytokine profiles for each of the 7 participants (n=7)**

| | Participant identifier number | | | | | | |
|---|---|---|---|---|---|---|---|
| | **#03** | **#16** | **#18** | **#23** | **#25** | **#26** | **#27** |
| Leptin* | 8143 | 7816 | -43 | 270 | 5792 | -1365 | -2514 |
| IL-1β* | -0.29 | 0.04 | -0.44 | 0.32 | -0.16 | -0.07 | -0.29 |
| MCP-1* | -406 | 260 | -47 | 140 | 324 | 63 | -8 |
| IL-6* | -2.8 | 0 | -17.3 | 4.6 | 1.1 | 2.6 | 0 |
| TNFα* | -4.8 | 0.3 | -3.3 | -1.3 | 3.2 | -1.9 | -2.6 |
| Osteonectin* | -0.20 | -0.18 | 0.70 | 0.16 | 0.23 | -0.26 | -0.17 |
| Osteocalcin* | -773 | -658 | -366 | -1031 | -300 | 673 | -926 |
| Cytokine composite (SD) | -0.34 | -0.20 | -0.18 | 0.04 | 0.35 | -0.13 | -0.23 |
| Inflammation composite (SD) | -0.61 | 0.35 | -0.12 | -0.15 | 0.32 | 0.03 | -0.12 |

**Values in pg/mL*

# Chapter 4: Discussion

## 4.1 Outcomes from the ENCOURAGEing Start study

The purpose of this thesis was to describe baseline cytokine profiles of CMBS patients, and to determine the effect of a 16-week pre-surgical physical activity intervention on the cytokine profiles of CMBS patients. Before discussing cytokine data, it is appropriate to discuss outcomes of the ENCOURAGEing Start intervention. The pre-post data demonstrates statistically significant positive health effects in measures related to fitness, health, and surgical outcomes. These results include body fat percentage, 6 minute walk test, 30 second chair stand, 1RM leg press, PHQ9, and the Laval QOL questionnaire. The post-intervention 6 minute walk test score (555 ± 54 m) is similar to 6-month post-operative scores of individuals who underwent surgery but did not receive a physical activity intervention, such as Hansen et al (504 m, no SD reported)[152] and Gallart-Aragon et al (372 ± 169 m)[153]. The ENCOURAGEing Start participants were able to achieve similar test scores to Gallart-Aragon's study despite having a significantly higher BMI (36.5 ± 4.5[153] versus 44.5 ± 6.6). ENCOURAGEing Start's improvement in 6 minute walk test score is also comparable to pre-surgical physical activity interventions with similar populations such as Simmons et al (560 ± 132 m)[154] and Baillot (492 m, no SD reported). As noted in the results section, it is unknown whether a minimum clinically significant difference for the 6 minute walk test exists for this population.

The mean 31.4% improvement seen in the indirect 1RM leg press is similar to the outcome of Daniels et al[155], whose intervention was a 3x/week hour-long resistance training session for bariatric patients and reported a 36.2% improvement in direct 1RM leg press. This is notable as ENCOURAGEing Start had only one session focused on resistance training machines, after which participants were free to use the equipment as desired, and reported a functionally

similar outcome to Daniel et al's far more time-intensive intervention. This may suggest that future iterations of ENCOURAGEing Start should continue to deliver education sessions to support skill acquisition so patients can become more physically active. The improvement reported in the 30 second chair stand was statistically significant unlike Kwok's study of CMBS patients[128]. The improvement ($3.7 \pm 1.9$) is similar to that seen in Baillot's more intensive 12 week, 3x/week intervention for pre-surgical patients (2, no SD given)[156].

Analysis of the Paffenbarger Physical Questionnaire revealed no statistically significant changes in physical activity levels. However, study participants tend to overestimate their own moderate-intensity physical activity by more than 100%[157], and obese subjects may overestimate by even more[158]. The extreme range of responses within groups (e.g: weekly kilometers walked at baseline ranged from 0 to 65.3) and between groups (e.g: one participant increasing from 0 to 5000 weekly minutes of weekly light-intensity activity) reinforces the value of the accelerometer data recorded during this intervention, which will be analyzed and published at a later date.

Pre-post measurements revealed the positive changes described above, though it is unclear whether the entire effect size is attributable to the intervention. This was a quasi-experimental study and therefore had no control group. It is likely that, in addition to the intervention's effects, participants continued to improve their physical activity behavior (and overall health behavior) in order to meet CMBS goals and receive a surgical consult. However, the ENCOURAGEing Start participants were drawn from the same pool of CMBS patients as Kwok's study[128], were demographically similar, and had the same exclusion criteria. Patients of the CMBS program during the time of both studies had identical eligibility criteria to enter the CMBS program.

Further discussion will focus on individual cytokines (baseline levels and correlations) before addressing methodological choices and other factors that may have contributed to these outcomes, and finally providing recommendations for future research. In this discussion, it is worthwhile to consider the pleiotropic nature of and the variability and range of cytokines, which are far greater than those of conventional markers such as HbA1c.

### 4.2a Leptin

The wide variance seen in leptin levels of human adults combined with the small sample size make it challenging to assess whether participants' leptin levels might be considered normal. Normative data is conflicting, with some studies of normal-weight and healthy subjects reporting mean serum leptin levels higher than those seen in ENCOURAGEing Start participants (as expected)[159]. However, several other studies reported serum leptin levels in normal-weight and healthy subjects lower than seen in ENCOURAGEing Start[160,161], with one study of n=100 having no crossover with the range in the ENCOURAGEing Start study (1618-38699 pg/mL)[162]. This wide range of leptin, and cytokines as a whole, is prevalent in the literature. For example, Rostas et al analyzed 19 articles studying the effect of exercise training on peripheral leptin in overweight individuals 45 years of age and older and found a range of 6300 - 38000 pg/mL[163] at baseline, which has an upper range quite close to ENCOURAGEing Start. Comparisons of serum leptin levels between ENCOURAGEing Start and other studies is documented in Table 16.

**Table 16: Interstudy serum leptin comparisons**

| | ENCOURAGEing Start | Segal et al[164] | Silha et al[160] | Isidori et al[165] | Terra et al[166] * |
|---|---|---|---|---|---|
| **Population** | Obese M/F, 49.9 ± 13.7 y | Obese M, 33 ± 3 y | Obese M/F, 45.2 ± 1.0 y | Obese M, 42 y (no SD given) | F, 47.2 ± 8.9 y |
| **Study purpose** | Physical activity and cytokines | Relationships to insulin sensitivity | Relationships with insulin resistance | Androgen levels | Leptin and ghrelin after RxY |
| **BMI** | 47.3 ± 5.6 | 32.5 ± 2.4 | 33.0 ± 1.3 | 32.7 ± 0.65 | 46.5 ± 5.0 |
| **Leptin (pg/mL)** | 19195 ± 9635 | 9270 ±1400 | 26900 ± 3900 | 11400 ± 2940 | 44010 ± 32560 |

**Subjects awaiting Roux-en-Y gastric bypass surgery*

As reported above, serum leptin levels of ENCOURAGEing Start participants are similar to other overweight and obese subjects recruited for select interventions, but with an increased standard deviation. No correlation was found between leptin and 6 minute walk test or 1RM leg press. However, there was a significant correlation between leptin and BMI ($p = 0.20$), which was predicted in Table 2.

Based on the results of a paired samples t-test there was no significant change (positive or negative) in the cytokine profiles of the 7 participants analyzed. These findings are in opposition to the hypothesis, which surmised that significant pre-post decrease in serum leptin values would be seen as forecasted in Table 2. No significance was found in the paired samples t-test when pre/post

measurements were $\log_{10}$ transformed. While ENCOURAGEing Start recruited diverse participants who experienced positive effects in their physical and mental health, the large dropout rate prior to the 16-week time point reduced the power of the study. Standard deviations in our cohort were larger than other pre-post cytokine studies[95,167] which may be because studies in this area of research tend to have larger sample sizes. For example, one review of 72 physical activity interventions on leptin found a mean sample size of $29.8 \pm 31.7$[95], and studies examining other analytes reported in this thesis (whether together or in isolation) have a similarly higher sample size[168,169].

In the specific case of leptin, a change would not have necessarily been expected. Although leptin is associated with adiposity, the participants' BMI did not change. Exercise training programs in the absence of weight loss have shown little effect on leptin[92], especially when the sessions are less than 60 minutes in length[170]. If this was a longer and post-operative physical activity program where the exercise intensity could have been elevated, a change may be possible.

As reported in Table 14 and graphed in Appendix E, bivariate correlations were performed between all cytokines with the 6 minute walk test, 1RM leg press, and BMI. Participant data was limited to those who completed both 16-week appointments (n = 7), and correlations were run at baseline and 16-week time points (N = 14). These outcomes were chosen specifically due to their relevance to Table 2, which described anticipated cytokine responses from stimuli of aerobic exercise, resistance training, and weight status. The 6 minute walk test, in addition to being a commonly used measurement of function pre- and post-bariatric surgery[171], is positively correlated with $V0_2max$ (a measure of aerobic fitness) in obese populations[172]. The

indirect 1RM leg press measures muscular strength, and BMI is an indicator of weight (because participant height did not change between baseline and 16-week time points).

Among the 21 correlations performed, 6 reached significance. Leptin was positively correlated with BMI ($p = .022$). This relationship is unsurprising, as it is well established in the literature that leptin is positively correlated with both indices of adiposity and BMI[173]. With this in mind, the statistically significant pre-post decrease in body fat percentage evidently did not disrupt the expected relationship between leptin and BMI. This may imply that the decrease in body fat percentage was not clinically significant, as adiposity is more tightly correlated to leptin than BMI is[159]. No correlation was seen between leptin and 1RM leg press or 6 minute walk test, nor was one expected. If a relationship had been found between leptin and 6 minute walk, the underlying mechanism would have likely been due to BMI and functional ability rather than aerobic fitness, as BMI is correlated to 6 minute walk test scores[174].

### 4.2b IL-1β

A comparison of ENCOURAGEing Start baseline IL-1β values compared to two other studies with a similar demographic is reported in Table 17.

**Table 17: Interstudy serum IL-1β concentrations**

| | **ENCOURAGEing Start** | **Stewart et al[175] (OPI group)** | **Damirchi et al[176]** (OB group) |
|---|---|---|---|
| **Population** | Obese M/F, 49.9 ± 13.7 y | Overweight M/F, 71 ± 4 y | Obese M/F, 43.2 ± 4.6y |
| **Study purpose** | Exercise and cytokines | Exercise and cytokines | Exercise and cytokines/insulin |
| **BMI** | 47.3 ± 5.6 | 27.9 ± 1.0 | 31.4 ± 1.6 |
| **IL-1β (pg/mL)** | 1.84 ± 2.23 | 4.9 ± 1.2 | 0.08 ± 0.01 |

We would expect to see relatively high levels of IL-1β in ENCOURAGEing Start participants, as it is an inflammatory cytokine and closely related in function (not structure) to TNFα[169] . However, there is a paucity of data from which to draw norms, even moreso than other cytokines examined in this thesis. IL-1β is rarely the primary outcome of a study in humans, and many studies using it as part of a panel do not report detectable levels[177,178], even in pre-bariatric surgery patients[179]. An observational study of n=1292 adults explored relationships between various chronic diseases and serum levels of IL-1β[169]. The group in that study with an IL-1β range closest to that of ENCOURAGEing Start was patients with heart failure, though no ENCOURAGEing Start participants had congestive heart failure. There is clearly more work to be done establishing IL-1β norms for different populations if it is to be used as a marker for chronic disease outcomes.

It is known that serum IL-1β is increased by chronic diseases associated with obesity such as type 2 diabetes [169], but this may have been offset by the recent lifestyle habits of

participants, who were mandated to be more physically active and maintain a healthy diet as a condition of their enrollment with CMBS.

Although a decrease was hypothesized, based on the results of a paired samples t-test there was no significant change (positive or negative) to IL-1β in the 7 participants analyzed. No significance was found in the paired samples t-test when pre/post measurements were $\log_{10}$ transformed.

A significant negative correlation ($p < .001$) was seen between IL-1β and BMI. This is the opposite of what was hypothesized, as IL-1β has been shown to correlate with both weight and adiposity[180]. It could not be explained by the participants' elevated absolute lean body mass, as IL-1β is not known to correlate with lean body mass[180]. IL-1β has been shown to decrease by approximately 0.5 pg/mL in a resistance training intervention[181], but it does not seem likely that there was a relationship between BMI and adherence to frequent resistance training (nor is it something that would be captured by accelerometer).

### 4.2c MCP-1

A comparison of ENCOURAGEing Start baseline MCP-1 values compared to 3 other studies with a similar demographic is reported in Table 18.

**Table 18: Interstudy serum MCP-1 concentrations**

| | ENCOURAGEing Start | Sams et al[182] * | Kim et al[183] | Christiansen et al[184] |
|---|---|---|---|---|
| **Population** | Obese M/F, 49.9 ± 13.7 y | Obese M/F, 37 ± 12 y | Obese M/F, 36.7 ± 5.6 y | Obese M/F, 34.5 ± 8.7 y |
| **Study purpose** | Exercise and cytokines | Pre-post RxY inflammatory markers | Observational: chemokines and obesity | Relation between tissue and circulating levels |
| **BMI** | 47.3 ± 5.6 | 47.24 ± 6.58 | 30.6 ± 2.00 | 51.1 ± 6.4 |
| **MCP-1 (pg/mL)** | 688 ± 198 | 500 (no SD) | 183 ± 112 | 189 ± 15 |

Similar to the already-discussed IL-1β, MCP-1 is a proinflammatory chemokine which is elevated in type 2 diabetic patients[185] and is associated with elevated BMI[184]. MCP-1 is secreted in response to other proinflammatory signals, and would therefore be elevated in ENCOURAGEing Start participants due to the chronic low-grade inflammation of obesity, a trait shared to varying

degrees of severity by all participants. Unlike IL-1β, MCP-1 has been reported in many human-subject obesity-related studies. MCP-1 levels in ENCOURAGEing Start participants are significantly higher than the other studies reported in Table 18. On one hand, this corroborates the high TNFα and IL-6 levels found in ENCOURAGEing Start participants (all 3 molecules are positively related[183]). However, it does not explain why ENCOURAGEing Start participants' MCP-1 was so much higher than values reported by other studies with morbidly obese and less physically active participants. The elevated MCP-1 does not appear to be a function of chance with a small sample size, as Kim et al[183] and Christiansen[184] et al used sample sizes of 50 and 23, respectively. It is possible that the timing of the blood draws raised these levels, as discussed in section 4.3d.

Although a decrease was hypothesized, based on the results of a paired samples t-test there was no significant change (positive or negative) to MCP-1 in the 7 participants analyzed. No significance was found in the paired samples t-test when pre/post measurements were $\log_{10}$ transformed.

MCP-1 was positively correlated to 1RM leg press ($p = .008$). MCP-1 is known to increase as a result of resistance training, but also known to decrease as a result of aerobic training. The net increase of MCP-1 as a result of an intervention offering both aerobic and resistance training components has been illustrated previously by Lucotti et al[109]. If a future implementation of ENCOURAGEing Start can be sufficiently powered to compare pre-post cytokine values, a novel question would be to assess the minimum effective dose of resistance training needed to achieve functional benefit while reducing inflammatory markers, which may help to increase adherence to an exercise program. Many resistance training interventions in the

literature consist of 3-5 sessions per week, whereas time is a barrier to exercise for the majority of people regardless of their weight[186].

No significant relationship was found between MCP-1 and BMI, though expected relationships were found between other inflammatory markers and BMI. A positive correlation was expected between MCP-1 and BMI[110], and it is unclear why this did not appear.

### 4.2d IL-6

A comparison of ENCOURAGEing Start baseline MCP-1 values compared to 4 other studies with a similar demographic is reported in Table 19.

**Table 19: Interstudy serum IL-6 comparisons**

| | ENCOURAGEing Start | Khanna et al[167] | Stewart et al[175] (OPI group) | Illan-Gomez et al[187] | Dekker et al[188] |
|---|---|---|---|---|---|
| **Population** | Obese M/F, 49.9 ± 13.7 y | Obese sedentary F, 46.7 ± 11.1 y | Overweight M/F, 71 ± 4 y | Obese M/F, 39.9 ± 10.4 y | Obese M, 47.5 ± 3.1 y |
| **Study purpose** | Exercise and cytokines | Diet-induced weight loss on inflammatory markers | Exercise and cytokines | Pre-post bariatric surgery | Exercise without weight loss |
| **BMI** | 47.3 ± 5.6 | 34.9± 6.4 | 27.9 ± 1.0 | 47.7 ± 7.1 | 32.4 ± 0.6 |
| **IL-6 (pg/mL)** | 31.4 ± 59.6 | 7.2 ± 1.5 | 4.1 ± 0.3 | 3.8 ± 1.7 | 5.2 ± 1.5 |

Values of IL-6 reported in ENCOURAGEing Start participants are disparate from values reported in other studies. This is not unexpected, as among all cytokines measured in this thesis IL-6 had the widest range. The highest value of IL-6 measured at baseline in ENCOURAGEing Start was 471 times greater than the lowest. Recall also that the reported IL-6 values display a left-censoring bias, as 3.2 pg/mL (the minimum detectable limit) was imputed for two values. Using this technique, rather than excluding those 2 samples from the analysis, lowered the mean.

IL-6 is unique among the analytes discussed thus far due to its properties as both an inflammatory cytokine and anti-inflammatory myokine[189] IL-6 is correlated with adiposity, yet also with the amount of muscle mass involved in exercise[190]. With this in mind, it is unlikely that exercise is the reason for the relatively high mean IL-6 seen in ENCOURAGEing Start participants.

Although circulating IL-6 can be increased 100-fold with exercise, participants were advised to avoid physical activity 8 hours prior to bloodwork, and the act of walking from the parking lot to the blood lab should not have been the intense exercise needed[62] to see those increases. Although IL-6 is commonly used as part of an inflammation panel for participants who are obese[187], its interpretation in the context of a physical activity intervention may have been clearer had the bloodwork been done immediately post-exercise, when we could use anticipated changes in TNFα and free fatty acids[62] as confirmatory variables. Alternately, it may warrant a discussion of which analytes are meaningful to monitor the health status of obese individuals versus which analytes are meaningful to monitor the effects of a physical activity intervention. Currently, it is difficult to interpret whether elevated IL-6 is a result of obesity-related inflammation or exercise. The distinction is important with obesity-related research, as IL-6 is a sensitive marker of the chronic inflammatory process and insulin resistance processes[187].

Had the comparisons in Table 19 been between ENCOURAGEing Start and healthy-weight study participants, the increased mean IL-6 may have been attributable to the proportionately larger volume of lean muscle mass used for ambulation and activities of daily living. However, this was not the case.

Although a decrease was hypothesized, based on the results of a paired samples t-test there was no significant change (positive or negative) to IL-6 in the 7 participants analyzed. No significance was found in the paired samples t-test when pre/post measurements were $\log_{10}$ transformed.

No correlation was seen between IL-6 and 6 minute walk test or leg press. This is somewhat surprising for the 6 minute walk test, as individuals who score higher on the 6 minute walk test would tend to be those who are more fit and active, and consequently circulate a larger quantity of anti-inflammatory cytokines[191]. No relationship was expected to 1RM leg press and none was found. Although the contraction of muscles, particularly at the intensity required for strength testing[62], would increase circulating levels of IL-6 acutely, blood draw appointments were separate from data collection appointments. The significant correlation ($p < .001$) between IL-6 and BMI was expected to be positive but was instead found to be negative. This may be attributable to the outliers clearly visible in Figures 8 and 10. It may also suggest that the anti-inflammatory effect elicited by the pleiotropic IL-6 when acting as an anti-inflammatory myokine confounds and outweighs its effect when acting as an inflammatory cytokine. Although it is known that a resistance training intervention can reduce IL-6 concentration without weight loss[188], it is unclear whether the net inflammatory profile of increased IL-6 from higher BMI may be offset by the anti-inflammatory effect of resistance training. This may be a novel query.

### 4.2e TNFα

A comparison of ENCOURAGEing Start baseline TNFα values compared to 4 other studies with a similar demographic is reported in Table 20.

**Table 20: Interstudy serum TNFα concentrations**

| | ENCOURAGEing Start | Khanna et al[167] | Stewart et al[175] (OPI group) | Sams et al[182] * | Whitson et al[192] * |
|---|---|---|---|---|---|
| **Population** | Obese M/F, 49.9 ± 13.7 y | Obese sedentary F, 46.7 ± 11.1 y | Overweight M/F, 71 ± 4 y | Obese M/F, 37 ± 12 y | Obese M/F, 42 ± 11 y |
| **Study purpose** | Exercise and cytokines | Diet-induced weight loss on inflammatory markers | Exercise and cytokines | Pre-post RxY inflammatory markers | RxY response in diabetics/non-diabetics |
| **BMI** | 47.3 ± 5.6 | 34.9± 6.4 | 27.9 ± 1.0 | 47.24 ± 6.58 | 50.0 ± 6.0 |
| **TNFα (pg/mL)** | 18.9 ± 7.3 | 7.2 ± 1.5 | 6.2 ± 0.4 | 8.5 (no SD) | 7.0 ± 0.88 |

TNFα is significantly higher in ENCOURAGEing Start than the values reported in the other 4 studies, one of which included a demographically similar cohort of pre-bariatric surgery patients. Considering the inflammatory markers of IL-1β, MCP-1 and TNFα, it is reasonable to state that ENCOURAGEing Start participants tend to express a higher degree of inflammation. Expected relationships between these markers are present. For example, TNFα is one of several stimuli for IL-6 production[83], and both of these analytes are higher than demographically similar peers in other studies.

Based on the results of a paired samples t-test there was no significant change (positive or negative) to TNFα in the 7 participants analyzed. No significance was found in the paired samples t-test when pre/post measurements were $\log_{10}$ transformed. A significant decrease was expected.

No significant correlations were seen between TNFα and 6 minute walk test, 1RM leg press, or BMI. In fact, these investigations yielded the lowest p values of any cytokine. Although TNFα might be expected to yield the same significant correlation to BMI as did IL-6 (as they are both correlated with BMI[193]), IL-6 has anti- and pro-inflammatory properties whereas TNFα does not. TNFα is secreted mainly by macrophages and lymphocytes, whereas IL-6 is produced by a wide range of cells including T cells, B cells, and myocytes[62]. However, given that TNFα levels in ENCOURAGEing Start participants are higher than demographically similar peers, the lack of correlation may be due to the sample size rather than any beneficial anti-inflammatory processes confounding the relationship.

### 4.2f Osteonectin

A comparison of ENCOURAGEing Start baseline Osteonectin values compared to 3 other studies with a similar demographic is reported in Table 21.

**Table 21: Interstudy serum osteonectin concentrations**

| | ENCOURAGEing Start | Lee et al[194] * | Lee et al[195] * | Kotani et al[196] |
|---|---|---|---|---|
| **Population** | Obese M/F, 49.9 ± 13.7 y | Obese M/F, most with T2DM, 40.8 ± 1.9 y | Obese Korean F, 32.5 ± 8.5 y | Obese Japanese M, 46.9 ± 6.9 y |
| **Study purpose** | Exercise and cytokines | Remodeling of adipose tissue after RxY | SPARC mRNA/ plasma association | SPARC and HbA1c during weight loss |
| **BMI** | 47.3 ± 5.6 | 34.7 ± 1.4 | 40.2 ± 5.7 | 26.1 ± 1.3 |
| **Osteonectin (ng/mL)** | 207 ± 131 | 165 ± 18 | 267 ± 40 | 33.0 ± 12.2 |

Compared to the cytokines already discussed, osteonectin is a relatively novel marker. It is pleiotropic, with relationships to $HbA_{1c}$[196], carcinogenesis[116], and collagen production[197]. It was included in this panel due to its strong relationship with adiposity and

leptin, both of which increase osteonectin secretion[89], and its contribution to adipose tissue fibrosis, which increases insulin resistance and systemic inflammation[198]. Based on Table 20, its relationship to BMI is evident.

The direction and magnitude of change was uncertain for this novel marker - it would be expected to decrease with participants' adiposity, but increase due to exercise response. However, based on the results of a paired samples t-test there was no significant change (positive or negative) to osteonectin in the 7 participants analyzed. No significance was found in the paired samples t-test when pre/post measurements were $\log_{10}$ transformed.

Osteonectin correlated positively with the 1RM leg press ($p = .044$). Strength training increases bone mineral density and bone remodeling[199], and osteonectin is a regulator of bone remodeling[200]. Though this relationship was not unexpected, its inclusion in this study of a pre-surgical cohort may be novel - there appears to be a paucity of data describing pre-post values in obese but physically active individuals. There appears to be a larger body of literature describing osteonectin's metabolic implications without an intervention, or post-surgery only. Similarly, osteonectin is known to correlate positively with BMI and that relationship was supported here. This may be especially true in individuals who are obese and active, as participation in physical activity would provide a greater stimulus to increase bone density than in lighter individuals.

### 4.2g Osteocalcin

A comparison of ENCOURAGEing Start baseline Osteonectin values compared to 3 other studies with a similar demographic is reported in Table 22.

**Table 22: Interstudy serum osteocalcin concentrations**

| | ENCOURAGEing Start | Pereira et al[201] * | Ivaska et al[202] * | Colleluori et al[203] |
|---|---|---|---|---|
| **Population** | Obese M/F, 49.9 ± 13.7 y | Obese F, 37.2 ± 3.1 y | Obese M/F, 44.9 ± 9.5 y | Obese M/F, 69 ± 1 y |
| **Study purpose** | Exercise and cytokines | Weight loss and bone health | Bone metabolism after RxY | Exercise/insulin control in frail older adults |
| **BMI** | 47.3 ± 5.6 | 47.7 ± 2.5 | 42.1 ± 4.0 | 37.3 ± 0.9 |
| **Osteocalcin (pg/mL)** | 3529 ± 2663 | 17800 ± 3000 | 5200 ± 1900 | 12400 ± 1000 |

**Subjects awaiting Roux-en-Y gastric bypass surgery*

Osteocalcin, an osteoblast-specific hormone, was included in this panel due to its association with insulin resistance and adiposity[118]. It is also regulated by leptin, whose downstream effects cause carboxylation of osteocalcin, a molecule containing up to 3 gamma-carboxyglutamic acid residues in its structure[204]. Total, carboxylated, and uncarboxylated levels of osteocalcin may have different effects on adiposity but this is not yet well understood[118]. Total osteonectin (measured in this thesis) is inversely related to

IL-6[118]. As such, we would expect a relatively low mean value of osteocalcin compared to demographically similar studies, as the mean of IL-6 was relatively high. Although the associations of osteocalcin with metabolic disorder are established, there is a paucity of data examining osteocalcin levels in obese adults undergoing health interventions - study subjects are more often older adults.

Osteocalcin would be expected to decrease as a result of decreased adiposity. However, based on the results of a paired samples t-test there was no significant change (positive or negative) to osteocalcin in the 7 participants analyzed. No significance was found in the paired samples t-test when pre/post measurements were $\log_{10}$ transformed.

No significant correlations were seen between osteocalcin and the 6 minute walk test, 1RM leg press, or BMI. There appears to be little research speaking to the association between osteocalcin and physical activity, but it would not have been surprising to see a relationship between osteocalcin and muscular strength (1RM leg press). Participants with higher leg strength could be expected to have more frequently engaged in resistance training (likely unstructured, and simply activities of daily living and ambulation), and resistance training does increase insulin sensitivity and interact with pathways relating to osteocalcin[205]. A relationship was expected with BMI[118] but none of significance was found.

### 4.2h Summary of analyte panel

Just as additional electrodes in an EKG provide more detailed information on heart function, this panel of analytes associated with insulin resistance and metabolic dysfunction has provided a more precise picture than any analyte could individually. For example, had leptin been the sole analyte we might have questioned whether acute psychological stress (e.g. finding

a parking spot, getting to the blood draw appointment on time) impacted its circulating levels. In this scenario, we have reduced the odds of this scenario by including osteonectin (leptin increases osteonectin secretion[89]), IL-1β and MCP-1 (both of which will have a relationship to BMI, and therefore leptin[184]). Indeed, the panel allows us to observe that the 20 participants were experiencing systemic inflammation consistent with obesity (as shown by TNFα and MCP-1), and increased insulin resistance (as shown by IL-6 and osteonectin). Although no significance was seen in pre-post cytokine data nor in the composite scores, it is likely that the small sample size of this dataset negatively impacted our power to detect a difference between time points. Nonetheless, the composite data can be used to inform a sample size calculation for further research.

This approach to building an analyte panel is common, and leptin and adiponectin in particular are chosen in many studies due to their strong correlations with metabolic function. The majority of studies cited throughout this section also reported leptin[203,167,192,206]. IL-6 and TNFα are frequently included as measures of inflammation in many studies, though fewer as a primary outcome as compared to leptin. These markers are also important for bariatric patients in post-operative scenarios, as they can mark diabetogenic inflammation. Because there is no clear consensus regarding the effect size of RxY on several of these markers[82], pre-surgical data from bariatric surgery patients may be helpful to contextualize the effects seen post-operatively.
In addition to IL-6 and TNFα, inflammatory markers typically used in a post-bariatric surgery panel include C-reactive protein and serum amyloid A[82]. Both of these markers were considered for inclusion in the panel but had incompatible dilution factors. A rationale for their inclusion in bariatric-related studies is found in Table 23.

**Table 23: Other commonly used analytes in post-bariatric surgery panels**

| ↑ Increases ↓ Decreases ↔ Unchanged | | | | |
|---|---|---|---|---|
| **Molecule Name** | **Description** | **Effects from exercise** | **Relation to obesity** | **Effects from roux-en-y gastric bypass surgery** |
| C-Reactive Protein (CRP) | Protein synthesized by the liver, known mainly as inflammation marker. Independent risk factor for atherosclerosis and associated with diabetes[207]. | ↓by aerobic, even without change in weight[208]. ↓by strength training[145]. | ↑ levels in obese individuals (it is the major determinant associated with ↑in obese individuals with metabolic syndrome[209]. | ↓ as soon as 3 weeks post-op[210]. It is also a good predictor of post-RxY complications[211]. |
| Serum amyloid A (SAA) | Protein synthesized by the liver, used as inflammation and immune function marker. Unlike CRP, its epigenetic pathways may lead to increased expression of other inflammatory factors[212]. | ↓by aerobic training program[213], but one study shows no association with leisure-time physical activity[214] | ↑ levels in obese individuals[215,216]. | ↓ by RxY [217] |

## 4.3 Directions for future research

### *4.3a Recruitment and sample size*

Though it produced no statistically significant results, the paired sample t-test is appropriate for this study where the mean values of a given participant's score are compared pre- and post-intervention. The lack of significance shown in the cytokine profile changes could be due to the small sample size or may also be due to a true absence of change. The improvements in six minute walk test, leg press and chair stand were statistically, and perhaps clinically significant, and would therefore be expected to elicit the changes in biomarkers described in

Table 2. This limitation of sample size was due to the thesis analyzing a subgroup of participants who fully completed both the baseline and 16-week appointments in ENCOURAGEing Start. Recruitment targets for ENCOURAGEing Start (n=48) were not met (n=26), and were also based on a primary outcome of the six minute walk test. The largest missed opportunity for recruitment may have been in the first stage (Figure 5). At this time point, CMBS staff were to identify suitable candidates for ENCOURAGEing Start and provide the option of signing a consent to contact form, after which a staff member from ENCOURAGEing Start would call the patient to provide more information. Only 21.5% of the patients going through CMBS at this time completed a consent to contact form, though CMBS staff opined during initial discussion that the majority of patients would meet criteria for ENCOURAGEing Start. This may be due in part to the rural status of many patients in the program (patients living outside Winnipeg were not eligible to join ENCOURAGEing Start). However, 83.9% of patients who were contacted by ENCOURAGEing Start after completing the consent to contact form agreed to participate in the intervention. The high rate of study enrollment after patients had the opportunity to ask questions of an ENCOURAGEing Start staff member suggest that a future recruitment phase would benefit from the attendance of a future researcher at the large group CMBS seminars. This would increase visibility for the study and reduce false impressions of eligibility criteria and time requirements. Additional resources for the CMBS, created by the ENCOURAGEing Start team, may have been useful to reduce demands on the CMBS team in regards to discussing the intervention with patients. Alternately, any selection bias could be eliminated by all patients of CMBS being presented with general study information, and self-selecting to contact the ENCOURAGEing Start team, at which time more detailed eligibility criteria could be discussed.

Ideally, this thesis will act as a pilot to suggest a sample size to power a larger study examining the effect of key cytokines in pre-surgical bariatric patients. This calculation could be done using tables presented by Cohen in *Statistical Power Analysis for the Behavioral Sciences*[218]. Using a primary outcome of leptin, we first calculate effect size index using Equation 1.

**Equation 2: calculation of d' in a two-tailed t-test without correlation**

$$d' = \frac{\overline{d}}{s_d}$$

Where d' is the effect size index, $\overline{d}$ is the mean of the difference scores, and $s_d$ is the standard deviation of the difference scores.

With a d' value of 0.809, power and sample size tables[218] can be used with an $\alpha_2$ value of 0.05 to determine that the power was 0.31. The indicated sample size for a subsequent study examining change in leptin levels in a similar population would be approximately n = 34 (90% power). As support for this target sample size, data reported by Cornish & Chilibeck[219] may be considered. Calculations based on their study's IL-6 outcomes suggest that a total sample size of 20 in each of the 2 time points is required to detect a 25% difference between groups (80% power, two-tailed alpha = 0.05; distribution of 9.5 ± 2.5 pg/mL [Mean ± SD]). Therefore, it could reasonably be stated that a target sample size for a future intervention focused on similar cytokines would be 20 or more. The recruitment number needed to attain the target of 20 post-intervention samples is difficult to estimate.

The largest setback to keeping participants in the study was, ironically, their success in achieving criteria to undergo surgery. Nearly half of ENCOURAGEing Start participants (11 of 26) dropped out for this reason. When the intervention was designed, the structure (Figure 4) allowed some clearance between the conclusion of ENCOURAGEing Start and their earliest

potential surgery date. However, several logistical delays such as the method to provide gym memberships and access cards to participants, accruing a sufficiently large first cohort, training research staff and completing data collection appointments meant that many participants would be entering the intervention at a later point in their CMBS journey than was optimal. Future interventions drawing from CMBS would be well advised to maintain a larger separation between inter-organizational time points where possible. A decreased time between receiving consent-to-contact forms and commencement of the intervention would likely reduce the dropout from participants undergoing surgery. The addition of a floating pre-surgery blood draw may also be useful to capture data from participants requiring dropout to undergo surgery.

The data of the 11 participants who were required to drop out due to surgery could reasonably have been expected to bolster the already-significant pre-post improvements. Successful adherence to healthy lifestyle choices, including physical activity and diet, were among the individualized eligibility criteria to be accepted for surgery from CMBS. It is recommended that future intervention protocols not remove any of ENCOURAGEing Start's tests that did not reach significance, as they were evidence-informed and may very well reach statistical significance when data from these individuals confirmed to be successful in lifestyle change is not removed from analysis.

### *4.3b Analytes*

The choice of biomarkers for analysis was selected due to the known relationships in obesity and fitness, and similar dilution factors used in the assay process. Additional biomarkers with extensive research into their properties, such as adiponectin, were identified as being of interest but excluded due to the different dilution ranges and the incremental cost of additional analysis kits. The absence of adiponectin in particular was a challenge, as it is a cytokine

frequently reported in cytokine and obesity-related studies. Other analytes identified as being of interest but removed from the project due to feasibility and cost were resistin, visfatin, vaspin, and gamma glutamyl transferase. Some of these analytes are described in Table 24, and may provide broader insight into the health benefits of an intervention. As this was a pilot, the quasi-experimental study was initiated not to examine every relevant cytokine, but to establish data regarding the physical activity intervention and inform future randomized controlled trials.

The relatively large number of cytokines analyzed in this thesis is greater than typically seen in studies examining cytokines in bariatric patients. As described in section 4.2h, this is because analysis of cytokines in bariatric patients within the context of a physical activity intervention is rare. It is more typical that the analyses focus on cytokines pre/post surgery. Therefore, this approach necessitated an inclusion of both the pro-inflammatory cytokines known to be associated with increased adiposity, and the anti-inflammatory cytokines known to be associated with physical activity. Had irisin not been below detectable ranges, an anti-inflammatory composite consisting or irisin and osteonectin could have been reported alongside the inflammatory composite consisting of IL-6, IL-1β, TNFα, MCP-1.

In my opinion, a researcher planning a randomized controlled trial spanning the period of time between a pre-surgical physical activity intervention to long-term post-operative follow-up should consider including, as a minimum, all analytes used within this thesis. There is uncertainty regarding the effect of bariatric surgery even on certain commonly-analyzed cytokines such as TNFα[82]. An analysis of these cytokines through the entire journey of the bariatric patient would be novel, and could potentially elucidate relationships or potential mechanisms heretofore unknown. An analysis of such longitudinal data may have the potential to

tease out individual analytes or composite scores that predict long-term success and weight management after surgery.

| **Table 24: Biomarkers of future interest** ↑ Increases ↓ Decreases ↔ Unchanged | | | | |
|---|---|---|---|---|
| **Molecule Name and *type*** | **Description** | **Effects from exercise** | **Relation to obesity** | **Effects from roux-en-y gastric bypass surgery** |
| Adiponectin<br>*Adipokine* | Hormone produced primarily by white adipose tissue[220]. Anti-inflammatory, protective role in development of atherosclerosis[221]. Increases insulin sensitivity[222] | ↑ by aerobic, though not nearly as much as the ↑ from diet interventions[220] ↔ strength training[223] | Paradoxically produced by adipose tissue but negatively correlated with obesity [224],and cardiovascular disease[220] | ↑ with a slightly stronger effect in non-diabetic patients[225] |
| Resistin<br>*Adipokine* | Hormone primarily secreted by peripheral-blood mononuclear cells (though it is secreted by adipose tissue in rodents)[226]. Increases insulin resistance[227] | ↓by aerobic in type 2 diabetes[228]. Associated with reduced exercise capacity[229] | Increases expression of pro-inflammatory cytokines including IL-6 and TNF-α. Obesity may decrease serum concentration[230] | No effect from non-surgical weight loss in adolescents[231] or adults[167]. ↓ post- RxY , stronger effect than banding[232] |
| Visfatin / Nicotinamide Phosphoribosyl-tranferase<br>*Adipokine* | Though the research describing it as an insulin mimetic cytokine has been retracted[233], this hormone may have a protective effect against skeletal and cardiac muscle dysfunction during metabolic stress[234] and is correlated to IL-6, TNF-α and other inflammatory markers[235] | Effects demonstrated thus far are variable and may be related to subject characteristics[236] | Shown to be a surrogate marker of inflammation in the obese and elderly[237], and is found in higher levels in cases of obesity with type 2 diabetes than obesity alone[238] | Some research shows its pre-surgical levels in obese subjects[239], but it appears there is no data on pre-post levels for any bariatric surgery type |
| Vaspin<br>*Adipokine* | Adipokine expressed in adipose tissue, stomach, liver, pancreas, skin[240]. Reported to be associated with obesity and impaired insulin sensitivity[241]. | ↑ by aerobic[241]. Strength training effect uncertain. | ↑ levels in obese individuals[241]. Insulin resistance status may affect the relationship between weight/weight loss/vaspin[242]. | ↓ by RxY[86] |

### *4.3c Method of analysis*

The analysis of cytokine profiles via magnetic bead-based multiplex assay was an efficient method by which to analyze groups of cytokines requiring similar dilution factors. However, peripheral (serum) measurements of these cytokines may be more prone to fluctuation than an examination within their secreting cells. For example, although leptin secretion rates in adipocytes correlate well with serum leptin levels[243], evidence on TNFα and other cytokines is conflicting[244]. Several studies analyze adipose tissue excised during bariatric or other surgeries[69], though this was not feasible for this project.

### *4.3d Appointment and Intervention Timing*

Because circulating levels of several analytes are strongly affected by acute stimuli, the structure of the blood draw appointments may have been an additional design weakness. For example, it is not impossible that a walk from a parking lot on a busy weekend morning caused a participant to reach 60% of their maximum heart rate shortly before the blood draw, which has been shown significantly lower serum leptin[245]. In order to accommodate the participants, the writer arranged a variety of appointment times, ranging from 6:30 AM weekday appointments to 10:30 AM on a Saturday. Appointment times were structured in 15 minute blocks. Participants were given standard instructions for fasting bloodwork, including avoidance of "exercise" for 24 hours. As the blood lab was located in the same building as the Active Living Centre and walking track, there was no way to verify that participants who arrived early did not engage in physical activity while waiting for their appointment. This risk seemed to be more prevalent on weekends, where several participants were observed to attend in gym clothes, stating that they had planned to build a workout into their visit, and even bringing family members to be active

with. Rather than giving specific time slots for their appointment, a more appropriate method may have been providing a window of time that would encourage participants to present to the lab promptly upon arrival and sit for a prescribed time, rather than "killing time" elsewhere in the building.

In addition to the demographic heterogeneity which comprises the normative cytokine data, the signaling between cytokines (even the few described in this thesis) has a confounding effect. For example, serum leptin is increased by the presence of TNFα[246], which is in turn increased by psychological stress[247]. It is entirely plausible that if the blood draw occurred within 45 minutes of the participant of trying to find a parking spot, or the lab itself, multiple variables would be affected. As it unlikely that every possible source of psychological stress could be eliminated prior to the blood draw, or that a participant could be monitored to confirm prior physical inactivity, the analysis of several cytokines is vital in any thorough study. Measuring both the relationship between cytokines and the properties each individual cytokine displays are necessary to elucidate trends and mechanisms of change and are a recommended part of any future study.

It is recommended for future implementations of this intervention increase the window of time prior to the blood draw during which participants are advised to avoid physical activity. ENCOURAGEing Start directed participants to avoid exercising for 8 hours prior to the appointment. Cornish and Chilibeck's study[219] measuring IL-6 and TNF-α after 12 weeks of resistance training directed participants to refrain from moderate to vigorous exercise for a minimum of 3 days before blood sample collection. Dekker et al's study measuring IL-6 and C-reactive protein (an inflammatory marker)[188] after 12 weeks of aerobic training directed participants to refrain from strenuous exercise for a minimum of 4 days before blood sample

collection. No protocol identified during the literature review described a no-exercise window as brief as the one used in ENCOURAGEing Start. Not controlling the temporal proximity of exercise to the blood draw, in addition to not defining what "exercise" meant for these instructions, quite likely effected cytokine measurements. It is recommended that the no-exercise buffer be a minimum of 72 hours, as serum leptin is known to decrease by approximately 30% 48 hours after moderate-intensity physical activity[248].

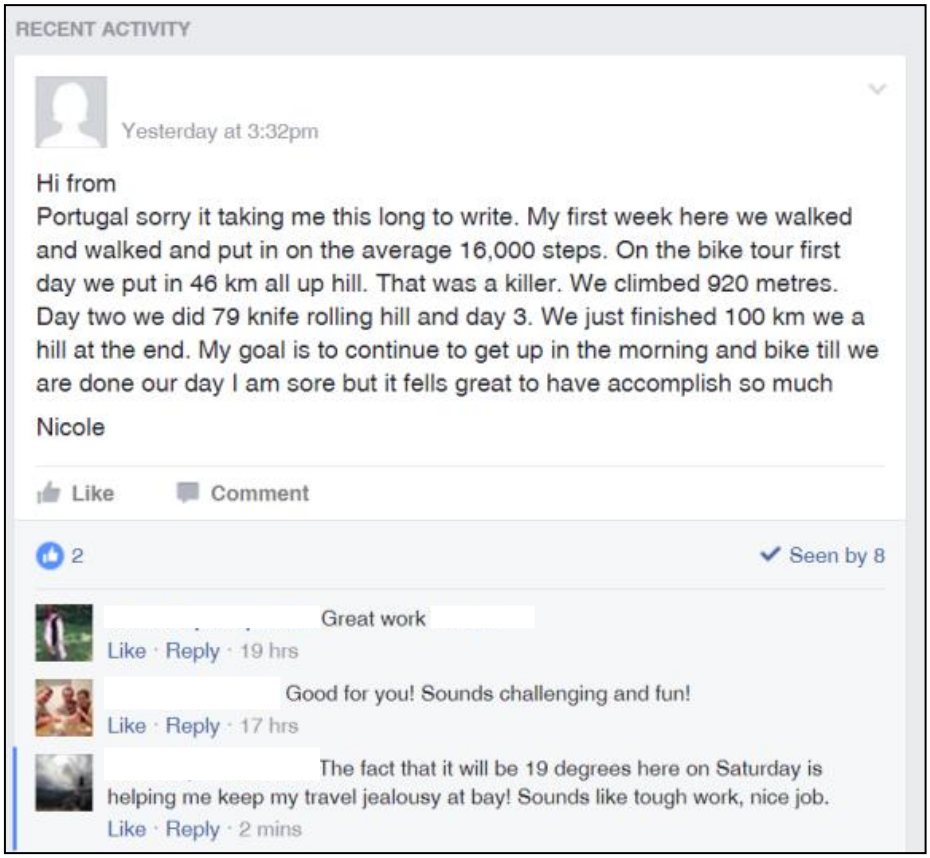
Finally, it is unclear whether the significant changes seen in body fat percentage can be attributed to the intervention, as participants also underwent Craving Change through CMBS, which is a cognitive-behavioral program for emotional eating. The presence of a control group to establish whether ES participants lost less lean body mass in this process would be valuable, as maintenance of lean body mass is associated with improved functional outcomes post-surgery. The absence of a control group is an inherent weakness of the quasi-experimental methodology.

### *4.3e Intervention structure and content*

The integration of social media may be an effective method of reducing dropout and increasing participation in health-related or behavior change interventions[249]. Social media can provide physical activity-related behavior change techniques not otherwise available, such as providing normative information about others' behavior and facilitating social comparison[250]. In early discussion with CMBS, the ENCOURAGEing Start team suggested the usage of social media as a component of the intervention in the form of a hidden, invitation-only Facebook group. This would serve to enhance the interactive nature of the weekly assignments and increase program adherence. For example, the participants would discuss their response to homework questions such as "*what activity did you try in the community this week, and do you*

*have any thoughts you would like to share? Take a look at what your classmates have chosen - are there any ideas that interest you?"*. This would also help participants stay connected with the program if they were forced to miss a week, as seen in Figure 13. This figure depicts a real post (with names removed) from an unrelated physical activity intervention.

**Figure 13: Facebook post from a physical activity intervention**




As CMBS expressed a desire not to use social media in any form, this component was not implemented. However, the majority of participants reported that they did "friend" each other on Facebook and join other Facebook groups which were observed to disseminate inaccurate health information. Researchers delivering a future iteration of ENCOURAGEing Start would be

encouraged to consider the integration of social media (with participation being optional) in whatever form is accessible and timely.

Although no formal questionnaire was administered to participants for evaluation of the components of ENCOURAGEing Start, participant feedback during the sessions indicated a desire for flexibility of exercise choices (rather than being bound only to the track for Walk & Talk), and high levels of enjoyment from the Thursday sessions, particularly the session focused on recreational sports.

## 4.4 Conclusion

The baseline cytokine profiles of the participants are broadly similar to other demographically similar cohorts, though the inherently wide range of cytokines resulted in large standard deviations. Although difficult to compare these participants to meaningful norms, they displayed inter-analyte trends suggesting a state of inflammation and insulin resistance consistent with obesity. Intervention and research goals, as well as the structure of data collection and blood draw appointments, should be considered while developing a cytokine panel for future studies.

The ENCOURAGEing Start intervention was associated with a statistically significant improvement in several functional and health measures, including the 6 minute walk test (10.7%), indirect 1RM leg press (31.5%), 30 second chair stand (29.3%), PHQ-9 (49.4%) and Laval QOL (19.0%). Although these outcomes would suggest that participants received a sufficient dose of physical activity to elicit measurable change in the cytokines (leptin, IL-1β, apelin, MCP-1, IL-6, TNFα, osteonectin and osteocalcin), pre-post analysis of these 7 analytes did not reach statistical significance. It is unknown whether the lack of significance is due to the small sample size or a true absence of change. Several statistically significant correlations were

seen at baseline and 16-week time points, with the highest significance being the established relationships between BMI and IL-1β, IL-6, and osteonectin.

Based on the positive health outcomes from the intervention, it is recommended that a large study be done using the existing ENCOURAGEing Start framework while incorporating the logistical and data collection changes described in this thesis. The intervention would appear to be broadly generalizable based on the participant demographics and their pre-surgical status. It is anticipated that the intervention could be carried out in other centers, perhaps with even greater results if the location was easily accessible and attended by diverse clientele.

# Appendix A:

# Informed Consent Form

## RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

**Title of Study: An Integrated Surgical and Physical Activity Program to Identify Bariatric Surgery Quality Indicators and Evaluate Patient Outcomes and Quality of Life**

**Principal Investigator: Dr. Krista M Hardy, MD, MSC, FRCSC, FACS**. University of Manitoba, Rady Faculty of Health Sciences, Max Rady College of Medicine, Department of Surgery, Assistant Professor; St. Boniface General Hospital, Z3049-409 Taché Avenue, Winnipeg, MB, Canada, R2H 2A6; Phone: (204) 237-2574, Fax: (204) 237-3429, Email: khardy@sbgh.mb.ca

**Co-Investigators:**

**(1) Dr. Todd Duhamel, PhD.** Associate Professor, University of Manitoba, Faculty of Kinesiology and Recreation Management. Principal Investigator, Institute of Cardiovascular Sciences, St.Boniface General Hospital Albrechtsen Research Centre. R4012 - 351 Taché Ave, Winnipeg, MB, Canada, R2H 2A6. Phone (204) 235-3589. Email: tduhamel@sbrc.ca

**(2) Dr. Kathleen M Clouston, PhD**. University of Manitoba, Rady Faculty of Health Sciences, Max Rady College of Medicine, Department of Surgery, Nil Appointment and Research Associate; St. Boniface General Hospital, 409 Taché Avenue, Z3033, Winnipeg, MB, Canada, R2H 2A6. Phone: (204) 258-1479, Fax: (204) 237-3429, Email: kclousto@cc.umanitoba.ca.

**(3) Dr. Ashley S Vergis, MD, MMEd, FRCSC, FACS**. University of Manitoba, Rady Faculty of Health Sciences, Max Rady College of Medicine, Department of Surgery, Associate Professor; St. Boniface General, St. Boniface General Hospital, Z3039-409 Taché Avenue, Winnipeg, MB, Canada, R2H 2A6; Phone: (204) 237-2574, Fax: (204) 237-3429, Email: avergis@sbgh.mb.ca

**Sponsor(s):** University of Manitoba Department of Surgery GFT; Victoria General Hospital Foundation.

You are being asked to participate in a Clinical Trial (a human research study). Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

The study doctors and the University of Manitoba are receiving financial support to conduct this study.

**Purpose of the Study**

This Clinical Trial is being conducted to study whether a physical activity and behavioral modification/education program, completed before surgery, improves outcomes and quality of life for patients who have bariatric surgery. Some of the outcomes include the amount of weight lost after the surgery, the amount of weight loss maintained over time, and improvements in obesity-related diseases you may have (example: diabetes and heart disease). You are being asked to take part in this study because the Centre for Metabolic and Bariatric Surgery has accepted you for publicly funded Roux-En-Y bariatric surgery. A total of 24 (minimum) to 48 (maximum) participants will participate in this program.

This research is being done because the role of physical activity and behavior modification/education in supporting successful patient outcomes and improved quality of life after bariatric surgery have not been established in Manitoba. The information obtained from this study will help inform, design and plan the implementation of such a program with the Winnipeg Regional Health Authority, Manitoba Health, and the Centre for Metabolic and Bariatric Surgery.

**Study Procedures**

In this study, you will receive the standard of care provided to all patients accepted for publically funded Roux-En-Y gastric bypass surgery at the Centre for Metabolic and Bariatric Surgery. This includes having two to four multidisciplinary visits over six months, receiving exercise counseling from a kinesiologist, completing a behavior modification program entitled Craving Change™, and achieving patient-tailored and specific lifestyle and dietary modification goals necessary to be scheduled for surgery. You will also take part in the 16 week pre-operative physical activity-behavioral modification/education program. This includes participating in a tailored physical activity and education program with follow-up about your experiences with the study coordinator by phone and/or email. The physical activity you will be involved in will be tailored to your specific capabilities and needs and will be safe. You will be asked to complete two supervised and structured exercise classes per week for eight weeks. The education sessions will be integrated into the bi-weekly physical activity sessions of the first eight weeks. Topics

will include how to reducing risk factors, healthy eating strategies, physical activity, stress management and promotion of self-managed care.

During the second eight weeks of the program, you will have access to drop-in exercise classes at the Active Living Centre (University of Manitoba Fort Gary Campus). Additional support will be provided to you to overcome barriers preventing you from being physically active and/or to address other areas of concern. In addition, you will have the opportunity to meet with the kinesiologist at least four times (for an hour) throughout the 16-week intervention for additional physical activity counseling. Attendance during the 16-week intervention will be recorded.

As part of standard care and the physical activity-education program, you will be asked to complete the following tests and procedures at various intervals over the course of the 16 week program (baseline, 8 weeks and/or 16 weeks) and after surgery (at one-year and five years):

1. Measurement of height, weight, waist, hip and neck circumference
2. Physical activity, strength, and endurance measurements using the 6 Minute Walk Test, Sit to Stand test, One-Repetition Maximum Leg Press, 5 meter gait-test, hand grip and arm curl test
3. Heart Rate Monitoring (self-monitored at certain times during physical activity)
4. Blood Pressure Measurement
5. Small and large artery elasticity measurement (non-invasive; similar to blood pressure measurement using a cuff)
6. Bioelectrical Impedance Analysis (InBody 270 Body Composition Analyzer)
7. Blood Analysis at baseline, 16 weeks and one year (total, LDL, and HDL cholesterol, triacylglycerides, blood glucose; $H_{A1c}$; total of two to four teaspoons of blood).
8. Wearing a waistband or wristband accelerometer, 24 hours a day for 7 days, to measure and record physical activity levels (at Baseline, 8 and 16 weeks and one year)
9. Quality of Life, Patient Satisfaction-Health-Depression, and select Self-Compassion-Anxiety-Physical Activity Questionnaires. It will take about 20 minutes to complete the surveys.

Study participants will have the option to participate in a Secret Facebook Group to facilitate interaction among study participants and their ALC exercise instructors outside of the bi-weekly sessions held at the ALC. This aspect of the program is also meant to provide an additional way to access and interact with some of the study education materials. It is meant to provide a supportive and encouraging environment that may help in learning and applying positive lifestyle choices and changes. Members of the Secret Facebook Group will be seen only by members of the group (who are study participants) and cannot be seen by the public. Therefore, patients choosing to participate in the Secret Facebook Group have the potential to be identified by those study participants who are also part of the Secret Facebook Group. Patient participation in this aspect of the program is optional and will not affect your acceptance to participate in this study. You will be asked to complete a brief questionnaire about the use of Facebook at the end of the study.

The study researchers may decide to take you off this study if you have or developed any orthopedic, neurologic or cardiopulmonary conditions that prevent you from participating in moderately strenuous exercise or if you are unable to commit to attending the regular training and education sessions of the intervention program**.** You can decide to stop participating in the program at any time. If you decide to stop participating in the study, we encourage you to talk to the study staff and your regular doctor first.

**Study results (group and/or individual) will be made available to participants upon request.**

**Risks and Discomforts**

Every effort will be made to ensure your safety and prevent injury during exercise. A kinesiologist with Certified Exercise Physiologist certification will tailor your exercise plan to your specific abilities, supervise your exercise sessions during the first eight weeks of the 16 week program, and be available to answer any questions and/or concerns you have during the second eight weeks of the program. While participating in the study, you may have certain minor physical risks (feeling short of breath, muscle strains, sprains) related to participation in the physical activity component of the program. Please immediately report any concerns about injury incurred as a result of the exercise intervention to the Principal Investigator (Dr. Krista Hardy). We will assess it and may request an independent evaluation by your physician or the appropriate health care professional.

Answering certain questionnaires may make you feel uncomfortable. If this occurs, you may opt out of completing them and still remain in the study. You can expect to be asked about whether you have any health conditions and how you think and feel about it. You will also be asked about your thoughts and feelings about your body and physical activity. You may also experience some bruising and discomfort associated with the blood draw which is also associated with a slight risk of infection.

**Medical Care for Injury Related to the Study**

In the case of injury or illness resulting from this study, necessary medical treatment will be available at no additional cost to you. If you should become physically injured as a result of any research activity, the study doctor will provide any necessary treatment, at no charge, to help you promptly recover from the injury.

**Benefits**

By participating in this study, you will be helping the study doctors collect information that will show whether the effects of the physical activity and behavior modification/education

program has beneficial effects for patients treated with Roux-En-Y gastric bypass surgery. There may or may not be direct medical benefits to you from participating in this study. We hope the information gathered and the knowledge learned from this study will benefit future participants undergoing Roux-En-Y gastric bypass by supporting the creation and implementation of a permanent pre-operative physical activity-education-behavior modification program.

**Costs and payment for participation**

All clinic and professional fees, diagnostic and laboratory tests performed as part of this study will be provided at no cost to you. There will be no parking costs for you at the Active Living Centre during the 16 week program. You will not receive payment or reimbursement for any other expenses related to taking part in this study (example travelling expenses).

**Confidentiality**

The information gathered from this research study may be published or presented in public forums, however your name and other identifying information will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. All study documents related to you will bear only your assigned patient identification number (called the study ID) and /or initials. With patient consent, HAMD questionnaire results for depression may be shared with a patient's primary care provider in order to support/facilitate necessary treatment for depression.With your permission, your family physician (general practitioner; GP) may be notified about your participation in this study.

De-identified/anonymous patient health information, study outcome measurements and questionnaire results will be entered into an electronically secure REDCap Database and coded by study ID. In order to ensure protection of personal health information and to maintain confidentiality, any information entered into the computer or transmitted electronically will be de-identified and coded with the patient specific study ID number only.

Medical and research study records containing personal health information will be accessed only by those individuals with permission to do so. This includes the study doctors and study coordinator only. The University of Manitoba Health Research Ethics Board may review research-related records for quality assurance purposes. All records will be kept in a locked, secure area. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave the University of Manitoba.

**Voluntary Participation/Withdrawal from the Study**

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. If you decide not to participate or to withdraw from the study, it will not effect any of your medical care at this site. If your study doctor feels that it is in your best interest to withdraw you from the study, your study doctor will remove you without your consent. We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

**Questions**

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact the study doctor and/or the study staff: Dr. Krista Hardy at (204) 237-2574 or Dr. Kathleen Clouston at (204) 258-1479.

For questions about your rights as a research participant, you may contact The University of Manitoba Health Research Ethics Board at (204) 789-3389**.**

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

**Statement of Consent**

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Krista Hardy and/or her study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements. Any relationship (such as employee, student or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my medical records by The University of Manitoba Health Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

I agree to being contacted in relation to this study. Yes ☐ No ☐

I agree that my family physician can be notified about my participation in this study.

Yes ☐ No ☐

Participant signature_____ Date _____

(day/month/year)

Participant printed name: _____

(First, Last)

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent.

Printed Name: _____Date _____

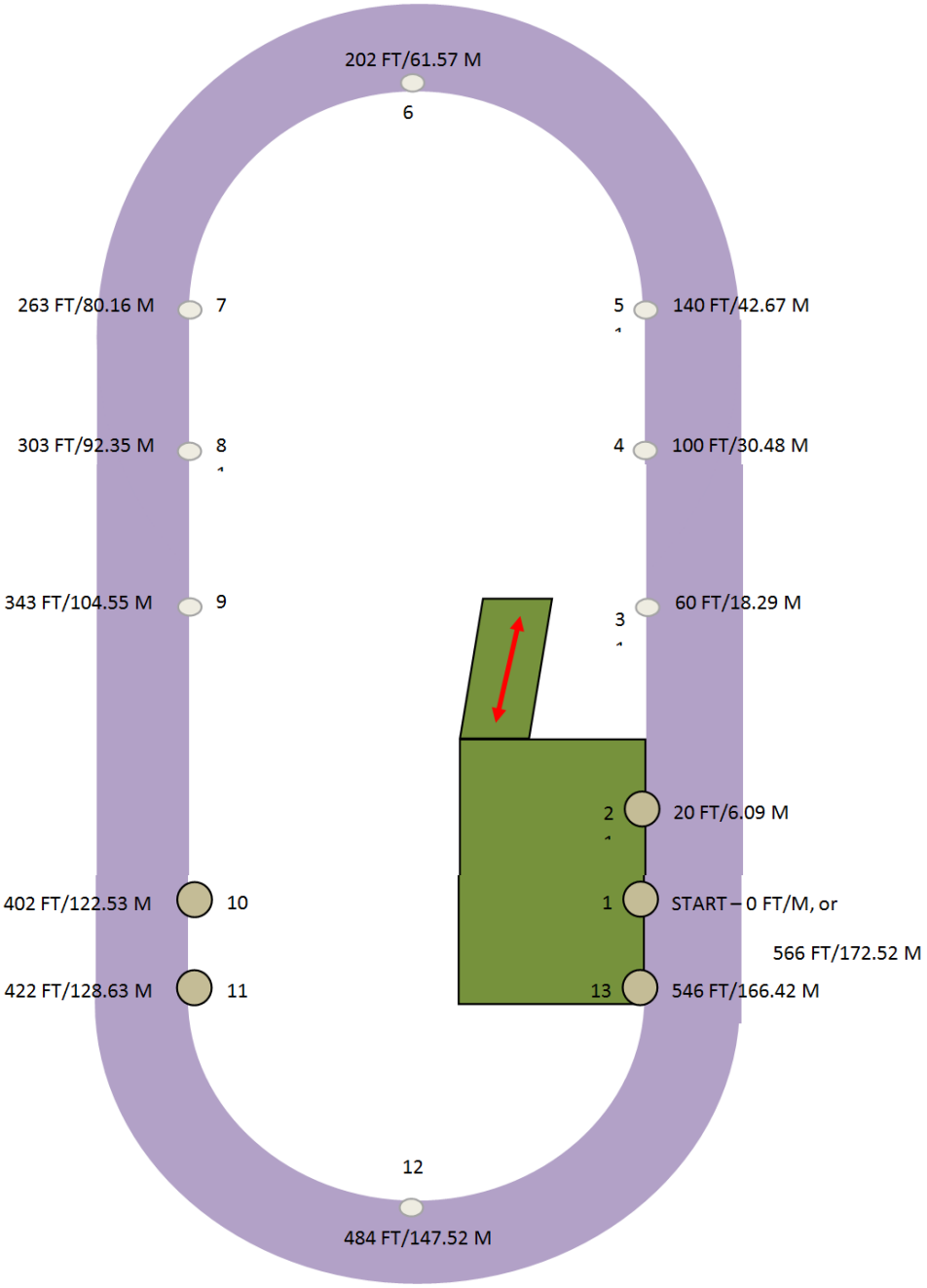
(First, Last) (day/month/year)

Signature: _____

Role in the study: _____

# Appendix B:

# Map for 6 minute walk test in Active Living Centre


202 FT/61.57 M
6
263 FT/80.16 M
7
5
140 FT/42.67 M
303 FT/92.35 M
8
4
100 FT/30.48 M
343 FT/104.55 M
9
60 FT/18.29 M
3
2
20 FT/6.09 M
402 FT/122.53 M
10
1
START – 0 FT/M, or
566 FT/172.52 M
422 FT/128.63 M
11
13
546 FT/166.42 M
12
484 FT/147.52 M

# Appendix C:

# Information sheet given to CMBS patients at seminar

ENCOURAGE

UNIVERSITY OF MANITOBA

## ENCOURAGEing Start
## Physical Activity Program

The University of Manitoba is proud to offer a free new healthy living and physical activity initiative designed for patients of the Centre for Metabolic and Bariatric Surgery. This research-informed 16 week program will help you develop skills to enjoy the benefits and overcome barriers towards a healthy lifestyle, and provide the knowledge and confidence to create or find your own physical activity opportunities as you strive for long-term change. The program takes place at the University of Manitoba's state-of-the-art Active Living Centre and is created by a Canadian Society for Exercise Physiology *Certified Exercise Physiologist* (CSEP-CEP): the highest standard of health and fitness certification in Canada. The program was developed in collaboration with physical activity researchers in the Faculty of Kinesiology and Recreation Management and clinicians working in the Bariatric and Metabolic Surgery Clinic.

Structured sessions will take place Tuesdays and Thursdays evenings beginning October 31 and finishing December 21.The program will continue for an additional 8 week (ending Feb 15) in a format which allows greater flexibility with no set times – follow-up with instructors will be done in person, by telephone, or by email (your choice).

Please contact Alex Edye-Mazowita at **204-474-7858** or **encourage@umanitoba.ca** to find out more about the program or to register for your first research appointment.

The exact time of classes depends on the number of initial registrants. It will start at a time between 6:30 and 7:45 on both Tuesday and Thursday, and we will confirm that time with you no later than October 18.

**Q: Besides the two sessions per week, are there any other time commitments to the study?**

A: Yes. Because this program is also a research study, we will be collecting data such as questionnaires and activity levels (measured by a small wearable device known as an accelerometer). At your first appointment, a researcher will go through a consent package to explain these procedures and answer any questions that you might have.

**Q: Are there any restrictions related to body weight?**

A: Yes. Because of the specifications of equipment that will be used throughout the program, we are only able to accommodate participants weighing less than 350 pounds.

**Q: Do I need to have a certain level of fitness to participate?**

A: You will get the most out of the program if you are able to walk for at least 5 minutes at a time, and transition from a standing position to a sitting or laying position on the ground.

**Q: Will we be exercising in the main gym area with other members such as university students?**

A: On Tuesdays we will mostly use the walking track on the third floor of the Active Living Centre, which is accessible to any user. You will be walking with a partner and occasionally stopping for skill-building instructor-led activities. Our Thursday exercise sessions will take place in more private areas. For example, you will learn to use exercise equipment in our Applied Research Facility, and will have the option in later weeks of using similar equipment in the main gym area or remaining in the Applied Research Facility.

**Q: Will this program provide us with nutritional information such as meal plans?**

A: No. The Centre for Metabolic and Bariatric Surgery will remain your source for dietary information – this program focuses on physical activity and general lifestyle.

# Appendix D:

# Magnetic bead panel manufacturer's guide (sample excerpt)

# Human Myokine Magnetic Bead Panel

**INTRODUCTION**

It is increasingly becoming apparent that skeletal muscle, besides being an organ for energy storage, is also actively involved in the synthesis and secretion of many proteins. These secretory proteins can be collectively termed as “myokines”.

Muscle contraction during physical activity is an important activator of the release of the myokines. Myokines not only can act in autocrine and/or paracrine manner in regulation of skeletal muscle growth, they can also act as endocrine hormones to mediate inter-organ crosstalk. The myokine theory provides molecular mechanistic explanations to the exercise-induced metabolic changes in liver and adipose tissue as well as the profound changes in immune and neuron systems.

MILLIPLEX® MAP offers the broadest selection of analytes across a wide range of disease states and species. Once the analytes of interest have been identified, you can rely on the quality that we build into each kit to produce results you can trust. In addition to the assay characteristics listed in the protocol, other performance criteria evaluated during the validation process include: cross-reactivity, dilution linearity, kit stability, and sample behavior (e.g. detectability and stability).

Each MILLIPLEX® MAP panel and kit includes:

- Quality controls (QCs) provided to qualify assay performance
- Comparison of standard (calibrator) and QC lots to a reference lot to ensure lot-to-lot consistency
- Optimized serum matrix to mimic native analyte environment
- Detection antibody cocktails designed to yield consistent analyte profiles within panel

In addition each panel and kit meets stringent manufacturing criteria to ensure batch-to-batch reproducibility. In order to meet the increasing need to provide quantification assays for myokines in pre-clinical and translational research models, EMD Millipore has developed the **MILLIPLEX® MAP** Human Myokine Magnetic Bead Panel. Coupled with the Luminex® xMAP® platform in a **magnetic bead** format, you receive the advantage of ideal speed and sensitivity, allowing quantitative multiplex detection of dozens of analytes simultaneously, which can dramatically improve productivity.

EMD Millipore’s MILLIPLEX® MAP Human Myokine Magnetic Bead Panel is part of the most versatile system available for myokine research. From our single to multiplex biomarker solutions, we partner with you to design, develop, analytically validate and build the most comprehensive library available for protein detection and quantitation.

- MILLIPLEX® MAP offersyou:
    - The ability to choose any combination of analytes from our panel of 15 analytes to design a custom kit that better meets your needs.
    - A convenient “all-in-one” box format that gives you the assurance that you will have all the necessary reagents you need to run your assay.

EMD Millipore’s MILLIPLEX® MAP Human Myokine Magnetic Bead Panel is a 15-plex kit to be used for the simultaneous quantification of any or all of the following analytes in serum or plasma samples: Apelin (APLN), Brain-derived Neurotrophic Factor (BDNF)*, Erythropoeitin (EPO), Fatty Acid-Binding Protein 3 (FABP3), Fibroblast Growth Factor 21 (FGF21), Fractalkine/CX3CL1, Follistatin-Like 1 Protein (FSTL-1), IL-6, IL-15, Irisin, Leukemia Inhibitory Factor (LIF), Myostatin (MSTN)/GDF8, Oncostatin M (OSM), Osteocrin/Musclin (OSTN), and Osteonectin (SPARC).

***For Research Use Only. Not for Use in Diagnostic Procedures.***
***Please read entire protocol before use.***

***It is important to use same assay incubation conditions throughout your study.***

## PRINCIPLE

**MILLIPLEX® MAP is based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life-sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded magnetic beads known as MagPlex®-C microspheres.**

- Luminex® uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, distinctly colored bead sets of 500 5.6 µm polystyrene microspheres or 80 6.45 µm magnetic microspheres can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of eachmicrosphere.
- EMD Millipore provides three Luminex® instruments to acquire and analyze data using two detection methods:
    - The Luminex® analyzers Luminex® 200™ and FLEXMAP 3D®, flow cytometry-based instruments that integrate key xMAP® detection components, such as lasers, optics, advanced fluidics and high-speed digital signal processors.
    - The Luminex® analyzer (MAGPIX®), a CCD-based instrument that integrates key xMAP® capture and detection components with the speed and efficiency of magnetic beads.
- Each individual microsphere is identified and the result of its bioassay is quantified

based on fluorescent reporter signals. EMD Millipore combines the streamlined data acquisition power of Luminex® xPONENT® acquisition software with sophisticated analysis capabilities of the new MILLIPLEX® Analyst 5.1, integrating data acquisition and analysis seamlessly with all Luminex® instruments.

**The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP® technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.**

## STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- For long-term storage, freeze reconstituted standards and controls at ≤-20°C. Avoid multiple (>2) freeze/thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibody,and Streptavidin-Phycoerythrin.**

### REAGENTS SUPPLIED

**Note: Store all reagents at 2 – 8°C**

| Reagents Supplied | Catalog Number | Volume | Quantity |
|---|---|---|---|
| Human Myokine Standard | HMY-8056 | Lyophilized | 1 vial |
| Human Myokine Quality Controls 1 and 2 | HMY-6056 | Lyophilized | 1 vial each |
| Bead Diluent | LBD | 3.5 mL | 1 vial |
| Serum Matrix<br>Note: Contains 0.08% Sodium Azide | MXHSM-10 | Lyophilized | 1 vial |
| Set of one 96-Well Plate with 2 sealers | ---------- | ---------- | 1 plate<br>2 sealers |
| Assay Buffer | L-AB | 30 mL | 1 bottle |
| 10X Wash Buffer<br>Note: Contains 0.05% Proclin | L-WB | 60 mL | 1 bottle |
| Human Myokine Detection Antibodies | HMY-1056 | 3.2 mL | 1 bottle |
| Streptavidin-Phycoerythrin | L-SAPE4 | 3.2 mL | 1 bottle |
| Mixing Bottle | ---------- | ---------- | 1 bottle |

**Included Human Myokine Panel Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel**

**Human Myokine Panel Antibody-Immobilized Magnetic Beads:**

| Bead/Analyte Name | Luminex® Magnetic Bead Region | Customizable 15 Analytes (20X concentration, 200 µL) Available | Cat.# |
|---|---|---|---|
| Anti-Apelin Bead | 13 | ✔ | HAPLN-MAG |
| Anti-Fractalkine Bead | 14 | ✔ | HMYFKN-MAG |
| Anti-BDNF Bead | 15 | ✔ | RBDNF-MAG |
| Anti-Erythropoeitin (EPO) Bead | 30 | ✔ | HEP0-MAG |
| Anti-Osteonectin Bead | 38 | ✔ | H0STNCTN-MAG |
| Anti-LIF Bead | 39 | ✔ | HMYLIF-MAG |
| Anti-IL-15 Bead | 42 | ✔ | HMYIL15-MAG |
| Anti-Myostatin (MSTN)/GDF8 Bead | 44 | ✔ | HMYSTN-MAG |
| Anti-FABP3 Bead | 45 | ✔ | HFABP3-MAG |
| Anti-Irisin Bead | 46 | ✔ | HIRISN-MAG |
| Anti-Follistatin-Like 1 Protein (FSTL-1) Bead | 51 | ✔ | HFSTL1-MAG |
| Anti-Oncostatin M Bead | 57 | ✔ | H0SM-MAG |
| Anti-IL-6 Bead | 61 | ✔ | HMYIL6-MAG |
| Anti-FGF21 Bead | 62 | ✔ | HFGF21-MAG |
| Anti-Osteocrin/Musclin Bead | 65 | ✔ | H0STCRN-MAG |

**MATERIALS REQUIRED BUT NOT PROVIDED**

## Reagents

**1. Luminex® Sheath Fluid (EMD Millipore Catalog #SHEATHFLUID) or Luminex® Drive Fluid (EMD Millipore Catalog #MPXDF-4PK)**

## Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000µL
2. Multichannel Pipettes capable of delivering 5 µL to 50 µL or 25 µL to 200µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Aluminum Foil
7. Absorbent Pads
8. Laboratory Vortex Mixer

9. Sonicator (Branson Ultrasonic Cleaner Model #B200 or equivalent)
10. Titer Plate Shaker (Lab-Line Instruments Model #4625 or equivalent)
11. Luminex® 200™, HTS, FLEXMAP 3D®, or MAGPIX® with xPONENT® software by Luminex® Corporation
12. Automatic Plate Washer for magnetic beads (BioTek® 405 LS and 405 TS, EMD Millipore Catalog #40-094, #40-095, #40-096, #40-097 or equivalent) or Handheld Magnetic Separation Block (EMD Millipore Catalog #40-285 or equivalent).

**Note: If a plate washer or handheld magnetic separation block for magnetic beads is not available, one can use a microtiter filter plate (EMD Millipore Catalog #MX-PLATE) to run the assay using a Vacuum Filtration Unit (EMD Millipore Vacuum Manifold Catalog #MSVMHTS00 or equivalent with EMD Millipore Vacuum Pump Catalog #WP6111560 or equivalent).**

## SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

**Note: See Full Labels of Hazardous components on next page.**

| Ingredient, Cat # | | Full Label | |
|---|---|---|---|
| 10X Wash Buffer | L-WB |  | **Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water. |
| Streptavidin-Phycoerythrin | L-SAPE4 |  | **Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
| Human MyokineStandard | HMY-8056 |   | **Danger.** Harmful if swallowed. Causes seriouseyedamage.Harmfultoaquatic life with long lasting effects. Avoid releasetotheenvironment.Weareye protection.IFINEYES:Rinsecautiously withwaterforseveralminutes.Remove contactlenses,ifpresentandeasytodo. Continuerinsing.Getmedicaladvice/ attention. |
| Human Myokine QualityControls1&2 | HMY-6056 |   | **Danger.** Harmful if swallowed. Causes seriouseyedamage.Harmfultoaquatic life with long lasting effects. Avoid releasetotheenvironment.Weareye protection.IFINEYES:Rinsecautiously withwaterforseveralminutes.Remove contactlenses,ifpresentandeasytodo. Continuerinsing.Getmedicaladvice/ attention. |
| Human MyokineDetection Antibodies | HMY-1056 |  | **Warning.**Causesseriouseyeirritation.IF INEYES:Rinsecautiouslywithwaterfor severalminutes.Removecontactlenses, ifpresentandeasytodo.Continue rinsing. |
| Serum Matrix | MXHSM-10 | | Harmfultoaquaticlifewithlonglasting effects. Avoid release to the environment. |

## TECHNICAL GUIDELINES

**To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.**

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTICPROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- The standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at
  **≤-20°C for 1 month and at ≤-80°C for greater than one month.**
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Mixing Bottle at 2-8°C for up to one month**.**
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay on Luminex® 200™, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the

recommended EMD Millipore filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height according to the protocols recommended by Luminex®to the kit solid plate or to the recommended EMD Millipore filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate using 1 alignment disc.

**For FLEXMAP 3D® when using the solid plate in the kit, the final resuspension should be with 150 µL Sheath Fluid in each well and 75 µL should be aspirated.**

- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard curve and control wells. If samples are diluted in Assay Buffer, use the Assay Buffer as matrix.
- For serum/plasma samples that require further dilution beyond 1:2, use the serum matrix provided in the kit.
- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue aggregates. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.

## SAMPLE COLLECTION AND STORAGE

**Note: Substantial amounts of BDNF are stored in circulating platelets and subsequently released upon platelet activation. Therefore, platelet-poor plasma is critical to ensure accurate measurement of circulating levels of BDNF. It should be noted many plasma preparation procedures, including those recommended by the Clinical Laboratory and Standards Institute (CLSI), would result in incomplete platelets removal from blood.**

**This will cause data variability and irreproducibility between assays.**

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤-20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.

- Serum samples should be diluted 1:2 in the Assay Buffer provided in the kit. For example, in a tube, 35 µL of serum may be combined with 35 µL of Assay Buffer. When further dilution beyond 1:2 is required, use Serum Matrix as the diluent.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. **Remove plasma and assay immediately or aliquot and store samples at ≤-20°C.**
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Plasma samples should be diluted 1:2 in the Assay Buffer provided in the kit. For example, in a tube, 35 µL of plasma may be combined with 35 µL of Assay Buffer. When further dilution beyond 1:2 is required, use Serum Matrix as the diluent.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤-20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue culture supernatant may require a dilution with an appropriate control medium prior to assay. Tissue/cell extracts should be done in neutral buffers containing reagents and conditions that do not interfere with assay performance. Excess concentrations of detergent, salt, denaturants, high or low pH, etc. will negatively affect the assay. Organic solvents should be avoided. The tissue/cell extract samples should be free of particles such as cells or tissue debris.

**NOTE:**

- A maximum of 25 µL per well of diluted serum or plasma can be used. Tissue culture or other media may also be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

## PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

For individual vials of beads, sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 150 µL from each antibody-bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Bead Diluent. Vortex the mixed beads well. Unused portion may be stored at 2-8°C for up to one month. (Note: Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.)

Example 1: When using 6 antibody-immobilized beads, add 150 µL from each of the 6 bead vials to the Mixing Bottle. Then add 2.1 mL Bead Diluent.

Example 2: When using 15 antibody-immobilized beads, add 150 µL from each of the 15 bead vials to the Mixing Bottle. Then add 0.75 mL Bead Diluent.

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL deionized water. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes. Unused portion may be stored at ≤-20°C for up to one month.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 60 mL of 10X Wash Buffer with 540 mL deionized water. Store the unused portion at 2-8°C for up to one month.

D. Preparation of Serum Matrix

**This step is required for serum or plasma samples only.**

Add 1.0 mL deionized water to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at ≤-20°C for up to one month.

E. Preparation of Human Myokine Standard

1.) Prior to use, reconstitute the Human Myokine Standard with 250 µL deionized water. Refer to table below for analyte concentrations. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes. This will be used as Standard 7; the unused portion may be stored at ≤-20°C for up to one month.

2). Preparation of Working Standards

Label 6 polypropylene microfuge tubes Standard 1 through Standard 6. Add 150 µL of Assay Buffer to each of the 6 tubes. Prepare serial dilutions by adding 50 µL of the reconstituted standard to the Standard 6 tube, mix well and transfer 50 µL of Standard 6 to the Standard 5 tube, mix well and transfer 50 µL of Standard5 to the Standard 4 tube, mix well and transfer 50 µL of Standard 4 to the Standard 3 tube, mix well and transfer 50 µL of Standard 3 to the Standard 2 tube, mix well and transfer 50 µL of

Standard 2 to the Standard 1 tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

| Standard # | Volume of Deionized Water to Add | Volume of Standard to Add |
|---|---|---|
| Standard 7 | 250 µL | 0 |

| Standard # | Volume of Assay Buffer to Add | Volume of Standard to Add |
|---|---|---|
| Standard 6 | 150 µL | 50 µL of Standard 7 |
| Standard 5 | 150 µL | 50 µL of Standard 6 |
| Standard 4 | 150 µL | 50 µL of Standard 5 |
| Standard 3 | 150 µL | 50 µL of Standard 4 |
| Standard 2 | 150 µL | 50 µL of Standard 3 |
| Standard 1 | 150 µL | 50 µL of Standard 2 |

## IMMUNOASSAY PROCEDURE

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Standards [0 (Background), [Standard 1 through 7], Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay induplicate.
- If using a filter plate, set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

A. Add 200 µL of Wash Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature(20-25°C).

B. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.

C. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 pg/mL standard (Background).

D. Add 25 µL of Assay Buffer to the sample wells.

E. Add 25 µL of appropriate matrix solution to the background, standards, and control wells. When assaying serum or plasma, use the Serum Matrix. When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution.

F. Add 25 µL of Sample (1:2 diluted) into the appropriate wells.

G. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)

H. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker overnight (16-18 hours) at 2-8°C. Alternatively, incubate for 2 hours at room temperature(20-25°C).

I. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.

J. Add 25 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)

**K.** Seal, cover with foil and incubate with agitation on a plate shaker for one hour at room temperature (20-25°C). **DO NOT ASPIRATE AFTERINCUBATION.**

L. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.

M. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature(20-25°C).

N. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.

O. Add 150 µL of Sheath Fluid (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5minutes.

P. Run plate on Luminex® 200™, HTS, FLEXMAP 3D® or MAGPIX® with xPONENT®software.

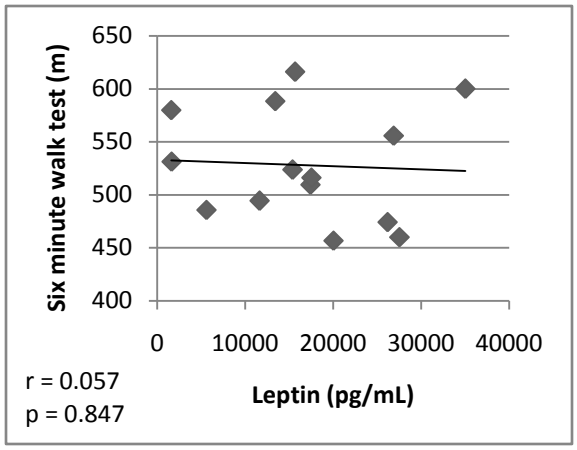
Q. Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples. (Note: For diluted samples, final sample concentrations should be multiplied by the dilution factor. For samples diluted as per protocol instructions, multiply by 2. If using another dilution factor, multiple by the appropriate dilution factor.)

# Appendix E:

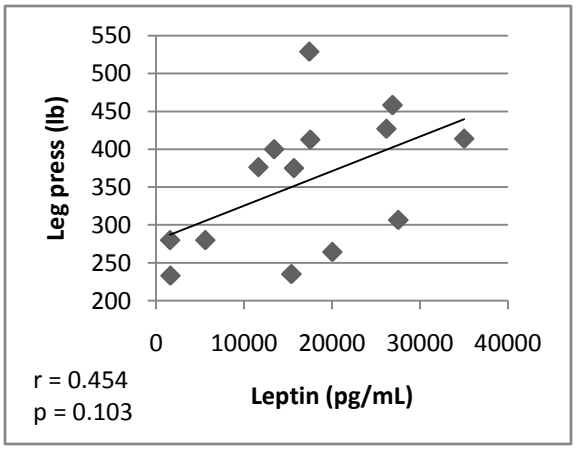
# Scatterplot graphs of bivariate correlations at baseline and 16-week time points

**Figure 7: Relationships between leptin and other outcomes at baseline and post (n=7 ; N=14)**

7A




7B




7C

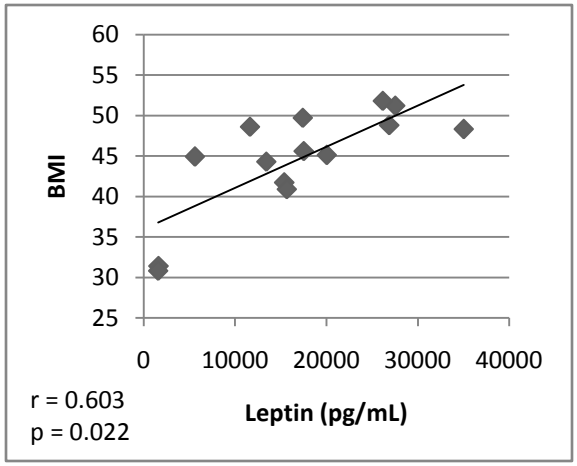
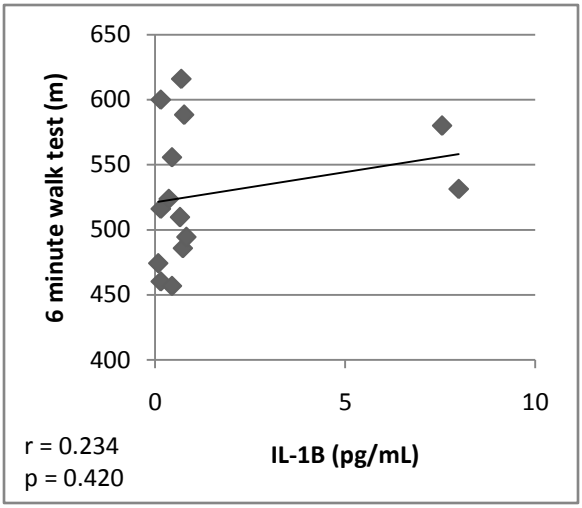
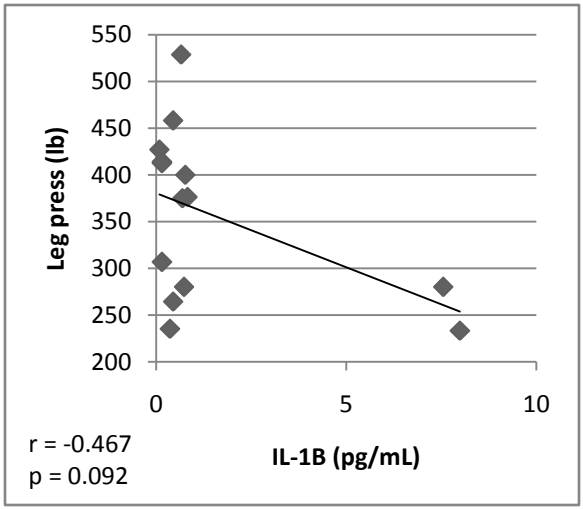


**Figure 8: Relationships between IL-1β and other outcomes at baseline and post (n=7 ; N=14)**

8A




8B




8C

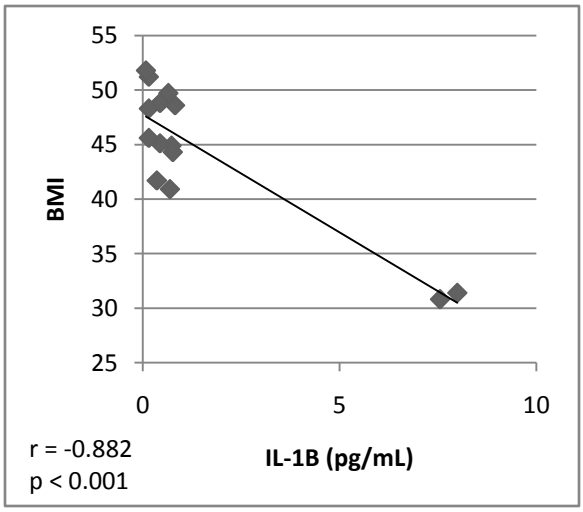
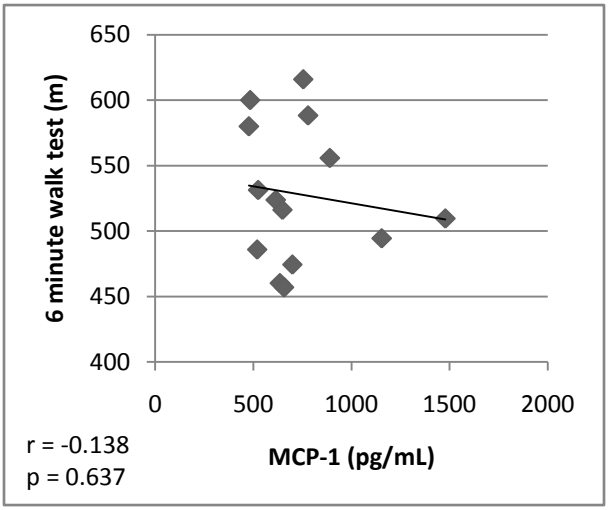
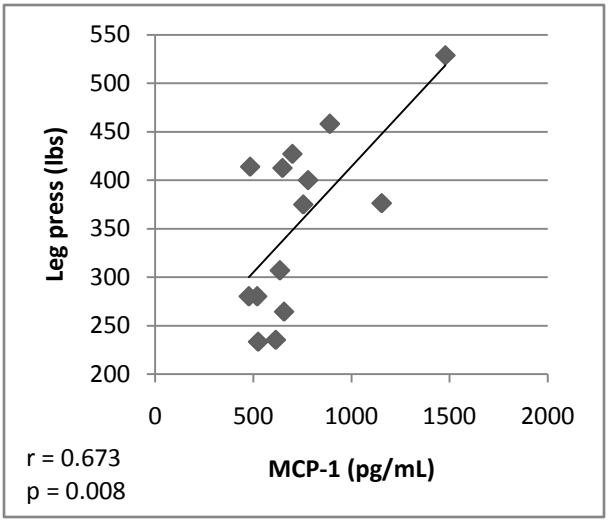


**Figure 9: Relationships between MCP-1 and other outcomes at baseline and post (n=7 ; N=14)**

9A




9B




9C

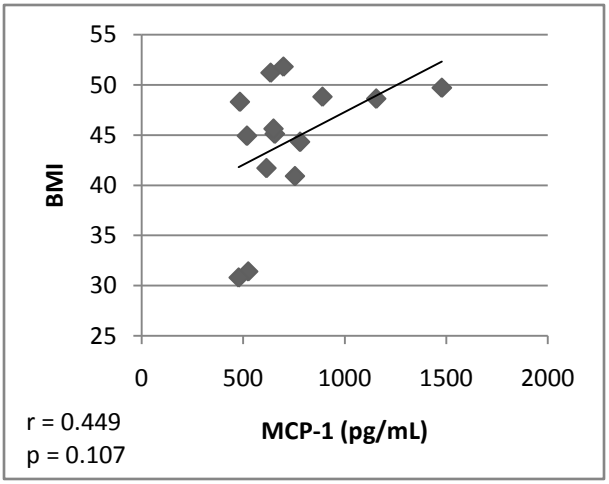
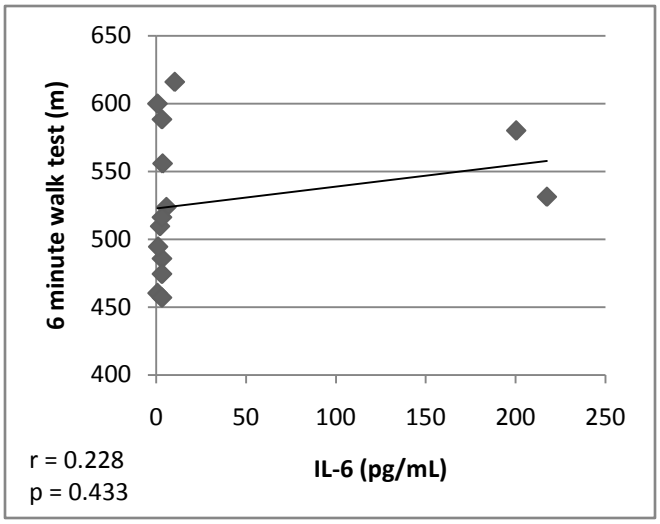
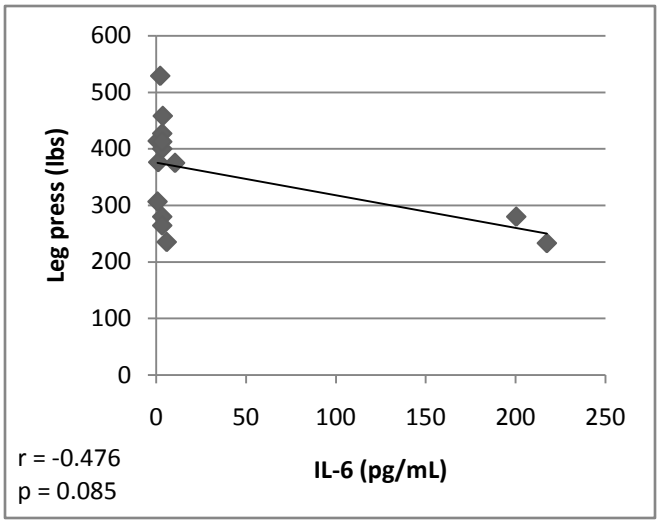


**Figure 10: Relationships between IL-6 and other outcomes at baseline and post (n=7 ; N=14)**

10A




10B




10C

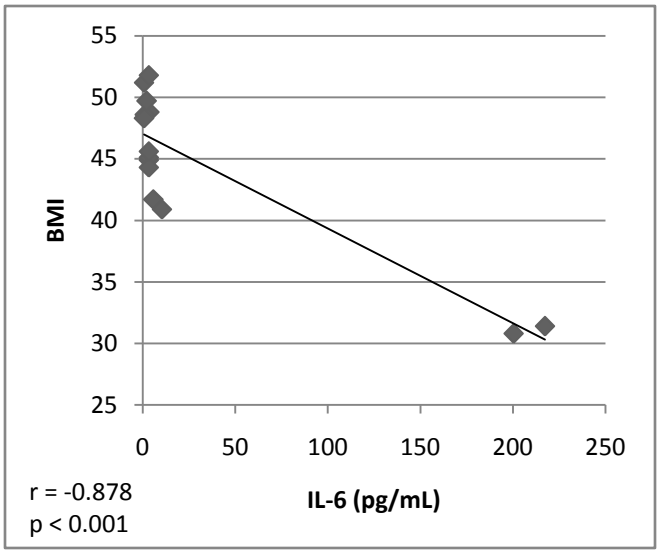
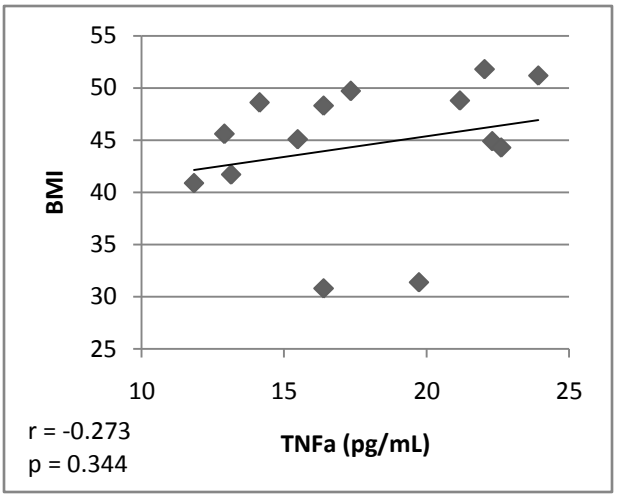
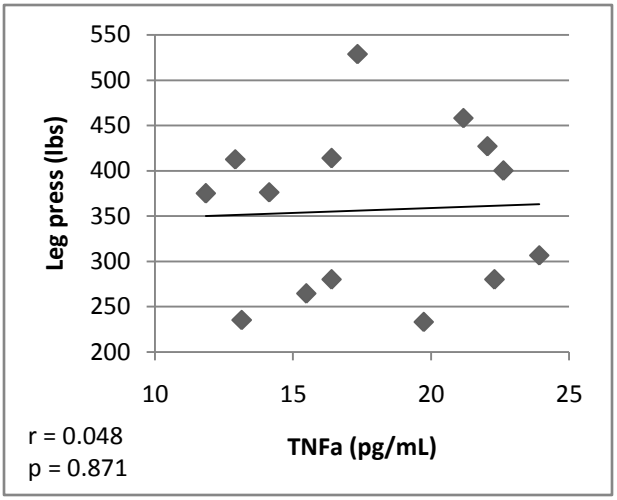


**Figure 11: Relationships between TNFα and other outcomes at baseline and post (n=7 ; N=14)**

11A




11B




11C

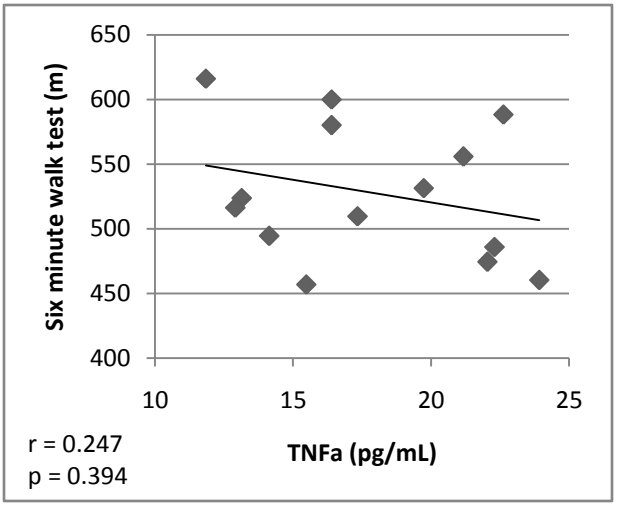
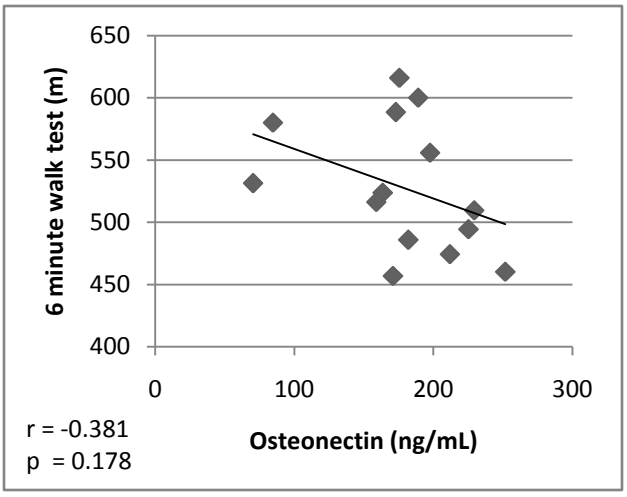
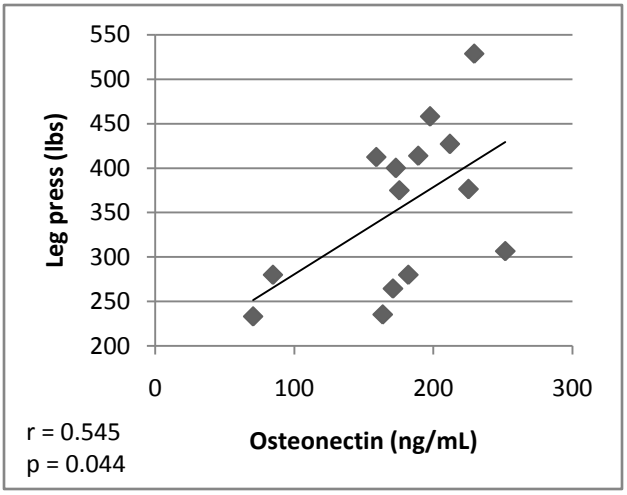


**Figure 12: Relationships between osteonectin and other outcomes at baseline and post (n=7 ; N=14)**

12A




12B




12C

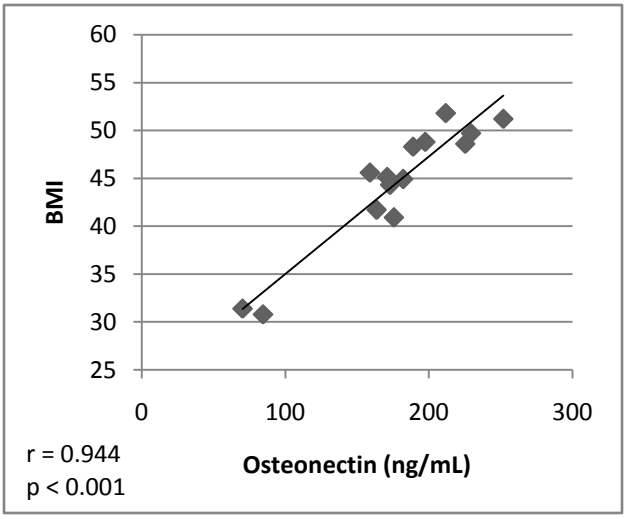
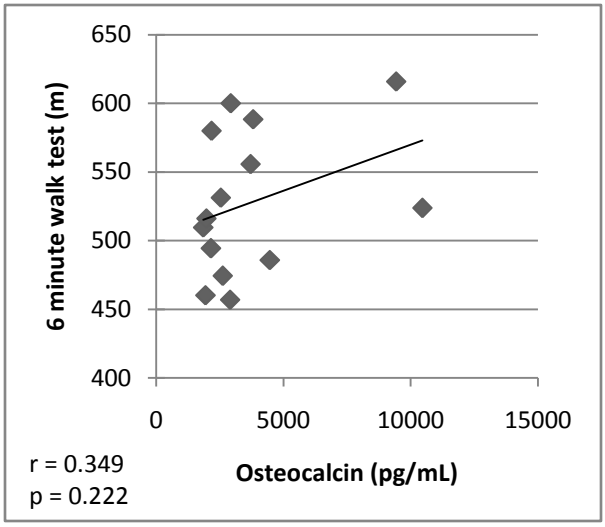
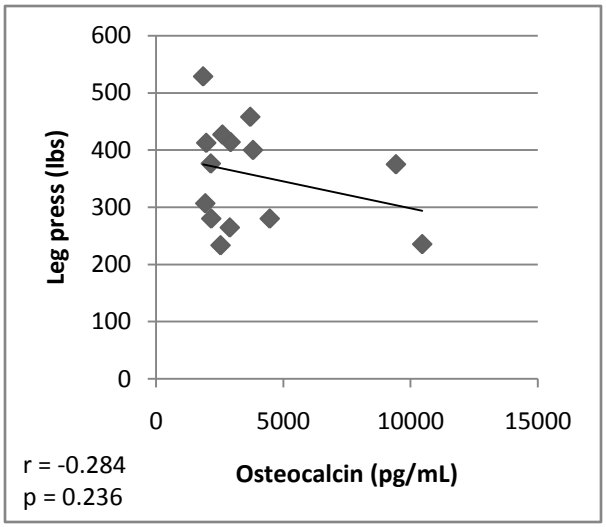


**Figure 13: Relationships between osteocalcin and other outcomes at baseline and post (n=7 ; N=14)**

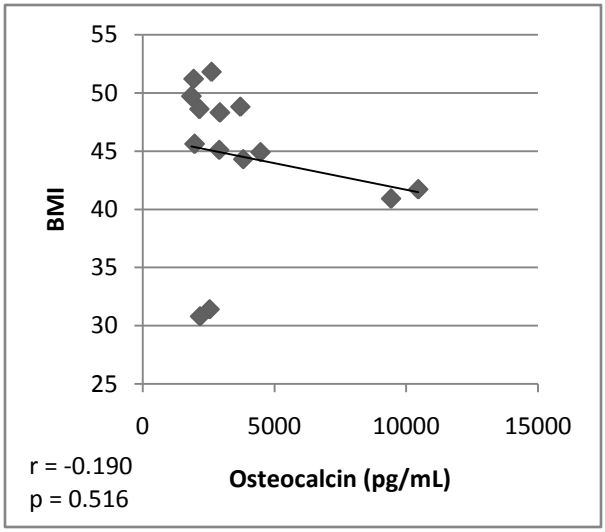
13A




13B




13C



# Appendix F:

# Bivariate correlations between delta values of cytokines and assessment measures

Table 25: Bivariate correlations between delta values of cytokines and assessment measures

| | **6 minute walk** | **Leg press** | **BMI** |
|---|---|---|---|
| Leptin | r = 0.081 | r = -0.585 | r = -0.429 |
| | p = 0.863 | p = 0.168 | p = 0.337 |
| IL-1β | r = 0.487 | r = 0.479 | r = -0.243 |
| | p = 0.268 | p = 0.277 | p = 0.600 |
| MCP-1 | r = 0.053 | r = 0.871 | r = 0.337 |
| | p = 0.910 | **p = 0.011** | p = 0.460 |
| IL-6 | r = 0.088 | r = 0.510 | r = 0.315 |
| | p = 0.852 | p = 0.242 | p = 0.492 |
| TNFα | r = -0.129 | r = 0.712 | r = 0.500 |
| | p = 0.783 | p = 0.073 | p = 0.253 |
| Osteonectin | r = 0.361 | r = -0.062 | r = -0.429 |
| | p = 0.426 | p = 0.864 | p = 0.337 |
| Osteocalcin | r = -0.725 | r = 0.088 | r = 0.495 |
| | p = 0.065 | p = 0.851 | p = 0.259 |

* Correlation is significant at the 0.05 level (2 tailed)

# Appendix G:

# ENCOURAGEing Start safety and emergency response document

# SAFETY AND EMERGENCY RESPONSE

The ENCOURAGEing Start will take place within the University of Manitoba's Active Living Centre (ALC) in the following areas:

- Main gym
- Applied Research Centre
- Multipurpose rooms

Below are existing safety and emergency response procedures for these areas, followed by specific considerations for the program.

## 1. Existing Safety Procedures for Active Living Centre patrons

Bison Active Living has the following procedures in place to respond to incidents and emergencies in the gym area of the Active Living Centre. All material in this indented section are the procedures given to Fitness Attendants, two of which are always present in the Active Living Centre.

Injury Assessment

An initial assessment is required for the staff member to determine whether the injury is a major or minor one.

**Minor Incidents**

1. Small cuts, bruises and scrapes.
2. Strains, sprains, bee/wasp stings, heat exhaustion, and dental injuries – although these injuries are usually dealt with easily, there is a potential risk for serious complications to arise

**Procedures for Minor Incidents**

1. When appropriate administer proper first aid using supplies in your first aid kit. Ice bags can be requested through the customer service desk.
2. Customers/members/participants can decide whether to continue or not.
3. Regardless of injury severity, an incident report must be completed accurately and signed by the [fitness attendant]. The incident forms can be found at the customer service desk and given to the [fitness attendant's] direct supervisor.

**Major Incidents**

1. Do not move the injured person (unless faced with a life-threatening situation).
2. Administer first aid to prevent further injury. An AED is available in the Active Living Centre.
3. If a phone is available, call 911 (4911 from a university phone) and call Security Services at 555 immediately after (or use the red phones). Time is crucial when dealing with life or death situations. You must notify security services so they can better direct EMS to the scene.
4. Help create an easy access/egress path for EMS, remain calm, and secure the surroundings.
5. Wait until EMS arrives and work in conjunction with EMS and security services.
6. An incident report must be completed accurately and signed by the staff member.

<u>Emergency Procedures</u>

**Fire Drill**

Work to ensure that all members have cleared the Active Living Centre in a calm and timely manner by sweeping all areas on level 300 and 400. Instruct members to access the first available exit and NOT to use the locker room prior to exiting the building. Assist individuals with mobility limitations to the nearest stairwell and inform the CFW of their exact location.

**First Aid Supplies**

Supplies are kept on the 3rd floor ALC at the fitness attendant desk and in the storage closet (319 ALC). These two locations are checked and replenished on a weekly basis. Additional supplies are kept at the customer service desk and in the Applied Research Centre.

**Automated External Defibrillators**

Our facilities are equipped with Automated External Defibrillators in all buildings (there are 5 in the ALC specifically). The fitness attendants will activate EMS before using an AED.

**Emergency Red Phones**

Emergency red phones/boxes can be found on every floor of the ALC. These phones provide 2-way voice communication with the Security Services Department.

When a Code Blue or Emergency Red Phone is activated, security guards will be priority dispatched to the location and in most cases will arrive in under 2 minutes. While the security guards are en route, the dispatcher continues to have 2-way voice communication with the caller.

**2. Questionnaire responses**

The Patient Health Questionnaire (PHQ-9) used in our questionnaire package asks participants questions about their mental health. It is a tool that can indicate clinical depression. If the total score is more than 20, staff should provide the participant with the Winnipeg Mental Health Resources pamphlet. The same guidelines apply if a participant circles higher than zero for question 9, regardless of overall score.

If a participant scores higher than zero for question 9, the staff should ask the participant whether they would like additional mental health resource information. If so, recommend one or both of:

- Crisis Response Centre: 24 hour help line 204-786-8686
- Manitoba Suicide Line 1-877-435-7170

Unfortunately, the clinic at University Centre does not guarantee the provision of assistance in a timely manner.

**3. Considerations for the ENCOURAGEing Start Program**

Applied Research Centre

The risk of an adverse event while in the Applied Research Centre boardroom is low as the area will be used for discussion rather than physical activity. Nonetheless, staff are familiar with the Standard Operating Procedures document for this area, which includes directions for AED and first aid supplies. The document can be found at http://umanitoba.ca/faculties/kinrec/research/arc.html

Staff are reminded to keep their access card with them at all times, as re-entry into 240 ALC necessitates a card.

Active Living Centre

Weight limits exist on the following exercise equipment:

Precor treadmills, bikes, stairclimbers and ellipticals: 350 lb

| | |
|---|---|
| Woodway Curve treadmill: | 400 lb run, 800 lb walk |
| Woodway 4Front treadmill: | 400 lb run, 800 lb walk |
| Concept 2 rowing ergometer: | 500 lb |
| TRX suspension trainer: | 350 lbs (strap) |
| | 500 lbs (ceiling mount) |

*Load testing was done in 2016 after an equipment failure in the multipurpose studio. All ceiling mounts have been fixed and confirmed to support 500 lbs – this enables more than one user per mount, but if used in the bariatric class there must be a one strap to one mount ratio.*

Staff

The BHLP will be conducted by both a CSEP Certified Exercise Physiologist (CSEP-CEP) and a student with experience in fitness assessment and lifestyle counseling. A CSEP-CEP's clientele can include individuals with functional limitations or disabilities associated with musculoskeletal, cardiopulmonary, metabolic, neuromuscular, and ageing conditions.

With a pilot project intake of twelve participants and two staff members, there will be at largest a 6:1 participant:instructor ratio. This ratio is classified by most commercial gyms as "small group training", and is more participant-focused than several exercise-based chronic disease programs currently operating in Winnipeg including the Reh-Fit Centre's Healthier Weigh and cardiac rehabilitation programs.

Waiver

Participants of the BHLP are subject to the same screening tools as other attendees of the ALC. Participants will complete a Physical Activity Readiness Questionnaire (PAR-Q). Positive responses do not automatically rule out the participant from ENCOURAGEing Start; the participant will discuss their health status with the CSEP-CEP to clarify their responses and determine an appropriate course of action which may include a Medical Information Release form to be completed by the participant's physician.

A summary of PAR-Q data will be kept on the instructor's person during exercise sessions for quick reference in case of emergency. The PAR-Q will be provided to the Active Living Centre as the part of the standard process to create a membership card and gain access to the facility.

As outlined by the American College of Sports Medicine, absolute contraindications to participation include uncontrolled severe hypertension, acute cardiac event, unstable angina, uncontrolled dysrhythmias, symptomatic severe aortic stenosis, uncontrolled symptomatic heart failure, acute pulmonary embolus, acute myocarditis, suspected or known dissecting aneurysm, and acute systemic infection. If a participant presents with any of the above symptoms, they will be informed that they cannot participate in ENCOURAGEing Start at that time.

Exercise Intensity

Sedentary individuals, particularly those who are overweight, beginning a physical activity program must do so in a gradual manner to avoid an increased risk of musculoskeletal injuries. The two BHLP instructors will assist participants in customizing their exercise intensity during group workouts and help participants learn how to self-select an intensity when exercising on their own. Exercise intensity during group workouts will be controlled through modification rather than modality, where possible.

## References


1. WHO | Obesity. *WHO*. 2014. https://www.who.int/topics/obesity/en/. Accessed November 17, 2018.

2. Navaneelan T, Janz T. Adjusting the Scales: Obesity in the Canadian population after correcting for respondent bias. *Stat Canada Heal a Glance*. 2014;(82):1-10. doi:10.1787/health_glance-2009-en

3. StatisticsCanada. Canadian Community Health Survey, 2000/01. *Dly*. 2002:2013-2016.

4. Tran BX, Nair A V, Kuhle S, Ohinmaa A, Veugelers PJ. Cost analyses of obesity in Canada: scope, quality, and implications. *Cost Eff Resour Alloc*. 2013;11(1):3. doi:10.1186/1478-7547-11-3

5. Lavie CJ, Arena R, Alpert MA, Milani R V., Ventura HO. Management of cardiovascular diseases in patients with obesity. *Nat Rev Cardiol*. 2017;15(1):45-56. doi:10.1038/nrcardio.2017.108

6. CDC-n.a.-2. Defining adult overweight and obesity. *Centers Dis Control Prev*. 2012:8-9. https://www.cdc.gov/obesity/adult/defining.html. Accessed May 26, 2019.

7. Ortega FB, Sui X, Lavie CJ, Blair SN. Body Mass Index, the Most Widely Used But Also Widely Criticized Index. *Mayo Clin Proc*. 2016;91(4):443-455. doi:10.1016/j.mayocp.2016.01.008

8. WHO | Obesity. *WHO*. 2014. https://www.who.int/topics/obesity/en/. Accessed March 24, 2019.

9. Smith GI, Mittendorfer B, Klein S. Metabolically healthy obesity: Facts and fantasies. *J Clin Invest*. 2019;129(10):3978-3989. doi:10.1172/JCI129186

10. Hamer M, Stamatakis E. Metabolically Healthy Obesity and Risk of All-Cause and Cardiovascular Disease Mortality. *J Clin Endocrinol Metab*. 2012;97(7):2482-2488. doi:10.1210/jc.2011-3475

11. Ortega FB, Cadenas-Sanchez C, Migueles JH, et al. Role of Physical Activity and Fitness in the Characterization and Prognosis of the Metabolically Healthy Obesity Phenotype: A Systematic Review and Meta-analysis. *Prog Cardiovasc Dis*. 2018;61(2):190-205. doi:10.1016/j.pcad.2018.07.008

12. Nguyen NT, Magno CP, Lane KT, Hinojosa MW, Lane JS. Association of Hypertension, Diabetes, Dyslipidemia, and Metabolic Syndrome with Obesity: Findings from the National Health and Nutrition Examination Survey, 1999 to 2004. *J Am Coll Surg*. 2008;207(6):928-934. doi:10.1016/j.jamcollsurg.2008.08.022

13. Cao JJ. Effects of obesity on bone metabolism. *J Orthop Surg Res*. 2011;6(1):30. doi:10.1186/1749-799X-6-30

14. Kopelman P. Health risks associated with overweight and obesity. *Obes Rev*. 2007;8(s1):13-17. doi:10.1111/j.1467-789X.2007.00311.x

15. Mitchell AB, Cole JW, McArdle PF, et al. Obesity Increases Risk of Ischemic Stroke in Young Adults. *Stroke*. 2015;46(6):1690-1692. doi:10.1161/STROKEAHA.115.008940

16. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation*. 1983;67(5):968-977. http://www.ncbi.nlm.nih.gov/pubmed/6219830. Accessed April 1, 2019.

17. Lavie CJ, Alpert MA, Arena R, Mehra MR, Milani R V., Ventura HO. Impact of Obesity and the Obesity Paradox on Prevalence and Prognosis in Heart Failure. *JACC Hear Fail*. 2013;1(2):93-102. doi:10.1016/j.jchf.2013.01.006

18. de Wit L, Luppino F, van Straten A, Penninx B, Zitman F, Cuijpers P. Depression and obesity: A meta-analysis of community-based studies. *Psychiatry Res*. 2010;178(2):230-235. doi:10.1016/j.psychres.2009.04.015

19. Peeters A, Barendregt JJ, Willekens F, Mackenbach JP, Mamun A Al, Bonneux L. Obesity in Adulthood and Its Consequences for Life Expectancy: A Life-Table Analysis. *Ann Intern Med*. 2003;138(1):24. doi:10.7326/0003-4819-138-1-200301070-00008

20. Yarborough CM, III, Brethauer S, et al. ACOEM Guidance Statement: Obesity in the Workplace: Impact, Outcomes, and Recommendations. *J Occup Environ Med*. 2018;60(1):97. doi:10.1097/JOM.0000000000001220

21. Williams EP, Mesidor M, Winters K, Dubbert PM, Wyatt SB. Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem. *Curr Obes Rep*. 2015;4(3):363-370. doi:10.1007/s13679-015-0169-4

22. Newton S, Braithwaite D, Akinyemiju TF. Socio-economic status over the life course and obesity: Systematic review and meta-analysis. *PLoS One*. 2017;12(5):e0177151. doi:10.1371/journal.pone.0177151

23. *OBESITY IN CANADA*. https://secure.cihi.ca/free_products/Obesity_in_canada_2011_en.pdf. Accessed March 24, 2019.

24. Ravussin E, Lillioja S, Knowler WC, et al. Reduced Rate of Energy Expenditure as a Risk Factor for Body-Weight Gain. *N Engl J Med*. 1988;318(8):467-472. doi:10.1056/NEJM198802253180802

25. StatisticsCanada. Leisure-time physical activity. https://www150.statcan.gc.ca/n1/pub/82-229-x/2009001/deter/lpa-eng.htm. Published 2017. Accessed April 1, 2019.

26. Church TS, Thomas DM, Tudor-Locke C, et al. Trends over 5 Decades in U.S. Occupation-Related Physical Activity and Their Associations with Obesity. Lucia A, ed. *PLoS One*. 2011;6(5):e19657. doi:10.1371/journal.pone.0019657

27. Ekelund U, Steene-Johannessen J, Brown WJ, et al. Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. *Lancet*. 2016;388(10051):1302-1310. doi:10.1016/S0140-6736(16)30370-1

28. McAuley P, Blaha M, Keteyian S, et al. Fitness, Fatness, and Mortality: The FIT (Henry Ford Exercise Testing) Project- ClinicalKey. *Am J Med*. 2016;129(9):960-965. doi:10.1016/j.amjmed.2016.04.007

29. Lee C Do, Blair SN, Jackson AS. Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. *Am J Clin Nutr*. 1999;69(3):373-380. doi:10.1093/ajcn/69.3.373

30. Baillot A, Audet M, Baillargeon JP, et al. Impact of physical activity and fitness in class II and III obese individuals: a systematic review. *Obes Rev*. 2014;15(9):721-739. doi:10.1111/obr.12171

31. Chlif M, Temfemo A, Keochkerian D, Choquet D, Chaouachi A, Ahmaidi S. Advanced Mechanical Ventilatory Constraints During Incremental Exercise in Class III Obese Male Subjects. *Respir Care*. 2015;60(4):549-560. doi:10.4187/respcare.03206

32. Ul-Haq Z, Mackay DF, Fenwick E, Pell JP. Meta-analysis of the association between body mass index and health-related quality of life among adults, assessed by the SF-36. *Obesity*. 2013;21(3):E322-E327. doi:10.1002/oby.20107

33. Marks R. Obesity Profiles with Knee Osteoarthritis: Correlation with Pain, Disability, Disease Progression*. *Obesity*. 2007;15(7):1867-1874. doi:10.1038/oby.2007.221

34. Weinsier RL, Schutz Y, Bracco D. Reexamination of the relationship of resting metabolic rate to fat-free mass and to the metabolically active components of fat-free mass in humans. *Am J Clin Nutr*. 1992;55(4):790-794. doi:10.1093/ajcn/55.4.790

35. Swift DL, Johannsen NM, Lavie CJ, Earnest CP, Church TS. The role of exercise and physical activity in weight loss and maintenance. *Prog Cardiovasc Dis*. 2014;56(4):441-447. doi:10.1016/j.pcad.2013.09.012

36. Curioni CC, Lourenço PM. Long-term weight loss after diet and exercise: a systematic review. *Int J Obes*. 2005;29(10):1168-1174. doi:10.1038/sj.ijo.0803015

37. Lifestyle Weight-Loss Intervention Outcomes in Overweight and Obese Adults with Type 2 Diabetes: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. *J Acad Nutr Diet*. 2015;115(9):1447-1463. doi:10.1016/J.JAND.2015.02.031

38. Elfhag K, Rossner S. Who succeeds in maintaining weight loss? A conceptual review of factors associated with weight loss maintenance and weight regain. *Obes Rev*. 2005;6(1):67-85. doi:10.1111/j.1467-789X.2005.00170.x

39. Wood L. The $72 Billion Weight Loss & Diet Control Market in the United States, 2019-2023 - Why Meal Replacements are Still Booming, but Not OTC Diet Pills - ResearchAndMarkets.com | Business Wire. BusinessWire. https://www.businesswire.com/news/home/20190225005455/en/72-Billion-Weight-Loss-Diet-Control-Market. Published 2019. Accessed May 12, 2019.

40. Bray G. Contemporary diagnosis and management of obesity. 2003. https://scholar.google.com/scholar_lookup?hl=en&publication_year=1998&author=GA.+Bray&title=Contemporary+Diagnosis+and+Management+of+Obesity. Accessed May 12, 2019.

41. Guidelines (2013) for managing overweight and obesity in adults. *Obesity*. 2014;22(S2):i-xvi. doi:10.1002/oby.20818

42. Gudzune KA, Doshi RS, Mehta AK, et al. Efficacy of Commercial Weight-Loss Programs. *Ann Intern Med*. 2015;162(7):501. doi:10.7326/M14-2238

43. Arguin H, Dionne IJ, Sénéchal M, et al. Short- and long-term effects of continuous versus intermittent restrictive diet approaches on body composition and the metabolic profile in overweight and obese postmenopausal women. *Menopause J North Am Menopause Soc*. 2012;19(8):870-876. doi:10.1097/gme.0b013e318250a287

44. Horne BD, Muhlestein JB, Anderson JL. Health effects of intermittent fasting: hormesis or harm? A systematic review. *Am J Clin Nutr*. 2015;102(2):464-470. doi:10.3945/ajcn.115.109553

45. Davis CS, Clarke RE, Coulter SN, et al. Intermittent energy restriction and weight loss: a systematic review. *Eur J Clin Nutr*. 2016;70(3):292-299. doi:10.1038/ejcn.2015.195

46. Gibson AA, Seimon R V., Lee CMY, et al. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obes Rev*. 2015;16(1):64-76. doi:10.1111/obr.12230

47. Yancy WS, Almirall D, Maciejewski ML, Kolotkin RL, McDuffie JR, Westman EC. Effects of two weight-loss diets on health-related quality of life. *Qual Life Res*. 2009;18(3):281-289. doi:10.1007/s11136-009-9444-8

48. Sumithran P, Proietto J. Ketogenic diets for weight loss: A review of their principles, safety and efficacy. *Obes Res Clin Pract*. 2008;2(1):1-13. doi:10.1016/J.ORCP.2007.11.003

49. Bueno NB, de Melo ISV, de Oliveira SL, da Rocha Ataide T. Very-low-carbohydrate ketogenic diet *v.* low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. *Br J Nutr*. 2013;110(7):1178-1187. doi:10.1017/S0007114513000548

50. Santos FL, Esteves SS, da Costa Pereira A, Yancy Jr WS, Nunes JPL. Systematic review and meta-analysis of clinical trials of the effects of low carbohydrate diets on cardiovascular risk factors. *Obes Rev*. 2012;13(11):1048-1066. doi:10.1111/j.1467-789X.2012.01021.x

51. Fothergill E, Guo J, Howard L, et al. Persistent metabolic adaptation 6 years after "The Biggest Loser" competition. *Obesity*. 2016;24(8):1612-1619. doi:10.1002/oby.21538

52. NHLBI Obesity Education Initiative. *The Practical Guide Identification, Evaluation, and Treatment of Overweight and Obesity in Adults NHLBI Obesity Education Initiative*.; 2000. https://www-nhlbi-nih-gov.uml.idm.oclc.org/files/docs/guidelines/prctgd_c.pdf. Accessed May 20, 2019.

53. Abdeen G, le Roux C. Mechanism Underlying the Weight Loss and Complications of Roux-en-Y Gastric Bypass. Review. *Obes Surg*. 2016;26(2):410-421. doi:10.1007/s11695-015-1945-7

54. van Rutte PWJ, Smulders JF, de Zoete JP, Nienhuijs SW. Outcome of sleeve gastrectomy as a primary bariatric procedure. *Br J Surg*. 2014;101(6):661-668. doi:10.1002/bjs.9447

55. Spaniolas K, Kasten KR, Brinkley J, et al. The Changing Bariatric Surgery Landscape in the USA. *Obes Surg*. 2015;25(8):1544-1546. doi:10.1007/s11695-015-1764-x

56. Maciejewski ML, Arterburn DE, Van Scoyoc L, et al. Bariatric Surgery and Long-term Durability of Weight Loss. *JAMA Surg*. 2016;151(11):1046-1055. doi:10.1001/jamasurg.2016.2317

57. Thomas EL, Parkinson JR, Frost GS, et al. The Missing Risk: MRI and MRS Phenotyping of Abdominal Adiposity and Ectopic Fat. *Obesity*. 2012;20(1):76-87. doi:10.1038/oby.2011.142

58. Klein S, Fontana L, Young VL, et al. Absence of an Effect of Liposuction on Insulin Action and Risk Factors for Coronary Heart Disease. *N Engl J Med*. 2004;350(25):2549-2557. doi:10.1056/NEJMoa033179

59. Blüher M. Adipose tissue dysfunction contributes to obesity related metabolic diseases. *Best Pract Res Clin Endocrinol Metab*. 2013;27(2):163-177. doi:10.1016/j.beem.2013.02.005

60. Kralisch S, Bluher M, Paschke R, Stumvoll M, Fasshauer M. Adipokines and adipocyte targets in the future management of obesity and the metabolic syndrome. *Mini Rev Med Chem*. 2007;7(1):39-45. http://www.ncbi.nlm.nih.gov/pubmed/17266636. Accessed April 29, 2019.

61. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: Action, regulation and association to insulin sensitivity. *Obes Rev*. 2005;6(1):13-21. doi:10.1111/j.1467-789X.2005.00159.x

62. Pedersen BK, Steensberg A, Fischer C, et al. *Searching for the Exercise Factor: Is IL-6 a Candidate?*

63. Fitzgibbons TP, Czech MP. Epicardial and perivascular adipose tissues and their influence on cardiovascular disease: basic mechanisms and clinical associations. *J Am Heart Assoc*. 2014;3(2):e000582. doi:10.1161/JAHA.113.000582

64. Messina G, Valenzano A, Moscatelli F, et al. Role of Autonomic Nervous System and Orexinergic System on Adipose Tissue. *Front Physiol*. 2017;8:137. doi:10.3389/fphys.2017.00137

65. Virtanen KA, Lidell ME, Orava J, et al. Functional Brown Adipose Tissue in Healthy Adults. *N Engl J Med*. 2009;360(15):1518-1525. doi:10.1056/NEJMoa0808949

66. Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. doi:10.1259/bjr/38447238

67. Seidell JC, Oosterlee A, Deurenberg P, Hautvast JG, Ruijs JH. Abdominal fat depots measured with computed tomography: effects of degree of obesity, sex, and age. *Eur J Clin Nutr*. 1988;42(9):805-815. http://www.ncbi.nlm.nih.gov/pubmed/3181112. Accessed May 7, 2019.

68. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous Adipose Tissue Releases Interleukin-6, But Not Tumor Necrosis Factor-α, *in Vivo* [1]. *J Clin Endocrinol Metab*. 1997;82(12):4196-4200. doi:10.1210/jcem.82.12.4450

69. Rakotoarivelo V, Lacraz G, Mayhue M, et al. Inflammatory Cytokine Profiles in Visceral and Subcutaneous Adipose Tissues of Obese Patients Undergoing Bariatric Surgery Reveal Lack of Correlation With Obesity or Diabetes. *EBioMedicine*. 2018;30:237-247. doi:10.1016/J.EBIOM.2018.03.004

70. Gavrilova O, Marcus-Samuels B, Graham D, et al. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest*. 2000;105(3):271-278. doi:10.1172/JCI7901

71. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994;372(6505):425-432. doi:10.1038/372425a0

72. Ahima RS, Flier JS. Leptin. *Annu Rev Physiol*. 2000;62(1):413-437. doi:10.1146/annurev.physiol.62.1.413

73. Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA*. 1999;282(16):1568-1575. http://www.ncbi.nlm.nih.gov/pubmed/10546697. Accessed April 29, 2019.

74. Koch CE, Lowe C, Pretz D, Steger J, Williams LM, Tups A. High-Fat Diet Induces Leptin Resistance in Leptin-Deficient Mice. *J Neuroendocrinol*. 2014;26(2):58-67. doi:10.1111/jne.12131

75. Paz-Filho G, Mastronardi CA, Licinio J. Leptin treatment: Facts and expectations. *Metabolism*. 2015;64(1):146-156. doi:10.1016/j.metabol.2014.07.014

76. Knuth ND, Johannsen DL, Tamboli RA, et al. Metabolic adaptation following massive weight loss is related to the degree of energy imbalance and changes in circulating leptin. *Obesity*. 2014;22(12):n/a-n/a. doi:10.1002/oby.20900

77. Korner J, Conroy R, Febres G, et al. Randomized double-blind placebo-controlled study of leptin administration after gastric bypass. *Obesity*. 2013;21(5):951-956. doi:10.1002/oby.20433

78. Romacho T, Elsen M, Röhrborn D, Eckel J. Adipose tissue and its role in organ crosstalk. *Acta Physiol*. 2014;210(4):733-753. doi:10.1111/apha.12246

79. Lehr S, Hartwig S, Sell H. Adipokines: A treasure trove for the discovery of biomarkers for metabolic disorders. *PROTEOMICS - Clin Appl*. 2012;6(1-2):91-101. doi:10.1002/prca.201100052

80. Dutheil F, Gordon BA, Naughton G, et al. Cardiovascular risk of adipokines: a review. *J Int Med Res*. 2018;46(6):2082-2095. doi:10.1177/0300060517706578

81. TRUJILLO ME, SCHERER PE. Adiponectin - journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *J Intern Med*. 2005;257(2):167-175. doi:10.1111/j.1365-2796.2004.01426.x

82. Hafida S, Mirshahi T, Nikolajczyk BS. The impact of bariatric surgery on inflammation: quenching the fire of obesity? *Curr Opin Endocrinol Diabetes Obes*. 2016;23(5):373-378. doi:10.1097/MED.0000000000000277

83. Febbraio MA. Role of interleukins in obesity: implications for metabolic disease. *Trends Endocrinol Metab*. 2014;25(6):312-319. doi:10.1016/j.tem.2014.02.004

84. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Metab*. 2001;280(5):E745-E751. doi:10.1152/ajpendo.2001.280.5.E745

85. Krist J, Wieder K, Klöting N, et al. Effects of Weight Loss and Exercise on Apelin Serum Concentrations and Adipose Tissue Expression in Human Obesity. *Obes Facts*. 2013;6(1):57-69. doi:10.1159/000348667

86. Handisurya A, Riedl M, Vila G, et al. Serum Vaspin Concentrations in Relation to Insulin Sensitivity Following RYGB-Induced Weight Loss. *Obes Surg*. 2010;20(2):198-203. doi:10.1007/s11695-009-9882-y

87. Howren MB, Lamkin DM, Suls J. Associations of Depression With C-Reactive Protein, IL-1, and IL-6: A Meta-Analysis. *Psychosom Med*. 2009;71(2):171-186. doi:10.1097/PSY.0b013e3181907c1b

88. Moreno M, Moreno-Navarrete JM, Serrano M, et al. Circulating Irisin Levels Are Positively Associated with Metabolic Risk Factors in Sedentary Subjects. Tauler P, ed. *PLoS One*. 2015;10(4):e0124100. doi:10.1371/journal.pone.0124100

89. Kos K, Wong S, Tan B, et al. Regulation of the Fibrosis and Angiogenesis Promoter SPARC/Osteonectin in Human Adipose Tissue by Weight Change, Leptin, Insulin, and Glucose. *Diabetes*. 2009;58(8):1780-1788. doi:10.2337/db09-0211

90. Nie J, Sage EH. SPARC Inhibits Adipogenesis by Its Enhancement of β-Catenin Signaling. *J Biol Chem*. 2009;284(2):1279-1290. doi:10.1074/jbc.M808285200

91. Pavlou KN, Steffee WP, Lerman RH, Burrows BA. Effects of dieting and exercise on lean body mass, oxygen uptake, and strength. *Med Sci Sports Exerc*. 1985;17(4):466-471. http://www.ncbi.nlm.nih.gov/pubmed/4033403. Accessed June 1, 2019.

92. Bouassida A, Zalleg D, Bouassida S, et al. Leptin, its implication in physical exercise and training: a short review. *J Sports Sci Med*. 2006;5(2):172-181. http://www.ncbi.nlm.nih.gov/pubmed/24259989. Accessed March 10, 2019.

93. Roca-Rivada A, Castelao C, Senin LL, et al. FNDC5/Irisin Is Not Only a Myokine but Also an Adipokine. *PLoS One*. 2013;8(4):e60563. doi:10.1371/journal.pone.0060563

94. Pandit R, Beerens S, Adan RAH. Role of leptin in energy expenditure: the hypothalamic perspective. *Am J Physiol Integr Comp Physiol*. 2017;312(6):R938-R947. doi:10.1152/ajpregu.00045.2016

95. Fedewa M V, Hathaway ED, Christie •, et al. The Effect of Chronic Exercise Training on Leptin: A Systematic Review and Meta-Analysis of Randomized Controlled Trials Key Points. *Sport Med*. 2018;48:1437-1450. doi:10.1007/s40279-018-0897-1

96. Monikh Kh, Kashef M, Azad A, Ghasemnian A. Effects of 6 weeks resistance training on Body Composition, serum Leptin and muscle strength in non-athletic men. *Q Horiz Med Sci*. 2015;21(2):135-140. doi:10.18869/acadpub.hms.21.2.135

97. Beckman LM, Beckman TR, Earthman CP. Changes in Gastrointestinal Hormones and Leptin after Roux-en-Y Gastric Bypass Procedure: A Review. *J Am Diet Assoc*. 2010;110(4):571-584. doi:10.1016/j.jada.2009.12.023

98. Rossi S, Sacchetti L, Napolitano F, et al. Interleukin-1  Causes Anxiety by Interacting with the Endocannabinoid System. *J Neurosci*. 2012;32(40):13896-13905. doi:10.1523/JNEUROSCI.1515-12.2012

99. Cannon JG, Fielding RA, Fiatarone MA, Orencole SF, Dinarello CA, Evans WJ. Increased interleukin 1 beta in human skeletal muscle after exercise. *Am J Physiol Integr Comp Physiol*. 1989;257(2):R451-R455. doi:10.1152/ajpregu.1989.257.2.R451

100. Calle MC, Fernandez ML. Effects of resistance training on the inflammatory response. *Nutr Res Pract*. 2010;4(4):259-269. doi:10.4162/nrp.2010.4.4.259

101. Moschen AR, Molnar C, Enrich B, Geiger S, Ebenbichler CF, Tilg H. Adipose and Liver Expression of Interleukin (IL)-1 Family Members in Morbid Obesity and Effects of Weight Loss. *Mol Med*. 2011;17(7-8):840-845. doi:10.2119/molmed.2010.00108

102. Boucher J, Masri B, Daviaud D, et al. Apelin, a Newly Identified Adipokine Up-Regulated by Insulin and Obesity. *Endocrinology*. 2005;146(4):1764-1771. doi:10.1210/en.2004-1427

103. Lee DK, Cheng R, Nguyen T, et al. Characterization of Apelin, the Ligand for the APJ Receptor. *J Neurochem*. 2001;74(1):34-41. doi:10.1046/j.1471-4159.2000.0740034.x

104. Vinel C, Lukjanenko L, Batut A, et al. The exerkine apelin reverses age-associated sarcopenia. *Nat Med*. 2018;24(9):1360-1371. doi:10.1038/s41591-018-0131-6

105. Gilbert JS. From apelin to exercise: emerging therapies for management of hypertension in pregnancy. *Hypertens Res*. 2017;40(6):519-525. doi:10.1038/hr.2017.40

106. Kechyn S, Barnes G, Howard L. Assessing dynamic changes in plasma apelin concentration in response to maximal exercise in man. In: *4.1 Clinical Respiratory Physiology, Exercise and Functional Imaging*. Vol 46. European Respiratory Society; 2015:PA2316. doi:10.1183/13993003.congress-2015.PA2316

107. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res*. 2009;29(6):313-326. doi:10.1089/jir.2008.0027

108. Becker D, Seul M, Lindemann S. *Strenuous Physical Exercise Induces Monocyte Chemoattractant Protein-1 Release in Patients with Coronary Artery Disease*. Vol 4.; 2005. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.523.8114&rep=rep1&type=pdf. Accessed April 21, 2019.

109. Lucotti P, Monti LD, Setola E, et al. Aerobic and resistance training effects compared to aerobic training alone in obese type 2 diabetic patients on diet treatment. *Diabetes Res Clin Pract*. 2011;94(3):395-403. doi:10.1016/j.diabres.2011.08.002

110. Panee J. Monocyte Chemoattractant Protein 1 (MCP-1) in obesity and diabetes. *Cytokine*. 2012;60(1):1-12. doi:10.1016/j.cyto.2012.06.018

111. Chen H, Qian L, Lv Q, Yu J, Wu W, Qian H. Change in gut microbiota is correlated with alterations in the surface molecule expression of monocytes after Roux-en-Y gastric bypass surgery in obese type 2 diabetic patients. *Am J Transl Res*. 2017;9(3):1243-1254. http://www.ncbi.nlm.nih.gov/pubmed/28386350. Accessed April 21, 2019.

112. Monte S V., Caruana JA, Ghanim H, et al. Reduction in endotoxemia, oxidative and inflammatory stress, and insulin resistance after Roux-en-Y gastric bypass surgery in patients with morbid obesity and type 2 diabetes mellitus. *Surgery*. 2012;151(4):587-593. doi:10.1016/j.surg.2011.09.038

113. Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exerc Immunol Rev*. 2002;8:6-48. http://www.ncbi.nlm.nih.gov/pubmed/12690937. Accessed April 21, 2019.

114. Stolzman S, Bement MH. Inflammatory Markers in Pediatric Obesity. *ICAN Infant, Child, Adolesc Nutr*. 2012;4(5):297-302. doi:10.1177/1941406412459344

115. Son JS, Kim JH, Kim H-J, et al. Effect of resistance ladder training on sparc expression in skeletal muscle of hindlimb immobilized rats. *Muscle Nerve*. 2016;53(6):951-957. doi:10.1002/mus.24940

116. Aoi W, Naito Y, Takagi T, et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut*. 2013;62(6):882-889. doi:10.1136/gutjnl-2011-300776

117. Kanazawa I, Tanaka S, Sugimoto T. The Association Between Osteocalcin and Chronic Inflammation in Patients with Type 2 Diabetes Mellitus. *Calcif Tissue Int*. 2018;103(6):599-605. doi:10.1007/s00223-018-0460-y

118. Polgreen LE, Jacobs DR, Nathan BM, Steinberger J, Moran A, Sinaiko AR. gam. *Obesity*. 2012;20(11):2194-2201. doi:10.1038/oby.2012.108

119. Levinger I, Zebaze R, Jerums G, Hare DL, Selig S, Seeman E. The effect of acute exercise on undercarboxylated osteocalcin in obese men. *Osteoporos Int*. 2011;22(5):1621-1626. doi:10.1007/s00198-010-1370-7

120. Maser RE, James Lenhard M, Balagopal P (Babu), Kolm P, Peters MB. Effects of surgically induced weight loss by Roux-en-Y gastric bypass on osteocalcin. *Surg Obes Relat Dis*. 2013;9(6):950-955. doi:10.1016/J.SOARD.2012.08.006

121. Hee Park K, Zaichenko L, Brinkoetter M, et al. Circulating Irisin in Relation to Insulin Resistance and the Metabolic Syndrome. *J Clin Endocrinol Metab*. 2013;98(12):4899-4907. doi:10.1210/jc.2013-2373

122. Huh JY, Siopi A, Mougios V, Park KH, Mantzoros CS. Irisin in Response to Exercise in Humans With and Without Metabolic Syndrome. *J Clin Endocrinol Metab*. 2015;100(3):E453-E457. doi:10.1210/jc.2014-2416

123. Blizzard LeBlanc DR, Rioux B V, Pelech C, et al. Exercise-induced irisin release as a determinant of the metabolic response to exercise training in obese youth: the EXIT trial. *Physiol Rep*. 2017;5(23). doi:10.14814/phy2.13539

124. Buscemi S, Corleo D, Vasto S, et al. Factors associated with circulating concentrations of irisin in the general population cohort of the ABCD study. *Int J Obes*. 2018;42(3):398-404. doi:10.1038/ijo.2017.255

125. Lee Y, Heo Y, Choi J-H, et al. Association of Circulating Irisin Concentrations with Weight Loss after Roux-en-Y Gastric Bypass Surgery. *Int J Environ Res Public Health*. 2019;16(4):660. doi:10.3390/ijerph16040660

126. HM A-H. Exercise Training Prior to Weight Reduction Surgery: A Brief Review. *Obes Open Access*. 2017;2(1). doi:10.16966/2380-5528.116

127. *ASBS Public/Professional Education Committee*.; 2007. https://asmbs.org/app/uploads/2014/05/bariatric_surgery_postoperative_concerns1.pdf. Accessed May 20, 2019.

128. Kwok K. The impact of a pre-operative exercise program on patients awaiting bariatric surgery. 2019. https://mspace.lib.umanitoba.ca/handle/1993/33662. Accessed May 20, 2019.

129. Eldridge SM, Chan CL, Campbell MJ, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. *BMJ*. 2016;355:i5239. doi:10.1136/bmj.i5239

130. Zimlichman R, Shargorodsky M, Boaz M, et al. Determination of arterial compliance using blood pressure waveform analysis with the CR-2000 system: Reliability, repeatability, and establishment of normal values for healthy European population - The Seven European Sites Study (SESS). *Am J Hypertens*. 2005;18(1):65-71. doi:10.1016/j.amjhyper.2004.08.013

131. Canadian Society for Exercise Physiology. *Canadian Society for Exercise Physiology-Physical Activity Training for Health (CSEP-PATH)*.; 2013. https://www.worldcat.org/title/canadian-society-for-exercise-physiology-physical-activity-training-for-health-csep-path/oclc/857771182. Accessed March 12, 2019.

132. Department of Health US, Services H, for Disease Control C. *Assessment 30-Second Chair Stand*. www.cdc.gov/steadi. Accessed March 12, 2019.

133. Wilson CM, Kostsuca SR, Boura JA. Utilization of a 5-Meter Walk Test in Evaluating Self-selected Gait Speed during Preoperative Screening of Patients Scheduled for Cardiac Surgery. *Cardiopulm Phys Ther J*. 2013;24(3):36-43. http://www.ncbi.nlm.nih.gov/pubmed/23997690. Accessed March 12, 2019.

134. Chaston TB, Dixon JB, O'Brien PE. Changes in fat-free mass during significant weight loss: a systematic review. *Int J Obes*. 2007;31(5):743-750. doi:10.1038/sj.ijo.0803483

135. Baechle TR, Earle RW, National Strength & Conditioning Association (U.S.). *Essentials of Strength Training and Conditioning*. Human Kinetics; 2000.

136. King WC, Bond DS. The Importance of Preoperative and Postoperative Physical Activity Counseling in Bariatric Surgery. *Exerc Sport Sci Rev*. 2013;41(1):26-35. doi:10.1097/JES.0b013e31826444e0

137. Bohannon RW, Glenney SS. Minimal clinically important difference for change in comfortable gait speed of adults with pathology: a systematic review. *J Eval Clin Pract*. 2014;20(4):295-300. doi:10.1111/jep.12158

138. Duprez DA, Florea N, Zhong W, et al. Vascular and cardiac functional and structural screening to identify risk of future morbid events: Preliminary observations. *J Am Soc Hypertens*. 2011;5(5):401-409. doi:10.1016/j.jash.2011.05.001

139. Baillot A, Boissy P, Tousignant M, Langlois M-F. Feasibility and effect of in-home physical exercise training delivered via telehealth before bariatric surgery. *J Telemed Telecare*. 2017;23(5):529-535. doi:10.1177/1357633X16653511

140. Cassin S, Sockalingam S, Hawa R, et al. Psychometric properties of the patient health questionnaire (PHQ-9) as a depression screening tool for bariatric surgery candidates. *Psychosomatics*. 2013;54(4):352-358. doi:10.1016/j.psym.2012.08.010

141. Casanova C, Celli BR, Barria P, et al. The 6-min walk distance in healthy subjects: Reference standards from seven countries. *Eur Respir J*. 2011;37(1):150-156. doi:10.1183/09031936.00194909

142. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. *JAMA*. 1999;282(18):1737-1744. http://www.ncbi.nlm.nih.gov/pubmed/10568646. Accessed February 23, 2020.

143. Lafleur B, Lee W, Billhiemer D, Lockhart C, Liu J, Merchant N. Statistical methods for assays with limits of detection: Serum bile acid as a differentiator between patients with normal colons, adenomas, and colorectal cancer. *J Carcinog*. 2011;10. doi:10.4103/1477-3163.79681

144. Catenacci VA, Wyatt HR. The role of physical activity in producing and maintaining weight loss. *Nat Clin Pract Endocrinol Metab*. 2007;3(7):518-529. doi:10.1038/ncpendmet0554

145. Ribeiro AS, Tomeleri CM, Souza MF, et al. Effect of resistance training on C-reactive protein, blood glucose and lipid profile in older women with differing levels of RT experience. *Age (Dordr)*. 2015;37(6):109. doi:10.1007/s11357-015-9849-y

146. Baillot A, Vallée CA, Mampuya WM, et al. Effects of a Pre-surgery Supervised Exercise Training 1 Year After Bariatric Surgery: a Randomized Controlled Study. *Obes Surg*. 2018;28(4):955-962. doi:10.1007/s11695-017-2943-8

147. Huck CJ. Effects of Supervised Resistance Training on Fitness and Functional Strength in Patients Succeeding Bariatric Surgery. *J Strength Cond Res*. 2015;29(3):589-595. doi:10.1519/JSC.0000000000000667

148. Wollner S, Adair J, Jones D, Blackburn G. Preoperative Progressive Resistance Training Exercise for Bariatric Surgery Patients. 2010:7(5):11–13. https://bariatrictimes.com/preoperative-progressive-resistance-training-exercise-for-bariatric-surgery-patients/. Accessed March 13, 2020.

149. Daniels P, Burns RD, Brusseau TA, et al. Effect of a randomised 12-week resistance training programme on muscular strength, cross-sectional area and muscle quality in women having undergone Roux-en-Y gastric bypass. 2017. doi:10.1080/02640414.2017.1322217

150. Buford TW, Rossi SJ, Smith DB, et al. *A COMPARISON OF PERIODIZATION MODELS DURING NINE WEEKS WITH EQUATED VOLUME AND INTENSITY FOR STRENGTH*. Vol 21.; 2007.

151. Larsson UE, Reynisdottir S. The six-minute walk test in outpatients with obesity: Reproducibility and known group validity. *Physiother Res Int*. 2008;13(2):84-93. doi:10.1002/pri.398

152. Hansen N, Hardin E, Bates C, Bellatorre N, Eisenberg D. Preoperative change in 6-minute walk distance correlates with early weight loss after sleeve gastrectomy. *J Soc Laparoendosc Surg*. 2014;18(3). doi:10.4293/JSLS.2014.00383

153. Gallart-Aragón T, Fernández-Lao C, Castro-Martín E, Cantarero-Villanueva I, Cózar-Ibáñez A, Arroyo-Morales M. Health-Related Fitness Improvements in Morbid Obese Patients After Laparoscopic Sleeve Gastrectomy: a Cohort Study. *Obes Surg*. 2017;27(5):1182-1188. doi:10.1007/s11695-016-2427-2

154. Simmons MC, Errickson SP, Endress G, Kolotkin R, Ostbye T, Eisenson H. Changes In Cardiovascular Fitness Using 6-minute Walk Test For Obese Patients Participating In A Diet And Fitness Lifestyle Intervention. *Med Sci Sport Exerc*. 2011;43(Suppl 1):766. doi:10.1249/01.mss.0000402129.66983.be

155. Daniels P, Burns RD, Brusseau TA, et al. Effect of a randomised 12-week resistance training programme on muscular strength, cross-sectional area and muscle quality in women having undergone Roux-en-Y gastric bypass. *J Sports Sci*. 2018;36(5):529-535. doi:10.1080/02640414.2017.1322217

156. Baillot A, Mampuya WM, Comeau E, Méziat-Burdin A, Langlois MF. Feasibility and impacts of supervised exercise training in subjects with obesity awaiting bariatric surgery: A pilot study. *Obes Surg*. 2013;23(7):882-891. doi:10.1007/s11695-013-0875-5

157. Kapteyn A, Banks J, Hamer M, et al. What they say and what they do: Comparing physical activity across the USA, England and the Netherlands. *J Epidemiol Community Health*. 2018;72(6):471-476. doi:10.1136/jech-2017-209703

158. Lichtman SW, Pisarska K, Berman ER, et al. Discrepancy between Self-Reported and Actual Caloric Intake and Exercise in Obese Subjects. *N Engl J Med*. 1992;327(27):1893-1898. doi:10.1056/NEJM199212313272701

159. Ostlund RE, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab*. 1996;81(11):3909-3913. doi:10.1210/jcem.81.11.8923837

160. Silha J V, Krsek M, Skrha J V, Sucharda P, Nyomba LG, Murphy LJ. *Plasma Resistin, Adiponectin and Leptin Levels in Lean and Obese Subjects: Correlations with Insulin Resistance*. Vol 149. www.eje.org. Accessed February 15, 2020.

161. Daniel Castracane V, Kraemer RR, Franken MA, Kraemer GR, Gimpel T. Serum leptin concentration in women: Effect of age, obesity, and estrogen administration. *Fertil Steril*. 1998;70(3):472-477. doi:10.1016/S0015-0282(98)00187-3

162. Ahsan Kazmi, Abdus Sattar, Rizwan Hashim, Shahida Parveen Khan MY and FAK. Serum Leptin values in the healthy obese and non-obese subjects of Rawalpindi Citation metadata. *J Pakistan Med Assoc*. 2013;63(2).

163. Rostás I, Pótó L, Mátrai P, et al. In middle-aged and old obese patients, training intervention reduces leptin level: A meta-analysis. *PLoS One*. 2017;12(8):e0182801-e0182801. doi:10.1371/journal.pone.0182801

164. Segal KR, Landt M, Klein S. Relationship Between Insulin Sensitivity and Plasma Leptin Concentration in Lean and Obese Men. *Diabetes*. 1996;45(7):988-991. doi:10.2337/diab.45.7.988

165. Isidori AM, Caprio M, Strollo F, et al. Leptin and Androgens in Male Obesity: Evidence for Leptin Contribution to Reduced Androgen Levels [1]. *J Clin Endocrinol Metab*. 1999;84(10):3673-3680. doi:10.1210/jcem.84.10.6082

166. Terra X, Auguet T, Guiu-Jurado E, et al. Long-term changes in leptin, chemerin and ghrelin levels following different bariatric surgery procedures: Roux-en-Y gastric bypass and sleeve gastrectomy. *Obes Surg*. 2013;23(11):1790-1798. doi:10.1007/s11695-013-1033-9

167. Khanna D, Riechman S, Murano P. *EFFECTS OF EXERCISE AND DIET-INDUCED WEIGHT LOSS IN SEDENTARY OBESE WOMEN ON INFLAMMATORY MARKERS, RESISTIN AND VISFATIN A Dissertation*.; 2016. https://oaktrust.library.tamu.edu/bitstream/handle/1969.1/157012/KHANNA-DISSERTATION-2016.pdf?sequence=1&isAllowed=y. Accessed April 19, 2019.

168. SEKIYAMA KD, YOSHIBA M, THOMSON AW. Circulating proinflammatory cytokines (IL-1β, TNF-α, and IL-6) and IL-1 receptor antagonist (IL-1Ra) in fulminant hepatic failure and acute hepatitis. *Clin Exp Immunol*. 2008;98(1):71-77. doi:10.1111/j.1365-2249.1994.tb06609.x

169. Di Iorio A, Ferrucci L, Sparvieri E, et al. , y'. *Cytokine*. 2003;22(6):198-205. doi:10.1016/S1043-4666(03)00152-2

170. Kraemer RR, Chu H, Castracane VD. Leptin and Exercise. *Exp Biol Med*. 2002;227(9):701-708. doi:10.1177/153537020222700903

171. King WC, Bond DS. The Importance of Preoperative and Postoperative Physical Activity Counseling in Bariatric Surgery. *Exerc Sport Sci Rev*. 2013;41(1):26-35. doi:10.1097/JES.0b013e31826444e0

172. Hulens M, Vansant G, Claessens AL, Lysens R, Muls E. Predictors of 6-minute walk test results in lean, obese and morbidly obese women. *Scand J Med Sci Sport*. 2003;13(2):98-105. doi:10.1034/j.1600-0838.2003.10273.x

173. Dagogo-Jack S, Fanelli C, Paramore D, Brothers J, Landt M. *Plasma Leptin and Insulin Relationships in Obese and Nonobese Humans*.

174. Donini LM, Poggiogalle E, Mosca V, Pinto A, Brunani A, Capodaglio P. Disability Affects the 6-Minute Walking Distance in Obese Subjects (BMI>40 kg/m2). *PLoS One*. 2013;8(10). doi:10.1371/journal.pone.0075491

175. Stewart LK, Flynn MG, Campbell WW, et al. The influence of exercise training on inflammatory cytokines and C-reactive protein. *Med Sci Sports Exerc*. 2007;39(10):1714-1719. doi:10.1249/mss.0b013e31811ece1c

176. Journal B. Brazilian Journal of Biomotricity EFFECT OF A SINGLE BOUT GRADED EXERCISE ON THE CYTOKINES RESPONSE AND INSULIN RESISTANCE INDEX. *of Biomotricity*. 2011;5(2):132-140. http://www.redalyc.org/articulo.oa?id=93018957008. Accessed March 24, 2020.

177. Ullum H, Haahr PM, Diamant M, Palmo J, Halkjaer-Kristensen J, Pedersen BK. Bicycle exercise enhances plasma IL-6 but does not change IL-1α, IL-1β, IL-6, or TNF-α pre-mRNA in BMNC. *J Appl Physiol*. 1994;77(1):93-97. doi:10.1152/jappl.1994.77.1.93

178. Circulating Levels of Interleukin-1 Family Cytokines in Overweight Adolescents. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2821754/. Accessed March 24, 2020.

179. Cottam D, Fisher B, Ziemba A, et al. Tumor growth factor expression in obesity and changes in expression with weight loss: another cause of increased virulence and incidence of cancer in obesity. *SOARD*. 2010;6:538-541. doi:10.1016/j.soard.2010.04.011

180. Strandberg L, Mellström D, Ljunggren Ö, et al. IL6 and IL1B Polymorphisms are Associated With Fat Mass in Older Men: The MrOS Study Sweden. *Obesity*. 2008;16(3):710-713. doi:10.1038/oby.2007.95

181. Salamat KM, Azarbayjani MA, Yusof A, Dehghan F. The response of pre-inflammatory cytokines factors to different exercises (endurance, resistance, concurrent) in overweight men. *Alexandria J Med*. 2016;52(4):367-370. doi:10.1016/j.ajme.2015.12.007

182. Sams VG, Blackledge C, Wijayatunga N, et al. Effect of bariatric surgery on systemic and adipose tissue inflammation. *Surg Endosc*. 2016;30(8):3499-3504. doi:10.1007/s00464-015-4638-3

183. Kim CS, Park HS, Kawada T, et al. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. *Int J Obes*. 2006;30(9):1347-1355. doi:10.1038/sj.ijo.0803259

184. Christiansen T, Richelsen B, Bruun JM. Monocyte chemoattractant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. *Int J Obes*. 2005;29(1):146-150. doi:10.1038/sj.ijo.0802839

185. Nomura S, Shouzu A, Omoto S, Nishikawa M, Fukuhara S. Significance of chemokines and activated platelets in patients with diabetes. *Clin Exp Immunol*. 2000;121(3):437-443. doi:10.1046/j.1365-2249.2000.01324.x

186. Welch N, McNaughton SA, Hunter W, Hume C, Crawford D. Is the perception of time pressure a barrier to healthy eating and physical activity among women? *Public Health Nutr*. 2009;12(7):888-895. doi:10.1017/S1368980008003066

187. Illán-Gómez F, Gonzálvez-Ortega M, Orea-Soler I, et al. Obesity and inflammation: Change in adiponectin, C-reactive protein, tumour necrosis factor-alpha and interleukin-6 after bariatric surgery. *Obes Surg*. 2012;22(6):950-955. doi:10.1007/s11695-012-0643-y

188. Dekker MJ, Lee SJ, Hudson R, et al. An exercise intervention without weight loss decreases circulating interleukin-6 in lean and obese men with and without type 2 diabetes mellitus. *Metabolism*. 2007;56(3):332-338. doi:10.1016/j.metabol.2006.10.015

189. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta - Mol Cell Res*. 2011;1813(5):878-888. doi:10.1016/j.bbamcr.2011.01.034

190. FEBBRAIO MA, PEDERSEN BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB J*. 2002;16(11):1335-1347. doi:10.1096/fj.01-0876rev

191. Kim KB. Effect of different training mode on Interleukin-6 (IL-6) and C-reactive protein (CRP) in type 2 diabetes mellitus (T2DM) patients. *J Exerc Nutr Biochem*. 2014;18(4):371-378. doi:10.5717/jenb.2014.18.4.371

192. Whitson BA, Leslie DB, Kellogg TA, et al. Adipokine Response in Diabetics and Nondiabetics Following the Roux-en-Y Gastric Bypass: A Preliminary Study. *J Surg Res*. 2007;142(2):295-300. doi:10.1016/j.jss.2007.03.036

193. Popko K, Gorska E, Stelmaszczyk-Emmel A, et al. Proinflammatory cytokines IL-6 and TNF-α and the development of inflammation in obese subjects. *Eur J Med Res*. 2010;15(2):120-122. doi:10.1186/2047-783X-15-S2-120

194. Lee YJ, Heo YS, Park HS, Lee SH, Lee SK, Jang YJ. Serum SPARC and matrix metalloproteinase-2 and metalloproteinase-9 concentrations after bariatric surgery in obese adults. *Obes Surg*. 2014;24(4):604-610. doi:10.1007/s11695-013-1111-z

195. Lee SH, Lee JA, Park HS, et al. Associations among SPARC mRNA expression in adipose tissue, serum SPARC concentration and metabolic parameters in Korean women. *Obesity*. 2013;21(11):2296-2302. doi:10.1002/oby.20183

196. Kotani K, Yamada T, Taniguchi N. *The Association between Circulating Secreted Protein Acidic and Rich in Cysteine (SPARC) and Glycosylated Haemoglobin (HbA 1c ) during Lifestyle-Modified Weight Reduction Intervention in Obese Male Subjects*. Vol 39.; 2011.

197. Trombetta JM, Bradshaw AD. SPARC/osteonectin functions to maintain homeostasis of the collagenous extracellular matrix in the periodontal ligament. *J Histochem Cytochem*. 2010;58(10):871-879. doi:10.1369/jhc.2010.956144

198. Buechler C, Krautbauer S, Eisinger K. Adipose tissue fibrosis. *World J Diabetes*. 2015;6(4):548. doi:10.4239/wjd.v6.i4.548

199. Menkes A, Mazel S, Redmond RA, et al. Strength training increases regional bone mineral density and bone remodeling in middle-aged and older men. *J Appl Physiol*. 1993;74(5):2478-2484. doi:10.1152/jappl.1993.74.5.2478

200. Delany AM, Hankenson KD. Thrombospondin-2 and SPARC/osteonectin are critical regulators of bone remodeling. *J Cell Commun Signal*. 2009;3(3-4):227-238. doi:10.1007/s12079-009-0076-0

201. Pereira FA, Castro JAS de, Santos JE dos, Foss MC, Paula FJ. Impact of marked weight loss induced by bariatric surgery on bone mineral density and remodeling. *Brazilian J Med Biol Res*. 2007;40(4):509-517. doi:10.1590/S0100-879X2006005000074

202. Changes in bone metabolism after bariatric surgery by gastric bypass or sleeve gastrectomy-ClinicalKey. https://www-clinicalkey-com.uml.idm.oclc.org/#!/content/playContent/1-s2.0-S875632821630326X?scrollTo=%23t0005. Accessed March 1, 2020.

203. Colleluori G, Napoli N, Phadnis U, Armamento-Villareal R, Villareal DT. Effect of Weight Loss, Exercise, or Both on Undercarboxylated Osteocalcin and Insulin Secretion in Frail, Obese Older Adults. *Oxid Med Cell Longev*. 2017;2017:4807046. doi:10.1155/2017/4807046

204. Lee D-H, Ha M-H, Kim J-H, et al. Gamma-glutamyltransferase and diabetes-a 4 year follow-up study. *Diabetologia*. 2003;46:359-364. doi:10.1007/s00125-003-1036-5

205. Ibanez J, Ibanez I, Izquierdo M, et al. *Twice-Weekly Progressive Resistance Training Decreases Abdominal Fat and Improves Insulin Sensitivity in Older Men With Type 2 Diabetes, 5*.; 2005.

206. Lee DC, Sui X, Artero EG, et al. Long-term effects of changes in cardiorespiratory fitness and body mass index on all-cause and cardiovascular disease mortality in men the Aerobics Center Longitudinal Study. *Circulation*. 2011;124(23):2483-2490. doi:10.1161/CIRCULATIONAHA.111.038422

207. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-Reactive Protein, Interleukin 6, and Risk of Developing Type 2 Diabetes Mellitus. *JAMA*. 2001;286(3):327. doi:10.1001/jama.286.3.327

208. Fedewa M V, Hathaway ED, Ward-Ritacco CL. Effect of exercise training on C reactive protein: a systematic review and meta-analysis of randomised and non-randomised controlled trials. *Br J Sports Med*. 2017;51(8):670-676. doi:10.1136/bjsports-2016-095999

209. Aronson D, Bartha P, Zinder O, et al. Obesity is the major determinant of elevated C-reactive protein in subjects with the metabolic syndrome. *Int J Obes*. 2004;28(5):674-679. doi:10.1038/sj.ijo.0802609

210. Miller GD, Nicklas BJ, Fernandez A. Serial changes in inflammatory biomarkers after Roux-en-Y gastric bypass surgery. *Surg Obes Relat Dis*. 2011;7(5):618-624. doi:10.1016/j.soard.2011.03.006

211. Williams MR, McMeekin S, Wilson RJT, et al. Predictive Value of C-Reactive Protein for Complications Post-Laparoscopic Roux-En-Y Gastric Bypass. *Obes Surg*. 2017;27(3):709-715. doi:10.1007/s11695-016-2349-z

212. Ye RD, Sun L. Emerging functions of serum amyloid A in inflammation. *J Leukoc Biol*. 2015;98(6):923-929. doi:10.1189/jlb.3VMR0315-080R

213. Tisi PV, Hulse M, Chulakadabba A, Gosling P, Shearman CP. Exercise training for intermittent claudication: Does it adversely affect biochemical markers of the exercise-induced inflammatory response? *Eur J Vasc Endovasc Surg*. 1997;14(5):344-350. doi:10.1016/S1078-5884(97)80283-3

214. Verdaet D, Dendale P, De Bacquer D, Delanghe J, Block P, De Backer G. Association between leisure time physical activity and markers of chronic inflammation related to coronary heart disease. *Atherosclerosis*. 2004;176(2):303-310. doi:10.1016/J.ATHEROSCLEROSIS.2004.05.007

215. Yang R-Z, Lee M-J, Hu H, et al. Acute-Phase Serum Amyloid A: An Inflammatory Adipokine and Potential Link between Obesity and Its Metabolic Complications. Lazar M, ed. *PLoS Med*. 2006;3(6):e287. doi:10.1371/journal.pmed.0030287

216. Zhao Y, He X, Shi X, et al. Association between serum amyloid A and obesity: a meta-analysis and systematic review. *Inflamm Res*. 2010;59(5):323-334. doi:10.1007/s00011-010-0163-y

217. Gómez-Ambrosi J, Salvador J, Rotellar F, et al. Increased Serum Amyloid A Concentrations in Morbid Obesity Decrease after Gastric Bypass. *Obes Surg*. 2006;16(3):262-269. doi:10.1381/096089206776116525

218. Cohen J. *Statistical Power Analysis for the Behavioral Sciences Second Edition*.

219. Cornish SM, Chilibeck PD. Alpha-linolenic acid supplementation and resistance training in older adults. *Appl Physiol Nutr Metab*. 2009;34(1):49-59. doi:10.1139/H08-136

220. Simpson KA, Singh MAF. Effects of Exercise on Adiponectin: A Systematic Review. *Obesity*. 2008;16(2):241-256. doi:10.1038/oby.2007.53

221. Hocking S, Samocha-Bonet D, Milner K-L, Greenfield JR, Chisholm DJ. Adiposity and Insulin Resistance in Humans: The Role of the Different Tissue and Cellular Lipid Depots. *Endocr Rev*. 2013;34(4):463-500. doi:10.1210/er.2012-1041

222. Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT. Adiponectin multimeric complexes and the metabolic syndrome trait cluster. *Diabetes*. 2006;55(1):249-259. http://www.ncbi.nlm.nih.gov/pubmed/16380500. Accessed March 17, 2019.

223. Ahmadizad S, Ghorbani S, Ghasemikaram M, Bahmanzadeh M. Effects of short-term nonperiodized, linear periodized and daily undulating periodized resistance training on plasma adiponectin, leptin and insulin resistance. 2014. doi:10.1016/j.clinbiochem.2013.12.019

224. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun*. 1999;257(1):79-83. http://www.ncbi.nlm.nih.gov/pubmed/10092513. Accessed March 14, 2019.

225. Yadav R, Hama S, Liu Y, et al. Effect of Roux-en-Y Bariatric Surgery on Lipoproteins, Insulin Resistance, and Systemic and Vascular Inflammation in Obesity and Diabetes. *Front Immunol*. 2017;8. doi:10.3389/fimmu.2017.01512

226. Huang X, Yang Z. Resistin's, obesity and insulin resistance: the continuing disconnect between rodents and humans. *J Endocrinol Invest*. 2016;39(6):607-615. doi:10.1007/s40618-015-0408-2

227. Barnes KM, Miner JL. Role of resistin in insulin sensitivity in rodents and humans. *Curr Protein Pept Sci*. 2009;10(1):96-107. http://www.ncbi.nlm.nih.gov/pubmed/19275676. Accessed April 19, 2019.

228. Kadoglou NP, Perrea D, Iliadis F, Angelopoulou N, Liapis C, Alevizos M. Exercise Reduces Resistin and Inflammatory Cytokines in Patients With Type 2 Diabetes. *Diabetes Care*. 2007;30(3):719-721. doi:10.2337/dc06-1149

229. Zhang MH, Na B, Schiller NB, Whooley MA. Resistin, exercise capacity, and inducible ischemia in patients with stable coronary heart disease: Data from the Heart and Soul study. *Atherosclerosis*. 2010;213(2):604-610. doi:10.1016/j.atherosclerosis.2010.09.015

230. Heilbronn LK, Rood J, Janderova L, et al. Relationship between Serum Resistin Concentrations and Insulin Resistance in Nonobese, Obese, and Obese Diabetic Subjects. *J Clin Endocrinol Metab*. 2004;89(4):1844-1848. doi:10.1210/jc.2003-031410

231. Gueugnon C, Mougin F, Simon-Rigaud M-L, Regnard J, Nègre V, Dumoulin G. Effects of an in-patient treatment program based on regular exercise and a balanced diet on high molecular weight adiponectin, resistin levels, and insulin resistance in adolescents with severe obesity. *Appl Physiol Nutr Metab*. 2012;37(4):672-679. doi:10.1139/h2012-045

232. Edwards C, Hindle AK, Fu S, Brody F. Downregulation of leptin and resistin expression in blood following bariatric surgery. *Surg Endosc*. 2011;25(6):1962-1968. doi:10.1007/s00464-010-1494-z

233. Fukuhara A, Matsuda M, Nishizawa M, et al. Retraction. *Science*. 2007;318(5850):565. doi:10.1126/science.318.5850.565b

234. Hsu C-P, Oka S, Shao D, Hariharan N, Sadoshima J. Nicotinamide Phosphoribosyltransferase Regulates Cell Survival Through NAD $^{+}$ Synthesis in Cardiac Myocytes. *Circ Res*. 2009;105(5):481-491. doi:10.1161/CIRCRESAHA.109.203703

235. Chang Y-C, Chang T-J, Lee W-J, Chuang L-M. The relationship of visfatin/pre–B-cell colony-enhancing factor/nicotinamide phosphoribosyltransferase in adipose tissue with inflammation, insulin resistance, and plasma lipids. *Metabolism*. 2010;59(1):93-99. doi:10.1016/j.metabol.2009.07.011

236. Mellick PF, Feger BJ, Oberlin DJ, Davis PG, Wideman L. High-Intensity Exercise and Carbohydrate Supplementation do not Alter Plasma Visfatin. *J Sports Sci Med*. 2017;16(1):69-76. http://www.ncbi.nlm.nih.gov/pubmed/28344453. Accessed April 21, 2019.

237. Owczarek AJ, Olszanecka-Glinianowicz M, Kocełak P, et al. The relationship between circulating visfatin/nicotinamide phosphoribosyltransferase, obesity, inflammation and lipids profile in elderly population, determined by structural equation modeling. *Scand J Clin Lab Invest*. 2016;76(8):632-640. doi:10.1080/00365513.2016.1230884

238. Martínez Larrad MT, Corbatón Anchuelo A, Fernández Pérez C, Pérez Barba M, Lazcano Redondo Y, Serrano Ríos M. Obesity and Cardiovascular Risk: Variations in Visfatin Gene Can Modify the Obesity Associated Cardiovascular Risk. Results from the Segovia Population Based-Study. Spain. Eckel J, ed. *PLoS One*. 2016;11(5):e0153976. doi:10.1371/journal.pone.0153976

239. Karbaschian Z, Hosseinzadeh-Attar MJ, Giahi L, et al. Portal and systemic levels of visfatin in morbidly obese subjects undergoing bariatric surgery. *Endocrine*. 2013;44(1):114-118. doi:10.1007/s12020-012-9821-x

240. Blüher M. Vaspin in obesity and diabetes: pathophysiological and clinical significance. *Endocrine*. 2012;41(2):176-182. doi:10.1007/s12020-011-9572-0

241. Youn B-S, Kloting N, Kratzsch J, et al. Serum Vaspin Concentrations in Human Obesity and Type 2 Diabetes. *Diabetes*. 2008;57(2):372-377. doi:10.2337/db07-1045

242. Chang HM, Lee HJ, Park HS, et al. Effects of Weight Reduction on Serum Vaspin Concentrations in Obese Subjects: Modification by Insulin Resistance. *Obesity*. 2010;18(11):2105-2110. doi:10.1038/oby.2010.60

243. Van Harmelen V, Reynisdottir S, Eriksson P, et al. Leptin secretion from subcutaneous and visceral adipose tissue in women. *Diabetes*. 1998;47(6):913-917. doi:10.2337/diabetes.47.6.913

244. Expression of tumor necrosis factor (TNF)-alpha protein in the subcutaneous and visceral adipose tissue in correlation with adipocyte cell volume, serum TNF-alpha, soluble serum TNF-receptor-2 concentrations and C-peptide level in: European Journal of Endocrinology Volume 149 Issue 2 (2003). https://eje.bioscientifica.com/view/journals/eje/149/2/129.xml. Accessed March 1, 2020.

245. Rahmani-Nia F, Rahnama N, Hojjati Z, Soltani B. Acute effects of aerobic and resistance exercises on serum leptin and risk factors for coronary heart disease in obese females. *Sport Sci Health*. 2008;2(3):118-124. doi:10.1007/s11332-008-0050-x

246. Zumbach MS, Wolfgang M, Boehme J, et al. *Tumor Necrosis Factor Increases Serum Leptin Levels in Humans*. Vol 82.; 1997.

247. Maydych V, Claus M, Watzl C, Kleinsorge T. Attention to Emotional Information Is Associated With Cytokine Responses to Psychological Stress. *Front Neurosci*. 2018;12(OCT):687. doi:10.3389/fnins.2018.00687

248. Essig DA, Alderson NL, Ferguson MA, Bartoli WP, Durstine JL. Delayed effects of exercise on the plasma leptin concentration. *Metabolism*. 2000;49(3):395-399. doi:10.1016/S0026-0495(00)90396-2

249. Patel R, Chang T, Greysen SR, Chopra V. Social Media Use in Chronic Disease: A Systematic Review and Novel Taxonomy CLINICAL RESEARCH STUDY. *Am J Med*. 2015;128:1335-1350. doi:10.1016/j.amjmed.2015.06.015

250. Michie S, Ashford S, Sniehotta FF, Dombrowski SU, Bishop A, French DP. A refined taxonomy of behaviour change techniques to help people change their physical activity and healthy eating behaviours: The CALO-RE taxonomy. *Psychol Health*. 2011;26(11):1479-1498. doi:10.1080/08870446.2010.540664