Anatomic, functional, and physiologic implications of altered body composition among people with Facioscapulohumeral muscular dystrophy

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DEDICATION

To my parents, who encouraged me in my academic pursuits, and stressed that an education is never wasted.

To my husband Rolando, and children, Petra, and Bruno. May this work allow us to follow our dreams and passions in the future.

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LIST OF ABBREVIATIONS

4q35—long arm of chromosome 4

aVO_2-difference—arteriovenous oxygen difference

ALM—appendicular LM

ALMI—appendicular LM index

BMI—body mass index

DUX4—double homeobox, chromosome 4

DXA—dual-energy x-ray absorptiometry scan

ECG—electrocardiogram/electrocardiography

FSHD—Facioscapulohumeral muscular dystrophy

FM—FM

GXT—graded exercise test(s)

HR—heart rate

kg—kilograms

LM—LM

m—meters

MET(s)—metabolic equivalent(s)

Q—cardiac output

RMR—resting metabolic rate

RPE—rating of perceived exertion

R_R—respiratory rate

V_E—minute ventilation

V_E/VCO_2—ventilatory inefficiency

V_T—tidal volume

VCO_2—carbon dioxide production

VO_2—oxygen consumption

VO_2max—maximal oxygen consumption

VO_2peak—peak oxygen consumption

Chapter 1: Introduction/Significance

Facioscapulohumeral muscular dystrophy (FSHD) is a dominantly-inherited myopathy, with estimated prevalence rates ranging from 1:8,000-20,000 [1]. As with other autosomal dominant diseases, alterations in gene expression have been shown to be responsible for the pathologic changes associated with FSHD. In fact, the overexpression of genes on the long arm of chromosome 4 (4q35), *particularly* of double homeobox chromosome 4 (DUX4) [2], are believed to lead to characteristic scapular winging and progressive lean mass (LM) atrophy in the face, shoulder girdle, and truncal regions among people with FSHD [3].

Despite continued research on the genetic etiology of FSHD in recent years, much remains to be learned regarding the functional and translational implications of the overexpression of the DUX4 gene. It has been shown that individuals with FSHD frequently exhibit pathophysiologic alterations in body composition, including high rates of LM atrophy [1, 3-5] and fatty tissue infiltration [6]. These factors are believed to contribute to the development of sarcopenic obesity, a medical condition characterized by the concurrent presence of sarcopenia, which has been described as an age-related loss of LM and strength or physical function [7], and obesity, or "abnormal/extensive fat accumulation" [8]. Along with contributing to the development of a number of other disadvantageous health consequences [9-12], sarcopenic obesity has been associated with impairments in lung function [13, 14], an increased risk of disability [15], and losses in functional and exercise capacity (i.e., peak oxygen consumption, VO_{2peak}) [7, 16]. Furthermore, the observed alterations in body composition among people with FSHD appear to lay the foundation for the development of exercise intolerance, a multifactorial

condition that has been associated with the presence of a reduced exercise capacity [17] and accompanying symptoms of exertional fatigue and dyspnea [18]. While exercise intolerance has not been previously identified in people with FSHD, it has been shown to be a strong indicator of mortality in some clinical groups [7, 16], thereby making its identification in an already high-risk population of vital importance.

In addition to having anatomic and functional repercussions, FSHD-driven alterations in body composition may also influence physiologic mechanisms. In fact, LM is known to be a primary contributor to metabolic function [19], whereby it may contribute to as much as 70-80% of inter-individual variability in resting metabolic rate (RMR) [20]. The previously observed low volume of LM among various dystrophic populations has led to speculation that RMR may be reduced within these groups [21]; while this theory has been confirmed in some forms of muscular dystrophy [22], the results are not unequivocal [21, 23], nor studied in FSHD. The potential for a lower measured RMR among people with FSHD, as compared to in controls, suggests that metabolic prediction equations – which are often used in the clinical setting to guide weight gain, loss, or maintenance, and based on generic measures of age, height and weight [24] – will likely *overestimat*e kilocalorie needs within this group. As people with FSHD have already demonstrated a greater propensity towards adiposity [5, 6], potential recommendations for caloric intake that is higher than necessary may result in the deposition of additional fat stores and hinder weight loss/maintenance attempts.

My dissertation was composed of three studies designed to examine the ways in which a pathologic body composition may contribute to anatomic, functional, and physiologic consequences among people with FSHD. **Aim 1** of this work identified

differences in the presence of sarcopenic obesity between adults with FSHD, and healthy controls. As people with FSHD have been shown to demonstrate an increased propensity towards LM atrophy [1, 3-5] and higher levels of overall adiposity [5, 6], *I hypothesized* that individuals within this group would demonstrate sarcopenic obesity with greater frequency, as compared to healthy controls. **Aim 2** explored variations in the severity of and mechanistic contributions to exercise tolerance, between adults with FSHD, and controls. For this purpose, I measured peak VO_2 and symptoms of exercise intolerance, including fatigue and dyspnea, in people with FSHD and in age- and sex-matched controls; to assess the extent to which measures of LM contribute to exercise intolerance, a regression model was used. The presence of exercise intolerance in other clinical populations [25-28] led to *my hypothesis* that this phenomenon would be more pronounced among people with FSHD. Furthermore, *I believed* that exercise intolerance would be linked to an observed low volume of LM among people with FSHD, though this relationship would not necessarily be present among controls. Finally, **Aim 3** investigated the impact of altered body composition, including a low volume of LM, on RMR in the FSHD population. Due to the strong relationship between LM and metabolic function [19, 20], *I hypothesized* that RMR would be lower among people with FSHD, among whom the volume of LM has similarly been shown to be reduced [1, 3-5]. By addressing these three aims, I have tangibly shown the ways in which an altered body composition may impact lifespan and quality-of-life among people with FSHD, and have created a launching pad from which translational exercise- and diet-based interventional strategies to address these alterations may be developed.

CHAPTER 2: Literature Review

FSHD is a dominantly-inherited myopathy, characterized by scapular winging, and progressive LM atrophy in the face, shoulder girdle, and truncal regions [3]. In addition to numerous anatomic changes, individuals with FSHD frequently exhibit pathophysiologic alterations in body composition, including high rates of LM atrophy [1, 3] and fatty tissue infiltration [6]. These factors are believed to contribute to the development of sarcopenic obesity, a condition which has been associated with impairments in functional capacity [7, 16] and greater exercise intolerance [29] in other clinical groups. Importantly, while exercise intolerance has been previously linked to increasing rates of morbidity and mortality [30], the extent to which exercise tolerance is impaired in people with FSHD is currently unknown. Furthermore, pathologic changes in body composition among people with FSHD – especially the loss of LM— has led to speculation that metabolic function may be altered within this group [21]. In fact, as LM is a known primary contributor to RMR [20], or the amount of energy required to sustain life within a resting state [31], it is possible that the loss of this anatomic component coincides with an RMR that is lower than expected, though evidence to back up this theory is currently lacking. As such, the purpose of this chapter is to more fully describe FSHD-driven changes in body composition, to address the ways in which these alterations may influence and contribute to pathophysiologic effects, to delineate the significance, mechanisms, and clinical observations of sarcopenic obesity, exercise intolerance, and RMR within the FSHD population, and to provide further rationale for the support of research in this area.

FSHD: Molecular to Whole-Body Observations

Clearly, FSHD is a complex genetic condition, with a number of disadvantageous changes ranging from the molecular to the whole-body level. In this section, I will review the mechanisms of genetic dysregulation in FSHD1 and FSHD2, and the implications of these "downstream effects" on the regulation of skeletal muscle growth/development. Furthermore, a translational analysis of the repercussions of DUX4 expression – including a review of anatomic and physiologic alterations among people with FSHD – is also included. By identifying the factors responsible for dysfunction and disability in males and females with FSHD, the development of effective therapeutic strategies designed to improve the functionality and quality of life among this population becomes more feasible.

Genetic Dysregulation in FSHD

As with other autosomal dominant diseases, alterations in gene expression are theorized to be responsible for the pathologic changes associated with FSHD. Namely, overexpression of the 4q35 gene DUX4 has been shown to exhibit overtly toxic effects, which appear to be specific to skeletal muscle [2, 32].

D4Z4 repeat contraction. EcoR1 fragments, which have been mapped to the 4q35 region [33] are widely believed to contain the gene involved in the development of FSHD1[34], and observations of regional DNA rearrangement, including a decrease in EcoR1 fragment size (<30 kilobases), have been shown to contribute to pathologic changes in this population. While the EcoR1 fragment appears to exhibit three distinct regions: a) 5.7 kilobase proximal segment b) 3.3 kilobase internal segment, composed of repeating units, c) 1.25/2.9 kilobase distal segment, only the central 3.3 kilobase repeat region appears to be altered among individuals with FSHD1 [34]. In fact, contraction in the

3.3 kilobase central region of D4Z4 repeat units is believed to result in transcriptional impairment or inadequate protein production in the FSHD1 population [34]. While healthy individuals may exhibit as many as 150 D4Z4 repeat units, a "critical threshold" of fewer than 11 units has been reported among those with FSHD1; it is theorized that additional reductions in the number of D4Z4 repeat units is associated with increasing clinical severity and rates of penetrance [2].

It is important to note that while non-pathogenic repeat contractions may be found in healthy control populations, the presence of this phenomenon, and the corresponding de-repression of DUX4 among individuals with FSHD1, appear to occur exclusively in the presence of the 4qA allele [35]. While it has been speculated that this may be driven by a greater intrinsic propensity towards DNA arrangement in 4qA, observations of an equal rearrangement of DNA in both 4qA and 4qB alleles in healthy populations has been found [35]. Together, these findings suggest that the pathogenic origins of FSHD1 are driven at least in part by functional—not anatomic—differences between 4qA and 4qB alleles.

D4Z4 hypomethylation. In addition to a loss of repeat units, individuals with FSHD1 exhibit increased rates of D4Z4 hypomethylation, on both wild-type and mutated chromosome 4 alleles; non-affected gene carriers show similar degrees of hypomethylation at the shortened, mutated D4Z4 allele [36]. Similarly, D4Z4 hypomethylation has been noted among those with FSHD2, though the mechanisms responsible for the presentation of this phenomenon are different [37] (see section titled "*Structural maintenance of chromosomes flexible hinge domain-containing protein 1 mutations.")* The hypomethylation associated with FSHD appears to be established early

and transmitted in a stable manner [36], and is theorized to contribute to impairments in gene silencing in the D4Z4 region. Notably, while D4Z4 contraction does not appear to be present among individuals with phenotypic FSHD2 (~5% of afflicted population), hypomethylation of this region has been observed [36].

Loss of transcriptional repression complex. The loss of a "silencing" complex appears to play a crucial role in the de-repression of 4q35 genes among individuals with FSHD1. This complex includes the D4Z4 binding element, a 27-base pair unit in the D4Z4 region, which is believed to exhibit transcriptional repression properties; in healthy tissue, the D4Z4 binding element is bound to a three-unit multiprotein complex (D4Z4 recognition complex), composed of ying yang 1, high-mobility group protein group 2, and nucleolin [2]. Importantly, while the presence of all three proteins is necessary for a functional silencing complex, the ability of the D4Z4 recognition complex to bind selectively to the D4Z4 binding element through ying yang 1, a known multifunctional repressor/activator, highlights the functional significance of this protein [2]. Notably, the loss of transcriptional repression complexes appears to be mediated by D4Z4 unit contraction, as individuals who exhibit the most profound 4q35 gene expression –and subsequently, manifestations of the disease –also display the greatest reductions in D4Z4 repeat unit size.

Presence of polydenylation signal. Along with D4Z4 contractions, a single nucleotide polymorphism, located distally to the terminal D4Z4 repeat and frequently termed the "pLAM" sequence, has been observed [38]. Lemmers et al. notes that the presence of the pLAM sequence is exclusive to 4qA alleles; alternatively, 4qB alleles are considered to be "non-permissive," due to an absent regulatory region and a

corresponding lack of D4Z4 contractions [35, 38]. pLAM appears to also contribute to greater rates of polyadenylation – and subsequently, stability – of the DUX4 transcript [38]; it is worthwhile to note that the pLAM sequence has been found among individuals with both FSHD1 and 2, and is hypothesized to confer pathogenicity to the D4Z4 repeat in the presence of additional contributing factors (repeat unit deletion or structural maintenance of chromosomes flexible hinge domain-containing protein 1 gene mutation).

Structural maintenance of chromosomes flexible hinge domain-containing protein 1 mutations. Like counterparts with FSHD1, individuals with FSHD2 exhibit de-repression of 4q35 genes; notably, however, this observed overexpression occurs despite the absence of D4Z4 repeat unit contraction [37]. Instead, it is theorized that the FSHD2 phenotype may present as a result of rare and pathogenic haploinsufficiency-mediated structural maintenance of chromosomes flexible hinge domain-containing protein 1 gene mutations, including splice-site defects, out-of-frame deletions, and missense mutations [37]. Furthermore, decreases in structural maintenance of chromosomes flexible hinge domain-containing protein 1 gene activity appears to contribute to a reduction in methylation rate – a finding that is similarly triggered by a decreased D4Z4 repeat unit size among individuals with FSHD1 – and corresponding increase in D4Z4 chromatin relaxation, which, in the presence of a permissive allele (4qA), result in DUX4 expression [37]. In combination, these findings highlight the divergent avenues by which unique forms of FSHD may manifest.

Effects of genetic dysregulation. The DUX4 gene has been widely theorized to have a profound influence on control of the cellular cycle and regulation of growth and development. Exposure to DUX4 results in a rapid downregulation of myoblast

determination protein 1 [32], a "master regulatory gene of skeletal muscle differentiation," [39]. A decrease in the presence of this functional gene is believed to contribute to a lower proportion of multinucleated myotubes in DUX4-induced samples [32]. Furthermore, the simultaneous competitive inhibition by DUX4 of paired-box genes 3 and 7 – which have a similarly important role in myogenic development and regeneration –likely contributes to a decreased viability and functionality of myocyte regulatory factors in afflicted muscle [32]. These alterations are likely to result in a decreased efficiency by the stem cell pool to promote the differentiation of myotubes into functional LM, thereby leading to reductions in muscular growth and development.

Along with impairments in the production of LM, DUX4 also appears to lower the capacity of myoblasts to withstand oxidative stress, even at low levels of gene expression [32]. While this response may be buffered by the presence of nutritional antioxidants – including ascorbic acid, and vitamins K2 and E – these compounds appear unable to completely prevent the morphologic changes associated with DUX4 exposure. By inducing a greater sensitivity to oxidative damage, an increase in the proportion of myoblasts lost to oxidative stress is likely to be observed; in combination with the aforementioned impairments in LM development, this alteration is hypothesized to result in a gradual exhaustion of LM regenerative potential [32].

Anatomic Alterations in FSHD

The amounts of fat mass (FM) and LM, and the ways in which they are distributed, are grossly altered among individuals with FSHD; therefore, the goal of this section is to consider the extent to which these changes occur. Identifying whether these

effects contribute to additional comorbidity or functional impairment in people with FSHD is an important step in the development of therapeutic strategies.

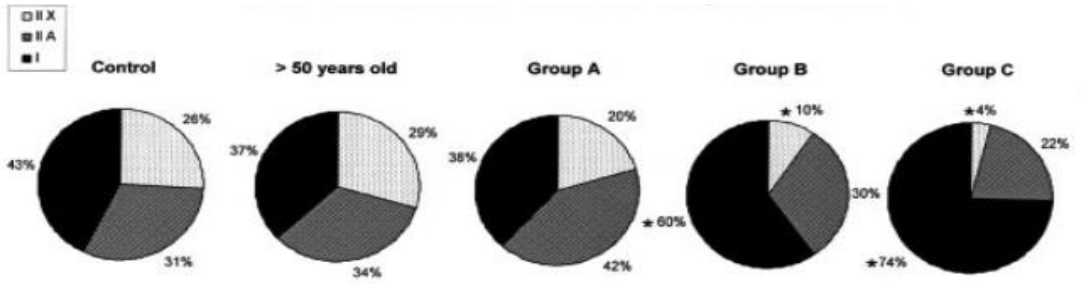


Figure 2.1: Fiber-type composite (%) in control, older groups, and among mildly (*Group A*), moderately (*Group B*), and severely affected (*Group C*) FSHD patients (Celegato et al., 2006).

Lean mass atrophy. LM atrophy, which may be defined as a decrease in LM [40], is a primary characteristic of muscular dystrophy. According to research by Skalsky et al., individuals with FSHD exhibit LM atrophy, as demonstrated by a mean reduction in the volume of whole-body LM by 17% [5]; furthermore, regional differences in the volume of LM between FSHD and control groups are even more profound, with adults afflicted with FSHD exhibiting a 27% reduction in arm LM [5]. Observations of LM atrophy are further supported by Marra et al., who note that both anatomic LM volume and contractile LM volume – or the proportion of LM able to generate force – are diminished among patients with FSHD [4]. The progressive loss of muscle fibers among this population appears to follow an asymmetrical pattern, with significant losses noted in the anatomic volume of the rectus femoris and vastus medialis, but not vastus lateralis or intermedius muscles of the quadriceps [4]. Similarly, while contractile volume of the vastus lateralis appears unaffected among individuals with FSHD, this measure is reduced among all other quadriceps muscle groups [4]. Whether dis-synchronous changes in anatomic and contractile volume are present amount other muscle groups, remains answered.

Fiber type transition. Along with high rates of LM atrophy, individuals with FSHD appear to exhibit a shift towards a slow muscle phenotype [6]. In fact, while the deltoid muscle in healthy adults is typically composed of approximately 43% type I, 31% type IIa, and 26% IIx muscle fibers, patients with FSHD show a progressive decrease in type IIx and a corresponding increase in type I muscle fibers [41] (**Figure 2.1**). Furthermore, losses in type IIa muscle fibers are largely absent until later stages of the disease, with severely affected patient groups demonstrating the most profound proportional changes in phenotype (total muscle composition: 74% type I, 22% type IIa, and 4% type IIx) (**Figure 2.1**) [41]. Though direct evidence is lacking, it is hypothesized that an altered regulation of downstream effectors in the calcineurin-NFAT pathway may be responsible for the fiber type transformation among individuals with FSHD [41]. *Fatty tissue infiltration.* People with FSHD appear to have an increased volume of FM [5], a finding that is believed to be driven primarily by muscular fat infiltration. In fact, while muscular fat infiltration among healthy adults generally does not exceed 10% of total muscle volume, rates are increased dramatically among individuals diagnosed with FSHD.

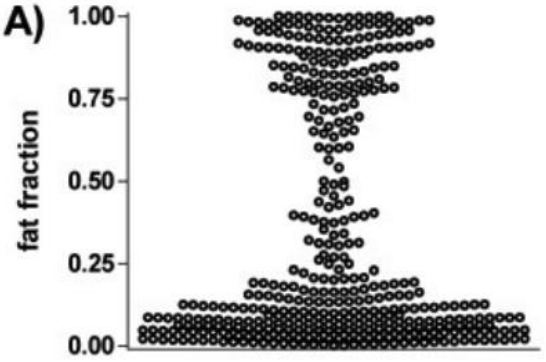


Figure 2.2: Distribution of fat fraction in the FSHD population (score of 0.00 reflects 100% muscle, 1.00 reflects 100% fat, each dot represents one muscle sample (*n*=427 leg muscle samples from 41 study participants)) (Janssen et al., 2014).

Fatty tissue infiltration.

According to Janssen et al., approximately 60% of patients with FSHD show normal/mild muscular fat infiltration (<25% infiltrated), with severe infiltration (>75% fatty tissue) occurring in as much as 26% of adults with FSHD (**Figure 2.2**) [6]. While

moderately infiltrated muscles (25-75% fatty tissue) are noted in only 12.6% of patients with FSHD, this subgroup also shows the most dramatic progression in infiltration rate [6]. In fact, moderately infiltrated muscles demonstrate an average increase in the fat-muscle ratio of approximately 10% in four months; at this rate, it is hypothesized that complete fat infiltration would occur within an average of three and a half years [6]. Further research is needed to understand why muscular fat infiltration occurs at such a rapid pace in moderately affected muscles, and whether this progression may be slowed through some form of intervention.

It is likely that fatty tissue infiltration contributes to increases in overall adiposity. In fact, high proportions of % FM have been noted among individuals with FSHD [5, 6] and in other dystrophic populations [42]–including Duchenne muscular dystrophy, a group in which fatty tissue infiltration has similarly been reported [43]. Though % FM has historically been estimated through calculations of body mass index (BMI) [8], this measure does not accurately reflect pathologic alterations in body composition in the FSHD population. In fact, despite greater overall adiposity, recent work has shown that individuals with FSHD appear to have a BMI that is similar to healthy controls [44].

Other anatomic alterations. Other notable changes among adults with FSHD include increases in interstitial fibrosis and rates of edema [4], which may contribute to reductions in fractional muscle area and decreased total muscle mass. Though the magnitude of their influence is unclear, it is likely that changes will alter the integrity of the musculoskeletal system, thereby contributing to losses in functionality. In the next section, I will outline the implications of both molecular and whole-body anatomic changes in FSHD, especially regarding their influence on exercise tolerance.

Physiologic Effects of FSHD

The pathogenic anatomic alterations exhibited by individuals with FSHD are theorized to contribute to the presentation of a number of disadvantageous physiologic changes, such as a reduced ability to generate force, low functional capacity, and impairments in cardiopulmonary function. Notably, the implications of these physiologic changes appear to include an increased propensity towards exercise intolerance, a condition which has been characterized by an inability to meet age- and sex-predicted values of physical performance [17], and which is accompanied by corresponding symptoms of exertional fatigue and labored breathing (dyspnea) during exercise. Identifying and quantifying the magnitude of physiologic impairment among individuals with FSHD may help estimate the extent to which exercise intolerance within this group is driven by the inherent effects of the disease.

Maximal force production. Individuals with FSHD exhibit reductions in both volitional and evoked strength when compared to a healthy cohort [45]. Additionally, specific strength – or strength per unit of physiological muscle cross-sectional area – are 56% lower in people with FSHD compared to healthy individuals [4]. According to Lassche et al., these pathologic changes appear to be restricted to type II fibers, in which maximal force-generating capacity is reduced by 70% [46]. This phenomenon is believed to occur in combination with a reported partial fast-to-slow muscle fiber type transition among individuals with FSHD [41] (**Figure 2.3**), thereby resulting in concurrent reductions in both force and velocity, and an even more pronounced loss in power in this population. These findings are hypothesized to reflect a combination of disadvantageous changes in dystrophic muscle, including increased fibrosis [4], lipid infiltration [6], and a loss of tendon-

fiber continuity during muscular regeneration [47], thereby resulting in impaired strength-volume relationship. The practical implications of these disadvantageous changes are expected to include major losses in function among individuals with FSHD.

Functional capacity. Measures of functional capacity, or "an individual's ability to perform work," [48] are reduced among adults with FSHD. In fact, Morse et al. report that distance traveled during the upper-body 6-minute cycle test, a frequent measure of functional capacity among individuals with neuromuscular disease, is diminished in people with FSHD [49]. It is hypothesized that these reductions may be partially driven by a reduced rate of handgrip strength ($r=0.61$, $p<0.05$) among individuals with FSHD, a finding which is believed to reflect a lower volume of available LM [41] in this patient group. Furthermore, an inverse association between FM and upper-body exercise capacity ($r=-0.57$, $p<0.05$) among individuals with FSHD appears to be driven primarily by the effects of fatty tissue infiltration, an insidious phenomenon that has been shown to trigger impairments in the contractile properties of type I and II muscle fibers [50].

Cardiopulmonary function. FSHD is frequently classified as a neuromuscular disease with infrequent cardiac involvement. In fact, while observations of structural cardiac involvement, including ventricular dilation, is reportedly rare in people with FSHD [1], electrophysiological abnormalities have been observed. According to van Dijk et al., as many as one-third of individuals with FSHD exhibit incomplete right bundle branch block [51]; similarly, other asymptomatic supraventricular arrhythmias have been reported in 5-10% of individuals with FSHD [52, 53]. Along with impairments in electrical conduction, autonomic dysregulation of the cardiovascular system does appear to be present in this group. Della Marca et al. have reported an inverse relationship between

disease severity among people with FSHD, and the power of the high-frequency bandwidth component – a known indicator of efferent vagal activity [54]– thus suggesting an impairment in parasympathetic activation with increasing disease severity [55]. This finding reflects the potential for an accentuated heart rate (HR) and blood pressure response during physical activity, thereby contributing to potential exercise limitations, especially among individuals who exhibit the most profound losses in muscular volume.

Reductions in cardiopulmonary function in FSHD also appear to be influenced by respiratory impairment, though the mechanisms underpinning this dysfunction remain unclear. Stübgen et al. notes that individuals with FSHD exhibit global impairments in respiratory muscle function, a finding that reportedly appears to be related to reductions in expiratory muscle function (maximal expiratory pressure and peak expiratory flow) [56]. Conversely, Scully et al. suggest that individuals with FSHD are at an increased risk for restrictive lung disease [57], a phenomenon that has been linked to losses in inspiratory capacity and reduced lung compliance. Furthermore, respiratory muscle endurance, as measured by maximal voluntary ventilation, also appears to be reduced in adults with FSHD [56]. Whether these changes are associated with atrophy of the diaphragm and accessory muscles of respiration or are purely a reflection of pulmonary pathophysiology among individuals with FSHD, remains unclear. Despite a lack of clarity regarding the origins of dysfunction, however, the possible contributions of respiratory impairment to the development of exercise limitation in people with FSHD must be acknowledged.

Exercise Intolerance

Exercise intolerance is a multifactorial condition, associated with the presence of a reduced exercise capacity—whereby age- and sex-predicted values of physical

performance (i.e., VO_2peak) are not reached [17]. It is important to note that the reduced exercise capacity is accompanied by symptoms of exertional fatigue and dyspnea [18], and is often considered a symptom-limited test in clinical populations. Reductions in exercise tolerance may be identified through the use of a number of objective tools, to include interviews, surveys and quantitative techniques. While exercise intolerance has been demonstrated in the elderly [28] and in certain clinical groups [25-27], it has yet to be elucidated in FSHD. Therefore, the purpose of this section is to outline the significance of exercise intolerance, as well as to identify known mechanisms of the condition. My dissertation work expands on existing findings and defines the ways in which differing factors may contribute to exercise intolerance in FSHD. Understanding the origins and effects of exercise intolerance in the FSHD population is a crucial first step in the development of therapeutic techniques for this group.

Significance of Exercise Intolerance

The identification of exercise intolerance is important, as it is believed to be a strong indicator of mortality among certain clinical groups. For example, among patients undergoing chronic hemodialysis with underlying non-diabetic nephropathy, increasingly severe exercise intolerance has been linked to a progressively greater likelihood of cardiovascular-related death [58]. Similarly, this observation has been mirrored among childhood cancer survivors, whereby exercise intolerance, as measured by a VO_2peak of <85% of age-predicted maximal levels, was associated with a 3.9-fold increase in overall mortality risk [59]. Together, these findings reinforce the importance of understanding exercise intolerance in clinical populations such as FSHD, a primary aim of the proposed PhD project.

It is important to note that while reductions in exercise capacity alone do not fully reflect the presence of exercise intolerance, it does play a major role in the pathogenesis of the condition. While low levels of exercise capacity have been linked to increased mortality rates among individuals with cardiovascular disease, a higher risk of death has reportedly also been observed among healthy controls with a low peak exercise capacity, as reflected by metabolic equivalents (METs) [30]. In fact, after adjusting for age, peak MET-load (odds ratio (each 1-MET increment): 0.84) is believed to be the strongest predictor of morality among both healthy individuals and those with known cardiovascular disease, and is theorized to be more influential than other established risk factors, including diabetes (1.30), left ventricular hypertrophy (1.22), dyslipidemia (1.21), or smoking (for each 10-year increment): 1.09) [30]. By identifying the presence of exercise intolerance among individuals with FSHD, we may aid in the identification of those who are at a greater risk for disease-related mortality.

Mechanisms of Exercise Intolerance

Aim 1 of this project was designed to examine if people with FSHD experience exercise intolerance, a phenomenon which may be partially reflected by a low maximal oxygen uptake (VO_2max) during exercise. While VO_2max has been consistently measured in healthy cohorts, the presence of concurrent dyspnea and fatigue during exercise among clinical populations often results in a symptom-limited test, thus precluding these individuals from reaching their "true" physiologic maximal capacity, and thus this test in clinical populations often results in a VO_2peak [60]. A number of mechanisms are hypothesized to contribute to the etiology of these symptoms, though the magnitude of their influence remains unclear. Therefore, identifying the factors which

contribute to *both* low VO_2peak *and* concurrent increases in dyspnea and fatigue during exercise will yield novel findings, which may serve as a foundation for treatments designed to target exercise intolerance.

Influence of cardiac output on exercise intolerance. VO_2peak has long been defined by the Fick equation [61], where:

$$VO_2peak = Q \times arteriovenous\ oxygen\ difference\ (a\text{-}VO_2\ difference)$$

and in which cardiac output (*Q)* is further reflected by the combined influences of HR and differences in left ventricular end-diastolic volume and left ventricular end-systolic volume, otherwise known as stroke volume [61]. Furthermore, stroke volume – or the volume of blood (mL) ejected from the left ventricle per heartbeat [62]— is driven primarily by changes in ventricular afterload, contractility, and preload, factors which are likewise influenced by alterations in heart size, fitness level, and the sex of the individual [62]. Stroke volume has been observed to be as much as 26% lower in clinical groups, as compared to controls, and is believed to be the primary factor responsible for a corresponding 48% reduction in VO_2peak [63]. Similarly, a blunted HR response by as much as 40% in patient populations has been linked to observations of low *Q* and corresponding exercise intolerance, and is theorized to be mediated at least in part by the presence of chronotropic incompetence [64]. While it is clear that in some clinical populations, a reduction in *Q* has a profound influence on VO_2peak, it is important to remember that cardiac dysfunction is reportedly rare among people with FSHD [1]. Therefore, the low VO_2peak exhibited by this group is unlikely to be driven by low *Q*, but rather, by a combination of peripheral factors (see **Figure 2.3)**. These potential

contributors to exercise intolerance will be discussed in the section titled "*Peripheral mechanisms of exercise intolerance.*

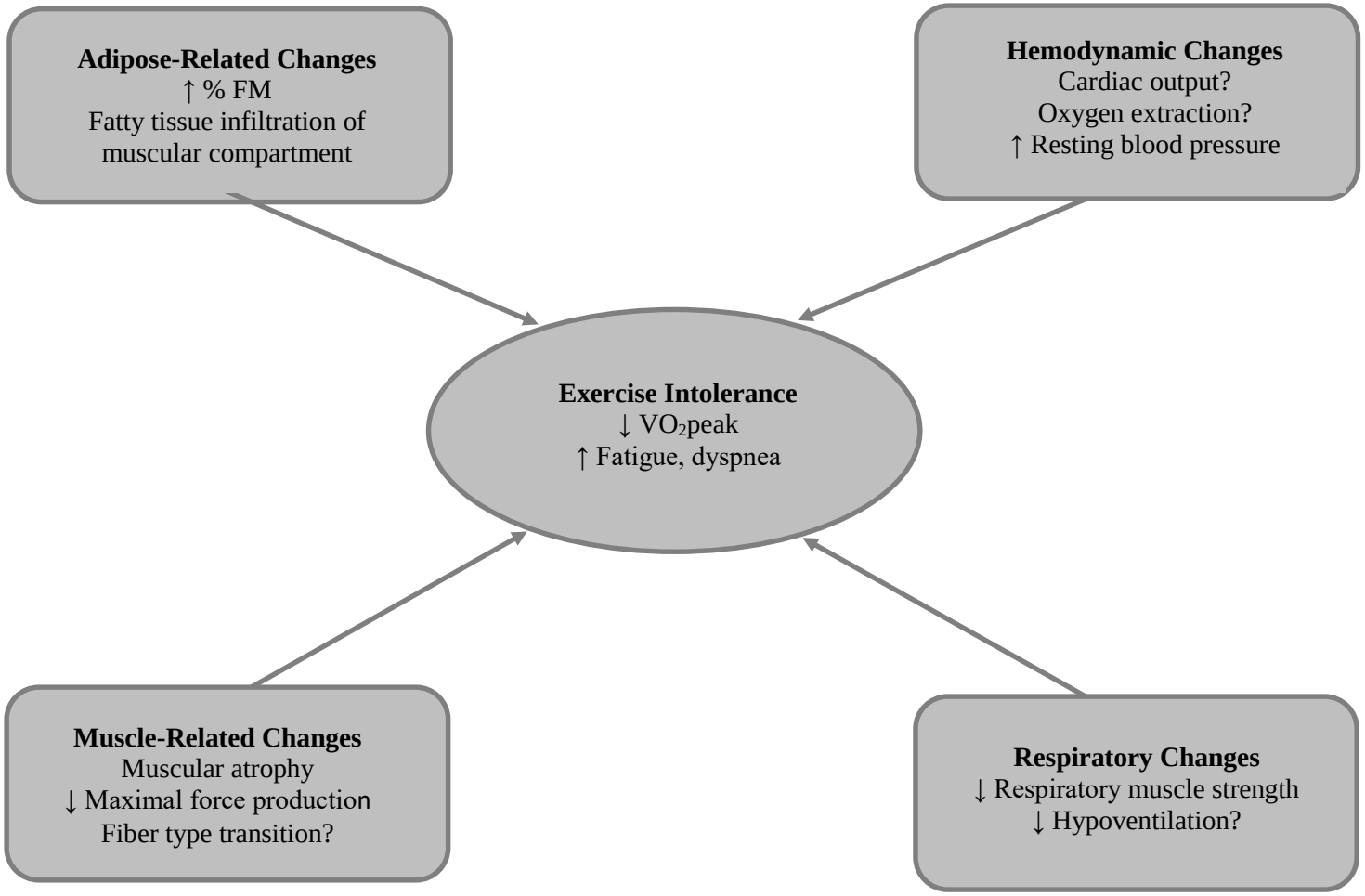


Figure 2.3: Mechanisms of exercise intolerance in FSHD. Observations of exercise intolerance in FSHD may be driven by a combination of fat mass- and lean mass-related changes, resulting in the presentation of sarcopenic obesity in this population.

Influence of a-VO_2 difference on exercise intolerance. As suggested by the name, a-VO_2 difference has been defined as the difference in oxygen content between arterial and venous blood [62]. Among healthy populations, resting a-VO_2 difference has been previously measured at approximately 5 mL/100 mL blood/min, a volume that correlates to oxygen extraction rates of approximately 25%; during exercise, however, this value reportedly increases to 15-20 mL/100 mL blood/min, an extraction rate that is estimated to approach 75-100% [65]. Alterations in this mechanism have been reported in groups whereby exercise intolerance is frequently observed—including among those with diastolic heart failure—as evidenced by *reductions* in a-VO_2 difference during peak exercise by as much as 13% [63]. Interestingly, observations of increases in this measure in patient groups during submaximal exercise have been noted [63], suggesting that impairments in the mechanism only appear with more intense stages of physical activity. Since symptom-limitation may prevent individuals with exercise intolerance from reaching maximal exercise thresholds, a nominal contribution of aVO_2-difference to genesis of the condition is expected (see **Figure 2.3**).

Ventilatory mechanisms of exercise intolerance. A number of ventilatory mechanisms have been theorized to underlie the presence of exercise intolerance among individuals with certain chronic diseases (**Figure 2.3**) [66]. According to research by Myers et al., clinical groups demonstrating exercise intolerance have been shown to exhibit lower tidal volumes (V_T), higher respiratory rates (R_R), and an increased, inverse relationship between VO_2max and the ratio of maximal estimated ventilatory dead space to maximal tidal volume during physical activity, as compared with healthy controls [66]. Furthermore, observations of ventilatory inefficiency, as reflected by an elevated

V_E/VCO_2 slope, beginning at the start of exercise, and continuing both below and above the ventilatory threshold, have been observed among individuals suffering from exercise intolerance [66]. While ventilatory inefficiency appears to be at least partially driven by ventilatory-perfusion mismatching, the activation of group III/IV skeletal muscle afferents also reportedly plays a role in the manifestation of this phenomenon. In fact, Keller-Ross et al. report that the pharmacologically-induced afferent inhibition of LM afferents results in a decrease in V_E/VCO_2 slope, particularly among clinical populations [25], highlighting the importance of changes within LM to ventilatory control, and thus tolerance to exercise. Furthermore, though V_E/VCO_2 slope does not appear to be influenced by body composition in healthy groups, a relationship between ventilatory inefficiency, leg LM (r=0.76) and leg FM (r=-0.86) among patients with heart failure has been reported [25], thus further reinforcing the purported influence of body composition on exercise intolerance in clinical populations. To determine whether these ventilatory parameters are associated with or predictive of exercise intolerance in the FSHD population, a secondary analysis has been included in my research.

Peripheral mechanisms of exercise intolerance. According to the "muscle hypothesis," a complex combination of peripheral factors is theorized to play a primary role in the development of exercise intolerance (**Figure 2.3**). This theory appears to be especially relevant among clinical populations, where a low volume of LM [67, 68] and a reduced rate of maximal force production [67, 69] have been widely reported. In fact, observations of an association between VO_2peak, calf LM (r=0.48) and mid-arm LM (4=0.36) among individuals with heart failure has furthermore been shown, thus underscoring the impact of LM and functionality within this group. Together, these

findings support the hypothesis that the presentation of sarcopenia among individuals with FSHD is a major contributor in the pathogenesis of exercise intolerance.

In addition to reductions in LM and strength, alterations in muscle fiber composition and corresponding changes to muscle metabolism are believed to contribute to symptoms of exercise intolerance. In fact, the presentation of a slow-to-fast (type I → type IIx) fiber type transition appears to be a frequent characteristic of certain chronic illnesses [70, 71], thereby leading to a decrease in citrate synthase and 3-hydroxyacyl-CoA dehydrogenase activity [71]. Ultimately, these histologic changes are believed to result in an increased utilization of glycolytic energy pathways and reductions in muscular endurance. Notably, among people with FSHD, a trend towards an increasing proportion of slow-twitch (type I) muscle fibers has been observed [41], thereby suggesting the capacity for an *increased* reliance on oxidative phosphorylation as an energy system and *greater* muscular endurance. Paradoxically, however, despite an increased proportion of type I muscle fibers, reductions in oxidative phosphorylation – including low levels of c oxidase activity and ATP synthesis [72]—have also been noted in adults with FSHD. Together, this suggests that while a fiber-type shift may occur, losses in functionality within the mitochondria of these fibers may be responsible for overall reductions in exercise capacity among this group.

Among individuals with exercise intolerance, it is possible that physical sensations of dyspnea and fatigue are peripherally-driven and occur in part by the over-activation of group III/IV LM afferents. According to Keller-Ross et al., an increase in glycolytic enzyme activity – such as that which may occur in patients with heart failure – results in an increased activation of mechano- (group III) and metaboreceptors (group IV)

within the LM [25]. Furthermore, the activation of group III/IV afferents is believed to trigger cardiorespiratory centers in the brainstem [73], resulting in an increase in ventilation (V_E) and blood pressure in the heart failure population. If individuals with FSHD have a shift toward glycolytic metabolic pathways, then an increase in metabolic activity and therefore greater activation of III/IV afferent activity, contributing to greater exercise cardiorespiratory responses and fatigue and dyspnea, is possible. However, confirmation of this theory requires further investigation.

Clinical Observations of Exercise Intolerance

Though the presence of exercise intolerance in certain clinical groups, including those with heart failure, is well-documented, whether it affects individuals with FSHD is unclear. It has been reported that while divergent forms of muscular dystrophy vary widely in etiology and presentation, nearly all are believed to cause exercise intolerance to some degree [74]. While observations of exercise intolerance have not been explicitly noted among individuals with FSHD, Morse et al. did find an exercise capacity, as reflected by distance traveled during the assisted 6-minute cycle test, a surrogate measure of VO_2peak, which was lower in the FSHD population, as compared to controls [75]. Importantly, since Morse et al. did not assess subjective measures of dyspnea or ratings of perceived exertion (RPE) during the assisted 6-minute cycle test, distinguishing between the presence of a reduced exercise capacity and exercise intolerance is not possible. However, measures of lung function, including forced expiratory volume in one-second and forced vital capacity, were lower in the FSHD group [75], and since reductions in these parameters are theorized to contribute to symptoms of breathlessness [76], it is plausible to hypothesize that people with FSHD experience exercise intolerance.

Despite findings of a reduced exercise capacity among individuals with FSHD, it is worthwhile to note that exercise training interventions do appear to elicit gains in this measure. In fact, research by Bankole et al. reports improvements in VO_2peak among people with FSHD by as much as 20% over baseline measures, following 6-weeks of strength, high-intensity interval, and low-intensity aerobic exercise training [77]. Similarly, measures of maximal voluntary contraction at rest – a value that is believed to reflect a combination of muscular volume and strength—increased by 15% in this group, following 24-weeks of combined exercise training [77]; distance traveled during a 6-minute walking test also increased by 14%, following the 6-week training modality, a measure which was correlated with both VO_2peak ($r=0.62$) and maximal voluntary contraction ($r=0.68$) [77]. Notably, Bankole et al. found post-intervention serum creatine kinase concentrations that were unchanged, as compared to baseline levels, indicating that key aspects of muscular structure, including sarcolemma integrity, are not degraded with exercise training [77]. Together, these finding suggest that the low exercise capacity exhibited by individuals with FSHD may not be solely related to the pathologic effects of the genetic condition. Instead, since a low exercise capacity among people with FSHD appears to be reversible in a safe and effective fashion through exercise training, it is likely that deconditioning is also a major contributor to exercise intolerance within this group. **Aim 1** of my dissertation was designed to build on this research, and has clearly defined that individuals with FSHD exhibit a physiologic response to exercise that differs from that seen in controls, a novel topic which has yet to be addressed in the literature.

Sarcopenic Obesity

Sarcopenic obesity is a unique medical condition, associated with the presence of two concurrent phenomenona – sarcopenia, an age-related loss of LM and strength or physical function [7], and obesity, or "abnormal/extensive fat accumulation" [8]. The presence of sarcopenic obesity has been widely observed in aging populations [7] and among those with chronic disease [10], whereby it has been associated with increases in disability and a greater risk of mortality. Though the exact mechanisms by which these relationships develop have not been fully defined, it is theorized that the anatomic changes associated with sarcopenic obesity, and their subsequent contribution to an increased propensity towards exercise intolerance, may provide at least a partial explanation [29]. In fact, since exercise intolerance has been associated with an increasing risk of disability [78] and mortality in some clinical groups [59, 79] I believe that these three factors – sarcopenic obesity, exercise intolerance, and disability/mortality – are inextricably linked.

The purpose of this section is to review the ways in which functionality, quality of life, and lifespan may be altered in those living with sarcopenic obesity. Furthermore, I will provide a detailed mechanistic background of the condition and discuss observations of sarcopenic obesity among individuals with various forms of muscular dystrophy, including FSHD. By obtaining a thorough understanding of sarcopenic obesity, confirming its presence in people with FSHD, and elucidating the extent to which it contributes to the development of functional impairment – including exercise intolerance – I aim to more clearly define the implications of this condition, both independently, and among clinical groups.

Significance of Sarcopenic Obesity

Identifying the presence of sarcopenic obesity is believed to be important when it comes to addressing impairments in functionality. Although a number of factors may contribute to the phenomenon, sarcopenic obesity has been shown to play a key role when it comes to losses in physical function and ability, reductions in gait speed [7], greater difficulty in the performance of tasks of independent living [16], and self-reported losses in health status [7]. Furthermore, research by Baumgartner, in which impairments in physical function were studied in an aging population (≥60 years), found that the odds ratio of developing three or more disabilities was greatest among individuals with sarcopenic obesity (odds ratio: 4.12), as compared to obese non-sarcopenic (2.33), non-obese sarcopenic (2.07), and healthy, non-obese counterparts with normal volumes of LM (1.00) [80]. Similarly, while abnormalities of gait, balance, or incidence of falls within the preceding year have been independently associated with the presence of both obesity and sarcopenia, the risk of these incidences are far greater among individuals with the combined manifestation of sarcopenic obesity [80]. Together, this evidence reinforces the hypothesis that sarcopenic obesity – a hypothesized comorbidity of FSHD – may lead to losses in functional capacity, thereby contributing to exercise intolerance within this clinical group.

Chronic illnesses – including osteoarthritis [81], kidney disease [82], and depression [83] – are increased among individuals with sarcopenic obesity. Furthermore, the presence of sarcopenic obesity has been strongly linked to cardiometabolic risk factors; according to research by Srikanthan et al., adults with sarcopenic obesity exhibit an increased propensity for both insulin resistance and dysglycemia, as compared to both those with a normal body composition, and non-obese, sarcopenic counterparts [12]. Similarly, hypertension [11], dyslipidemia [9], and as much as 8-fold increase in the risk for metabolic

syndrome has been reported in people with sarcopenic obesity [10]. Based on these observations, it is not surprising that the risk of cardiovascular disease among individuals with sarcopenic obesity is believed to be increased by as much as 23% [84], though it is interesting to note that in prospective cohort study by Atkins et al., no associations between sarcopenic obesity and either cardiovascular event or mortality were found [85].

As with cardiovascular disease, impairments in lung function appear to be associated with the presence of sarcopenic obesity. Research by Moon et al. reports that both forced expiratory volume in one-second and forced vital capacity are lowest among individuals with sarcopenic obesity, as compared to those with sarcopenia only, obesity only, and those with normal volumes of LM and body composition [13]. This finding was further supported by work by Lee et al., in which the odds ratio of restrictive lung disease was greatest among people with sarcopenic obesity (odds ratio: 2.81), as compared to counterparts with sarcopenia only (2.00), obesity only (1.88), or a normal body composition (1.00) [14]. As restrictive lung disease has been previously reported among individuals with FSHD, and as pulmonary function is theorized to contribute to exercise intolerance, this finding provides further support for the hypothesis that sarcopenic obesity may contribute to exercise intolerance among individuals with FSHD, though it is important to note that the exact etiology and pathways by which these phenomena present have not been fully defined.

Mechanisms of Sarcopenic Obesity

A combination of biologic, hormonal, and behavioral influences are believed to result in the development of sarcopenic obesity. Investigating the extent to which the components of sarcopenic obesity may contribute to observations of exercise intolerance

among individuals with FSHD – both independently, and in combination with each other – is a key aspect of this research (**Aim 2**).

Sarcopenia. The factors that contribute to decreases in LM volume and functionality – and ultimately, lead to the development of sarcopenia –are complex in nature. In fact, a combination of mechanisms, to include an increased presence of pro-inflammatory cytokines [86], an altered production of growth and sex hormones [87], and impairments in the anabolic response of protein metabolism to nutrients (i.e., essential amino acids) [88] have all been proposed as possible mechanisms in the development of this condition. These factors have reportedly been linked to decreases in both the size (atrophy) and number (hypoplasia) of LM fibers [89], thereby distinguishing sarcopenia from disuse atrophy, whereby a decrease in muscle fiber size—but not number—has been reported. Furthermore, the development of sarcopenia appears to be mediated primarily via the loss and atrophy of type II muscle fibers [87], a finding which is believed to contribute to decreases in muscular power. **Aim 2** of this study was designed, in part, to identify the extent to which sarcopenia contributes to exercise intolerance in people with FSHD. Since exercise intolerance is theorized to be at least partially driven by losses in muscular power, I hypothesized that a strong association between the presence of sarcopenia and exercise intolerance among people with FSHD would be observed.

Obesity. As with sarcopenia, there are a number of pathophysiologic mechanisms associated with the development of obesity, including impairments in nutritional feedback and neuroendocrine signaling, altered gut microbiota, desynchronization of circadian rhythms, and imbalances in energy intake and expenditure [90]. While obesity has been classically associated with high stores of FM, individuals with *sarcopenic*

obesity are believed to exhibit unique anatomic alterations – to include a greater propensity towards fatty infiltration of skeletal muscle [91]. Along with contributing to reductions in the contractile strength of type I and II muscle fibers [50], fatty tissue infiltration is believed to result in a loss in muscular power. Together, these observations provide further support that individuals with sarcopenic obesity are likely to exhibit impairments in exercise capacity, a theory which will be further clarified in subsequent chapters.

Clinical Observations of Sarcopenic Obesity

Though not exclusive to particular demographic groups, sarcopenic obesity has been widely reported in aging populations [80]. However, since people with FSHD that met the criteria for sarcopenic obesity within our study were, on average, 10 years younger (50±11 years) than those studied in aging research, it is likely that another factor is responsible for the presentation of abnormal body composition within this population. Sarcopenic obesity has been previously reported among individuals with various forms of muscular dystrophy, including Bethlem myopathy, Ullrich congenital muscular dystrophy, rigid spine syndrome, limb girdle muscular dystrophy type 2d, Duchenne muscular dystrophy, and Becker muscular dystrophy [42, 92, 93]. Similarly, research by Skalsky et al. did report alterations in body composition among individuals with FSHD, including lower amounts of whole-body LM (FSHD: 40.44±11.07 kg; control: 48.76±12.33, $p<0.001$) and higher amounts of whole-body FM (FSHD: 25.41±8.33 kg; control: 17.98±6.65 kg, $p<0.001$) [5]. Since assessments of traditional indicators of sarcopenic obesity – including appendicular LM (ALM), appendicular LM index (ALMI), and % FM were not measured in this research, identifying the presence of

sarcopenic obesity, even as a retrospective analysis, is not possible. Despite the limitations of investigations completed among individuals with FSHD, these observations still provide an important theoretic framework, on which the hypothesis of an expected presence of sarcopenic obesity among individuals with FSHD is well-founded.

Resting Metabolic Rate

An individual's RMR has previously been defined as the volume of calories burned, while in a resting state [94]. Furthermore, RMR is a key component of total daily energy expenditure, whereby it is believed to account for approximately 70% of total calorie expenditure within a 24-hour timeframe [95]. While RMR has been shown to be strongly – but not exclusively – driven by the presence of metabolically-active tissue, it is notable that alterations in body composition, and parallel changes in RMR, have been associated with the emergence of certain pathologies [96]. In fact, a steep decline in RMR among people with heart failure, independent of age and sex, has been reported [96], likely as a result of cardiac cachexia, a deleterious wasting syndrome. As cachexia has been shown to be a strong prognostic factor among people with heart failure [97], it is possible that a similar relationship may be observed in adults with FSHD; as such, it is vitally important to study the ways in which body composition and RMR may intersect in a vulnerable, high-risk group.

In this section, I will outline the biologic and environmental factors believed to contribute to RMR. Furthermore, this section will outline previous research on RMR in dystrophy groups and provide possible explanations for disparate findings. By identifying and understanding the implications of an altered RMR with a clinical population, I hope

to highlight the need for targeted exercise and nutritional intervention among people with FSHD.

Significance of Resting Metabolic Rate

RMR is frequently used as an objective marker of the minimum number of calories needed to sustain life, and therefore, is of high significance as a practical tool in the field of clinical nutrition. While in general, RMR has been shown to contribute to a high percentage of total daily energy expenditure (**Figure 2.4A**), it is notable that the influence of this parameter is especially high among people who are sedentary [98] (**Figure 2.4B**), of which individuals with FSHD potentially may be included. In fact, the absence of structured physical activity among sedentary populations means that the remaining two components of total

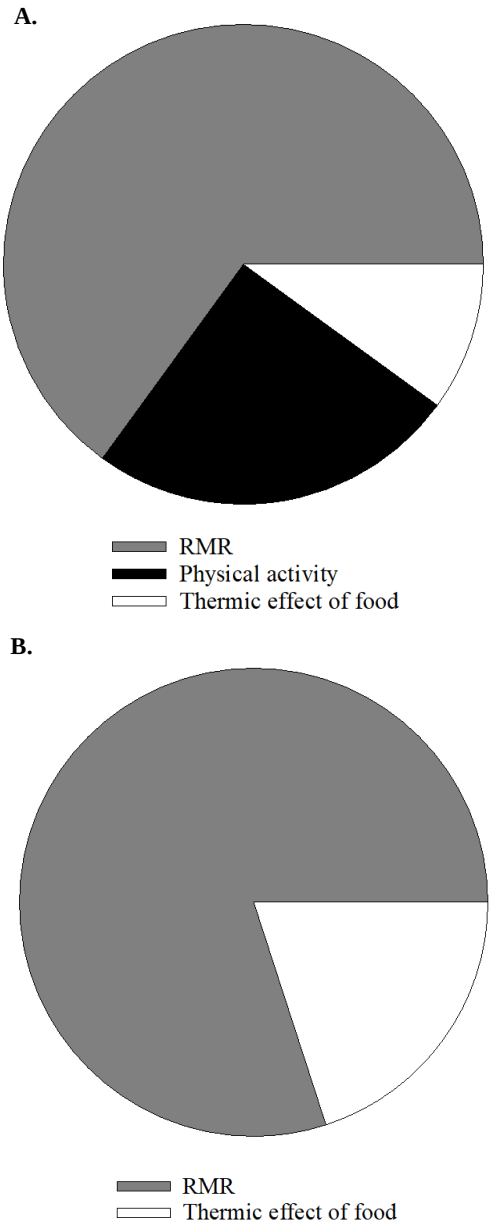


Figure 2.4: Components of total daily energy expenditure in an (A) active and (B) sedentary population. Among people who are physically active, components of total daily energy expenditure are as follows: resting metabolic rate, 65-70%; physical activity, 25-30%; thermic effect of food: 10%. In sedentary populations, components of total daily energy expenditure are reflected in the following way: resting metabolic rate, 70-80%, thermic effect of food, 20-30%.

daily energy expenditure – RMR and thermic effect of food – are significantly altered, resulting in the contribution of RMR to total daily energy expenditure of up to 80% [98]. Overall, in sedentary groups, RMR is subsequently believed to be more impactful – and as such, a measured value which is lower than expected, such as might be found among people with FSHD, means that total daily energy expenditure is also likely to be disadvantageously low. As measures of RMR are frequently used to guide recommendations for weight loss, gain, or maintenance [98], it has been speculated that attempts at weight maintenance or loss may be especially difficult among groups where RMR is low, resulting in the potential for excessive weight gain and adiposity, both of which have previously been documented in the FSHD population [5, 6, 44]. This research was designed to further elucidate the extent to which RMR may be altered in the FSHD population **(Aim 3)**, thereby providing translational evidence to support altered nutritional guidelines for clinicians working with this group.

Mechanisms of Resting Metabolic Rate

A complex combination of biologic and environmental factors, including body composition, age, sex, race, hormonal level, physical activity patterns, and diet are theorized to influence RMR, in both healthy and clinical populations. The ways in which these variables may intersect and impact RMR among people with FSHD is a primary focus on my dissertation.

Body composition. Body composition, or the relative proportion of FM and LM, appears to be strongly influential as a primary driver of RMR [99]. According to Sparti et al., LM appears to be especially impactful on RMR, as this parameter has been found to contribute to as much as 70-80% of the variability in metabolic function [20]. While not

consistently observed, some studies have also found that the absolute volume of FM is positively correlated with RMR [100, 101]; most notably, research by Svendsen et al. found that among overweight, post-menopausal women, whole-body FM accounted for approximately 4% (r=0.20) of the inter-individual variability in RMR [100]. In my research, I hypothesized that losses in LM would significantly impact RMR in people with FSHD. As such, the extent to which body composition was altered and the degree to which it influenced RMR among people with FSHD is further discussed in **Chapter 5**.

Age. After the age of 20 years, a progressive decline in RMR of approximately 1-2% per decade, has been reported [102]. Age-related declines in metabolically-active LM are theorized to be a primary contributor to this phenomenon [103], though it is striking that after adjusting for LM, older adults continue to display an RMR that is significantly lower than that seen in younger counterparts [104]. This observation may be at least partially explained by the use of a two-compartment model in many RMR studies, in which the volumes of LM and body organs are combined into the single category of fat-free mass. In fact, while the brain, liver, heart, and kidneys comprise only 7% of fat-free mass, they are believed to account for approximately 60% of RMR in adults [105]; moreover, all of these organs, excluding the heart, have been shown to decrease in mass with advancing age [106]. Therefore, it appears that while decreases in organ size with aging may have a profound impact on metabolism overall, their proportionately small contribution to body composition means that their influence may be "lost" when RMR is normalized to the total volume of fat-free mass. Finally, it has been theorized that age-related declines in RMR may be due not only to changes in the *volume* of fat-free mass, but also, to differences in the *quality* of the LM itself [105]. In fact, research by Cree et al.

shows that among older adults, intramuscular fatty infiltration is increased [107], a phenomenon that is believed to contribute to as much as 32-36% of total variation in muscle strength [108]; additional contributors to muscle dysfunction in aging include a reduced mitochondrial oxidative capacity, a factor which has been associated with muscle strength, quality, and walking speed [109]. Together, these observations show that while metabolism may be loosely associated with fat-free mass, vague generalizations on the relationship do not truly reflect the ways in which the atrophy of particular components of fat-free mass, and the functionality of remaining tissue, affect RMR. Moreover, they show the interconnection of age and body composition, and the ways in which these two factors combine to profoundly impact RMR among people across the lifespan.

Sex. Males have been shown to have an absolute RMR that is as much as 33% higher than age-matched female counterparts [110], thereby highlighting the influence of sex on metabolic rate. As with age, alterations in body composition between males and females appear to be the primary driver of sex-driven differences in metabolic function, as research by Buchholz et al. notes that the adjustment of RMR for volume of LM resulted in a non-significant difference between sexes of only 4% ($p=0.22$) [110]. Moreover, these findings hold true among men and women across a large age range, as previous research has shown that both pre- and postmenopausal women have an RMR that is lower than age-matched male counterparts, even when corrected for body composition and fitness status [111]; while this evidence suggests that sex hormones may have a limited influence on RMR, the implications of this research are far from equivocal. In fact, it has been noted that during the luteal phase of menstruation, pre-menopausal females have a metabolic rate that is higher than post-menopausal counterparts, though this same

observation is not made when post-menopausal females are compared to females in the follicular menstrual phase [112]. Although these observations implicate progesterone as a driver of intra-individual differences in metabolic function in women, it is likely that other sex hormones play a contributory role, as the chronic suppression of E_2 has likewise elicited widespread reductions in RMR among premenopausal women [113]. Finally, differences in muscle fiber composition between males and females may partially explain variations in metabolic rate between sexes, though research on this topic has not been fully elucidated [112]. Due to the low proportion of female participants in the study, sex-driven differences in RMR were not examined; FSHD-control pairs were matched by sex, though I did not control for menstrual cycle phase or menopause status, and therefore, it is possible that hormonal differences may have had an impact, albeit minor, on some outcome measures among female participants.

Race. Race has also been shown to be influential as a mechanistic driver of RMR [114]. While African-Americans have been repeatedly shown to have an RMR that is lower than in non-Hispanic white counterparts [115], it is noteworthy that sex is a strong modifier of this relationship. In fact, research by Weyer et al., in which metabolic rate was measured over a period of 24-hours via direct calorimetry, found that the lower energy expenditure in African Americans occurred primarily in female study participants [116]. As with African American men, Asian adults of both sexes appear to have an RMR that is similar to non-Hispanic whites, even after adjusting for alterations in body composition. Notably, the way in which RMR is reflected among Hispanic males and females, and differences that may exist between this group and non-Hispanic white counterparts, has not been fully elucidated [117].

Physical activity. While RMR is classically measured at rest, a thermic effect of exercise, in which calorie expenditure is increased following participation in physical activity [118], is believed to exert a strong influence on metabolic function [114]. Interestingly, the thermic effect of exercise is believed to be most impactful in the immediate, post-exercise period, though regular exercise participation also appears to have a chronic effect on RMR, even if physical activity has not recently occurred. In fact, Tremblay et al. report that even after adjusting for differences in LM, RMR remains 11% higher in people who are regularly physically active, as compared to those who do not engage in a consistent exercise regimen [119]. Finally, it is noteworthy that a relationship between RMR and VO_2max has been previously reported, an association that is likely mediated by regular participation in structured physical activity [114].

Diet. In addition to physical activity, diet is also believed to have an influence on RMR. This theory is based in part on research by Thompson et al., in which endurance athletes who followed a low-energy diet burned 158 fewer kilocalories per day, than counterparts on an adequate-energy diet, despite being of a similar weight, LM volume, and activity level. Moreover, as the thermic effect of food was similar between groups, it appeared that the disparity in total daily energy expenditure was exclusively due to observations of a lower RMR, among the low-energy diet participants [120]. It is notable that while energy intake appears to be influential as a predictor of RMR in both sexes, it is especially impactful among women, where it has been shown to explain as much as 36% of the variability for this factor [121]. In this study, I did not ask participants to perform a dietary recall, and therefore, it is possible that some disparities in energy intake between individuals may have occurred. I believe that by asking participants to fast for at

least 5 hours before beginning the protocol, diet-related confounding effect to a large extent were minimized.

When viewed together, it becomes clear that the mechanisms which contribute to RMR are highly interwoven, thereby making it difficult to study and understand the "weight" which each individual component may carry. While body composition, for instance, is a known driver of RMR [99], it, in turn, may also be influenced to varying degrees by age, sex, physical activity level, and a combination of other factors. In this dissertation, I have attempted to isolate the effect of primary contributing factors on RMR as much as possible, though it is unlikely that the impact of secondary factors has been completely removed. An identification of the mechanisms which appear to exert the greatest influence on RMR among people with FSHD, and the ways in which these may be interconnected with other biologic and environmental factors, is discussed in further detail in **Chapter 5**.

Clinical Observations of Altered Resting Metabolic Rate

Despite the presence of a reduced volume of LM among dystrophic groups, current research on the ways in which RMR may be affected among people with muscular dystrophy has yielded contradictory findings. Gonzalez-Bermejo et al. have reported that absolute RMR among 20 males (age: 25±4 years) with Duchenne muscular dystrophy was 39% lower than in controls [122]; furthermore, this finding disappeared after the correction of RMR by the total volume of LM [122]. Similarly, my hypothesis is supported from work by Shimizu-Fujiwara et al., in which RMR was significantly lower among people with Duchenne muscular dystrophy vs. controls; notably, as body composition was not assessed in this study, it is difficult to know the degree to which

RMR was affected by the volume of LM [123]. Finally, a lower RMR has been noted among obese males with Duchenne muscular dystrophy, as compared to control counterparts with multifactorial obesity, a condition in which an increased presence of adiposity is believed to be derived from a variety of environmental and biologic factors unrelated to neuromuscular disease [22]. This finding is especially interesting, as it suggests that differences in absolute RMR between dystrophic and control populations may hold true across a variety of body weights and compositions.

While there is strong evidence to support my hypothesis that RMR is lower in people with FSHD, it is important to note that paradoxical findings have been reported in other muscular dystrophy phenotypes. For example, Okada et al. noted that people with Duchenne muscular dystrophy have an absolute basal metabolic rate that is 20-30% higher than documented normative values for individuals of a similar age and sex [23]. Additionally, Jacques et al. have reported an RMR that does not differ between people with Becker muscular dystrophy (n=21) and controls (n=12) [21], though it is possible that a high-degree of variability and small sample size could explain these findings. Similarly, this powering-error may explain why Vaisman et al. did not find differences in RMR between young males with Emery-Dreifuss muscular dystrophy (n=6) and controls (n=4), despite observations of a caloric expenditure that was greater in the dystrophic group, following normalization to LM [124]. Another explanation for these inconsistent findings may be related to wide-ranging inter-individual variability and/or altered physiologic function within dystrophic subgroups.

Despite the relative abundance of research on RMR among people with various alternative forms of muscular dystrophy, a targeted focus on this relationship in adults

with FSHD appears to be lacking. In fact, existing evidence within this population appears to be found only in animal research, whereby mice with FSHD demonstrated periodic episodes of hypermetabolism, as characterized by alternating phases of high and precipitously low volumes of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) [125]. As such, the research outlined in **Chapter 5** of this work is especially important, as it is the first to define the ways in which RMR is affected in humans with FSHD. Furthermore, I believe that the formal documentation of alterations in metabolic function could serve as the first step in the development of more accurate nutritional guidelines, such as those designed to promote the preservation of LM and assist in lowering morbidity and mortality rates.

Conclusions and Aims of Thesis Work

FSHD is a complex genetic disease, with implications ranging from the molecular to the whole-body level. In fact, individuals with FSHD demonstrate wide-ranging anatomic alterations, including those associated with the presence of sarcopenic obesity. Furthermore, the consequence of these changes in body composition appear to coincide with disadvantageous physiologic effects, including a propensity towards exercise intolerance and the potential for impaired metabolic function. To further investigate these observations, three aims were developed and studied:

AIM 1: Identify differences in the presence of sarcopenic obesity between individuals with FSHD and controls. People with FSHD have been shown to have a higher propensity towards LM atrophy, and a greater proportion of overall adiposity, as compared to controls. Local and regional measures of LM and FM were collected, and markers of sarcopenic obesity were compared between groups. *My primary hypothesis*

was that the anatomic characteristics associated with the presence of sarcopenic obesity would be more pronounced among people with FSHD, thereby making it more likely that diagnostic criteria for the condition would be met in the clinical group.

AIM 2: Explore variations in the severity of and mechanistic contributions to exercise tolerance, between adults with FSHD, and control groups. Exercise intolerance has been observed in other clinical groups, whereby the phenomenon has been linked to measures of body composition. Peak VO_2 and symptoms of exercise intolerance, including fatigue and dyspnea, were compared between people with FSHD and age- and sex-matched controls; to assess the extent to which measures of LM contribute to exercise intolerance, a regression model was used. *My primary hypothesis* was that rates of exercise intolerance would be greater among people with FSHD. My *secondary hypothesis* was that among people with FSHD, exercise intolerance would be driven by disease-related LM atrophy, though this relationship would not necessarily be present among controls.

AIM 3: Identify differences in RMR between individuals with FSHD and controls. FSHD has been shown to cause atrophy of LM, a key component of body composition and primary contributor to RMR. RMR was measured with indirect calorimetry and compared to those of age- and sex-matched controls; RMR values were also normalized to total and regional measures of LM and FM, to account for the influence of LM on caloric expenditure. My *primary hypothesis* was that absolute RMR would be lower among people with FSHD, due to a lower volume of LM. My *secondary hypothesis* was that the normalization of RMR, whereby the number of calories burned per gram of LM was calculated, would be similar between people with FSHD and control counterparts.

Chapter 3: Sarcopenic Obesity in Facioscapulohumeral Muscular Dystrophy

The contents of this chapter have been published in Frontiers in Physiology (August 2020).

OVERVIEW

Sarcopenic obesity has been observed in people with neuromuscular impairment and is linked to adverse health outcomes. It is unclear however, if sarcopenic obesity develops in adults with FSHD. The work in this chapter was designed to determine if adults with FSHD meet criteria for sarcopenic obesity, as reflected by ALMI index scores of <7.26 kg/m^2 or 5.45 kg/m^2, and corresponding proportions of FM of ≥28% or 40% in men/women, respectively. Ten people with FSHD (50±11 years, 2 females) and ten age/sex-matched controls (47±13 years, 2 females) completed one visit, which included a full-body DXA scan. Regional and whole body total mass, FM, and LM were collected, and BMI and sarcopenia measures were computed.

People with FSHD and controls had a similar whole body total mass (84.5±12.9 vs. 81.8±13.5 kg, respectively; p=0.65). Though BMI was 2% lower in the FSHD group (p=0.77), the relative proportion of FM was 46% higher in FSHD, compared with controls (p<0.01). FSHD participants also exhibited a greater absolute volume of total body FM (p<0.01) and total leg FM (p<0.01) but were similar in volume of total arm FM compared with controls (p=0.09). The absolute volume of whole-body LM trended to be lower in FSHD vs. controls (p=0.05), and arm and leg LM were both lower in FSHD compared with controls (p<0.05). In addition, ALM volume was 23% lower (p=0.02) and ALMI was 27% lower in FSHD compared with controls (p<0.01). Furthermore, the

relative proportion of whole-body LM was 18% lower in FSHD vs. controls ($p<0.01$). Overall, this chapter will show that people with FSHD, although similar in BMI and total body mass compared with controls, commonly meet the definition of sarcopenic obesity. Adults with co-existing FSHD and sarcopenic obesity may be at risk for significant impairments in quality of life and encounter additional challenges in the management of FSHD manifestations.

INTRODUCTION

FSHD is one of the most common dominantly-inherited muscular dystrophies, with prevalence frequencies ranging from 1:15,000 to 1:21,000 worldwide [126]. Classically, FSHD manifests in progressive, often asymmetrical muscular weakness, most prominently in the face, shoulder girdle, and upper-arm region [1, 3]. Unlike other forms of muscular dystrophy, in which the presence of cardiomyopathy and pulmonary impairment frequently results in a heightened mortality rate [127], life expectancy among individuals with FSHD appears to be preserved [1]. However, impairments in functional capacity, as measured by a decreased capacity for independent ambulation [3], and a greater reliance on assistive devices among older adults with FSHD (>50 years), have been reported in this population [128]. It is speculated that this functional impairment may be partially driven by alterations in body composition, which likewise have been linked to high rates of LM atrophy [1, 3] and corresponding increases in fatty tissue infiltration of the muscular compartments [6]. For example, Janssen et al. demonstrated that up to 26% of individuals with FSHD may experience severe rates of fatty infiltration, whereby as much as 75% of LM in certain muscular compartments is replaced by FM [6], an observation that may have clinical and functional implications for people with FSHD.

With the manifestation of LM atrophy and increased proportion of FM, it is likely that people with FSHD exhibit a medical condition known as sarcopenic obesity. Sarcopenic obesity combines the key features of sarcopenia (losses in LM, declining strength, and/or impairments in physical performance [129]) with an increased presence of adiposity [130]. Adults who meet diagnostic criteria for sarcopenic obesity have been reported to exhibit an increased risk of mortality (hazard ratio: 1.44) as compared to control counterparts [82], and show a greater propensity towards physical disability [80]. It is believed that sarcopenic obesity contributes to physical disability via a combination of concurrent changes, including the loss and atrophy of type II muscle fibers [87] and a greater propensity towards fatty infiltration of skeletal muscle [91]. These LM alterations lead to impairments in the contractile strength of type I and II muscle fibers [50], and overall reductions in muscular power.

While sarcopenic obesity has been noted among individuals with various types of muscular dystrophy (i.e., Duchenne, Becker, and Ullrich congenital muscular dystrophies [42]), it is unclear if people with FSHD exhibit this condition. By identifying sarcopenic obesity as a potential comorbidity of FSHD, the development of effective preventative and therapeutic strategies designed to address the condition may be incorporated as part of the medical treatment plan, thereby leading to gains in functional capacity, a greater ability to perform activities of daily living, and an overall improvement in quality of life. Therefore, we aimed to determine if people with FSHD meet the diagnostic criteria for sarcopenic obesity. We hypothesized that the anatomic characteristics associated with the presence of sarcopenic obesity are more pronounced among people with FSHD, as compared to age- and sex-matched controls.

MATERIALS AND METHODS

Subjects

Ten adults with genetically-confirmed FSHD and ten age- and sex-matched healthy control participants (n=20 combined; men: 16, women: 4) completed the study. Inclusion criteria consisted of an age of ≥18 years, and no prior history of cardiovascular, pulmonary, orthopedic, or neuromuscular disorders other than FSHD; female participants were excluded if they were currently pregnant or breastfeeding [131, 132]. Physical activity levels were assessed via the Modified Minnesota Leisure Time Physical Activity Questionnaire, and reported as an activity metabolic index score [133]. Severity of disease burden and perceptions of functional ability among individuals with FSHD was evaluated through completion of the FSHD Health Index survey, a self-reported measure, whereby a score of 100 reflects the highest degree of disease-related impairment, and 0 reflects no disease-related impairment [134, 135]. The study was approved by the University of Minnesota Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

Experimental Protocol

Study participants attended one study session, which included a written informed consent following a description of the study design and a DXA scan (Lunar iDXA, GE Healthcare, Chicago, IL, USA); female participants took a urine human chorionic gonadotropin (hCG) test (Clinical Guard, Atlanta, GA, USA) to confirm the absence of pregnancy.

Data Collection Techniques

Body composition was obtained from the DXA scan; an estimation of regional and whole body total mass (g), FM (g, %), LM (g, %), and bone mineral content (g) was provided by enCORE v16 (GE Healthcare, Chicago, IL, USA). As FSHD is primarily a disease that affects the upper extremity, differences between upper and lower LM and FM were also obtained. ALM was quantified as the sum of fat- and bone-free tissue in the arms and legs, and was normalized to height to control for fluctuations in body size [42]. An ALMI index [ALM weight (kilograms (kg))/height^2 (meters (m), m^2)] was utilized as an index of sarcopenia [42], whereby the presence of sarcopenia was defined by an ALMI that is two standard-deviations lower than ALMI from the means observed in sex-specific reference groups [15]. Sarcopenic obesity was defined by the combined presentation of an ALMI of <7.26 kg/m^2 and proportion of whole-body FM to whole-body total mass (% FM) of >28%, or an ALMI of <5.45 kg/m^2 and % FM of >40%, in men and women, respectively [15]. BMI was calculated from manual measurements of height (m) and weight (kg); study participants were categorized by BMI status into standard body composition categories (World Health Organization, 2019).

Statistical Analysis

Data is reported as group averages (mean ± standard deviation), distribution normality was assessed and parametric vs. non-parametric methods were used as appropriate. Independent samples t-tests were used to compare differences in body composition between FSHD and control participants; in cases where the data was not normally distributed, the Mann-Whitney U test was performed. Pearson product moment correlation was used to determine relationships between continuous variables. Statistical

analyses were performed using SPSS v24.0 (SPSS, Inc., Chicago, IL, USA) with significance defined as an α-level of $p<0.05$ for all comparisons.

RESULTS

Subject Characteristics

FSHD and control participants were similar in age, weight, height, and BMI ($p>0.05$ for all, **Table 3.1**). In the FSHD group, all 10 participants self-reported as non-Hispanic white; among controls, self-reported race was as follows: non-Hispanic white: 7 (5 men, 2 women), Black: 1, Asian: 1, Hispanic: 1. Overall, the whole body total mass [calculated as sum of whole-body FM (kg), whole-body LM (kg), and bone mineral content (kg)] of individuals with FSHD was similar to that of healthy controls (84.5 ± 12.9 vs 81.8 ± 13.5 kg, respectively, p=0.65, **Table 3.2**).

Measures of Sarcopenic Obesity

Adults with FSHD were found to have an ALM that was 23% lower, as compared to the control group (p=0.02, **Table 3.2**). This observation was further accompanied by an ALMI score that was 27% lower among individuals with FSHD, as compared to healthy controls ($p<0.01$, **Figure 3.1**). Furthermore, % FM was 46% greater in FSHD, compared with controls ($p<0.01$, **Figure 3.2**). Mean alterations in ALMI (6.3 ±1.3 kg/m^2) and % FM (40.0 ± 6.4%) among men with FSHD were sufficient to meet the diagnostic criteria for sarcopenic obesity; furthermore, six of eight men with FSHD were individually found to meet compositional requirements for the condition. Conversely, the same criteria were not met in female FSHD counterparts (ALMI: 6.2 ±1.0 kg/m^2, % FM: 44.1 ±11.4%), and neither of the two FSHD females individually met the diagnostic requirements. Sarcopenic obesity was not observed in any of the control participants.

	FSHD	Range (FSHD)	Control	Range (Control)	Significance (*p*-value)
Age (years)					
Males and females combined	50±11	36-72	47±13	31-75	0.60
Males	51±12	36-72	48±14	31-75	0.68
Females	45±9	38-51	41±14	31-51	0.80
Weight (kg)					
Males and females combined	85.4±12.9	65.5-105.7	81.8±13.4	65.3-103.7	0.55
Males	86.8±12.0	67.9-105.7	81.6±15.1	65.3-103.7	0.46
Females	79.8±20.1	65.5-94.0	82.7±4.5	79.5-85.9	0.87
Height (m)					
Males and females combined	1.80±0.07	1.67-1.91	1.74±0.08	1.63-1.78	0.09
Males	1.84±0.04	1.78-1.91	1.76±0.09	1.68-1.87	0.06
Females	1.70±0.04	1.67-1.73	1.70±0.10	1.63-1.78	0.95
BMI (kg/m^2)					
Males and females combined	26.1±4.4	21.4-33.7	26.7±3.6	21.5-32.2	0.77
Males	25.7±3.7	21.4-29.9	26.2±3.4	21.5-31.8	0.80
Females	27.8±8.3	21.9-33.7	28.7±5.0	25.1-32.2	0.92

Table 3.1: Subject characteristics (mean±SD). kg, kilograms, m, meters; $p<0.05$.

	FSHD	Range (FSHD)	Control	Range (Control)	Significance (*p*-value)
Measures of Sarcopenia					
ALM (kg)					
Males and females combined	20.5±4.4	15.3±27.5	26.5±5.9	7.2-12.0	0.02
Males	21.2±4.7	15.3±27.5	27.7±6.1	7.3-12.0	0.03
Females	17.7±1.9	16.4±19.1	22.0±1.1	7.2-8.0	0.15
Additional Measures of Lean Mass					
Whole-body lean mass (kg)					
Males and females combined	47.6±6.0	40.5-57.3	56.6±11.1	45.9-78.8	0.05
Males	49.1±5.8	40.5-55.0	58.8±11.5	45.9-78.8	0.05
Females	41.7±1.7	42.9-57.3	47.7±0.6	47.2-48.1	0.10
Total arms lean mass (kg)					
Males and females combined	5.4±1.1	43.9-73.9	7.5±2.1	4.4-12.5	<0.01
Males	5.7±1.1	44.6-73.9	8.1±2.1	5.1-12.5	0.01
Females	4.4±0.04	43.9-44.5	5.5±0.6	4.4-5.9	0.22
Total legs lean mass (kg)					
Males and females combined	15.1±3.5	10.8-20.5	19.0±3.9	14.0-26.5	0.03
Males	15.5±3.8	10.8-20.5	19.6±4.2	14.0-16.5	0.06
Females	13.3±2.0	11.9-14.7	16.4±0.6	16.0-16.8	0.25
Additional Measures of Adiposity					
Whole-body fat mass (kg)					
Males and females combined	33.7±10.1	21.8-49.6	22.0±6.9	11.2-49.6	<0.01
Males	33.5±9.4	21.8-49.6	19.7±5.3	11.2-49.6	<0.01
Females	34.7±17.0	22.7-46.7	31.1±4.9	27.7-34.7	0.82
Total arms fat mass (kg)					
Males and females combined	3.2±0.9	2.1-5.0	2.5±0.9	1.4-4.9	0.09
Males	3.1±0.9	2.1-5.0	2.2±0.5	1.4-4.9	0.02
Females	3.4±1.2	2.5-4.3	3.8±0.6	3.4-4.2	0.75
Total legs fat mass (kg)					
Males and females combined	10.7±2.4	7.2-14.9	6.0±1.6	3.1-12.1	<0.01
Males	10.3±2.1	7.2-13.1	5.4±1.1	3.1-12.1	<0.01
Females	12.2±3.9	9.4-14.9	8.5±0.1	8.4-8.5	0.40

Table 3.2. Measures of body composition. When males and females are combined, individuals with FSHD had lower ALM, arm LM and leg LM, but greater body and leg FM. When separated by sex, only males with FSHD exhibited a significant difference in these measures. kg: kilogram; data are shown in mean±SD.

Additional Measures of Lean Mass

Additional measures of LM are located in **Table 3.2**. The absolute volume of whole-body LM was 15% lower in FSHD, compared with controls, trending towards significance (p=0.05). In addition, individuals with FSHD demonstrated a relative proportion of whole-body LM to whole-body total mass (% LM) that was 18% lower, compared with controls (p<0.01, **Figure 3.3**). Furthermore, both the absolute volume of total arm (p<0.01) and total leg (p=0.03) LM were lower in FSHD by 29% and 21%, respectively, as compared with controls.

Additional Measures of Adiposity

Additional measures of adiposity are located in **Table 3.2.** Absolute volume of whole-body FM was 53% greater in FSHD compared with controls (p<0.01). While the

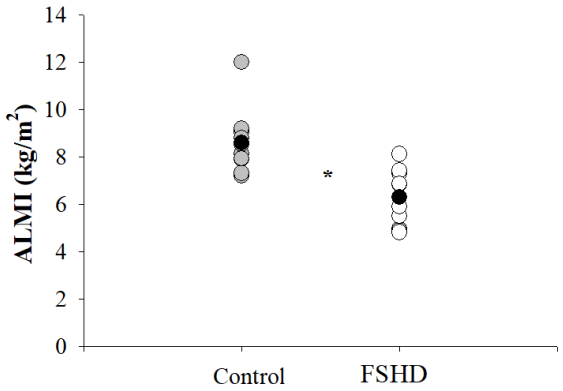


Figure 3.1. Appendicular lean mass index (ALMI, kg/m^2) in FSHD and controls. Individuals with FSHD had lower LM, as compared to controls. Black circles indicate average data for each group. *p<0.01.

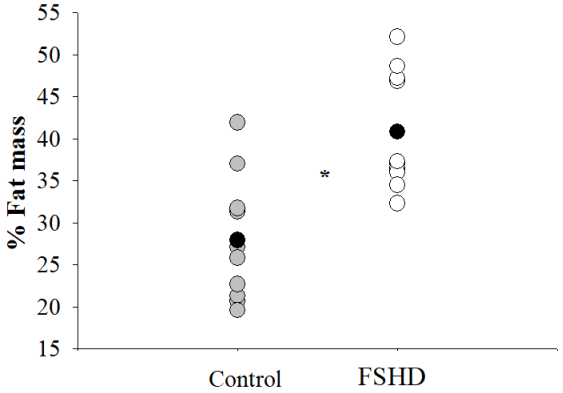


Figure 3.2. % fat mass (FM) in FSHD and controls. Individuals with FSHD had a higher % FM, as compared to controls. Black circles indicate average data for each group. *p<0.01.

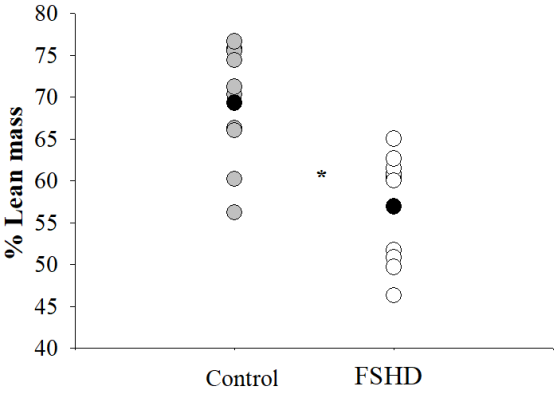


Figure 3.3. % lean mass in FSHD and controls. Individuals with FSHD had a lower % LM, as compared to controls. Black circles indicate average data for each group. *p<0.01.

absolute volume of total leg FM among individuals with FSHD was 78% greater ($p<0.01$), the arms were somewhat less affected, with mean total arm FM only 28% greater in the FSHD group, which did not reach statistical significance ($p=0.09$).

Self-Reported Measures of Functional Ability and Severity of Disease

Self-reported FSHD Health Index (HI) scores are located in **Table 3.3**, and range in value from 8.0 to 53.4 arbitrary units. Total FSHD-HI ($r=-0.60$), mobility and ambulation ($r=-0.50$), and activity limitation ($r=-0.62$) trended to be correlated with ALMI ($p=0.07$), but not % FM or BMI ($p>0.05$ for all). Activity limitation also trended to be correlated with age ($r=0.62$, $p=0.06$). Other self-reported measures included an attenuated amount of physical activity completed each day among individuals with FSHD (activity metabolic index score; FSHD: 28.0±33.6 kcal/day, control: 184.3±152.7 kcal/day; $p<0.01$). Activity metabolic index score was not related to measures of body composition (% FM, ALMI) in either FSHD or control groups ($p>0.05$ for all). Conversely, the same criteria were not met in female FSHD counterparts (ALMI: 6.2 ±1.0 kg/m^2, % FM: 44.1 ±11.4%), and neither of the two FSHD females individually met

FSHD Health Index Category	Mean Score (n=10)
Shoulder and arm function	53.4±27.4
Mobility and ambulation	48.3±22.3
Fatigue	44.9±15.9
Social performance	44.0±24.6
Core strength and function	43.0±19.8
Activity limitation	40.1±14.6
Body image	35.0±22.1
Social satisfaction	33.1±22.8
Emotional health	28.6±18.5
Pain	19.9±11.8
Hand and finger function	19.7±22.3
Communication	19.5±19.9
Gastrointestinal function	10.3±14.8
Cognitive function	8.0±12.8
Total FSHD-HI Score	20.1±8.8

Table 3.3. FSHD Health Index (HI) Survey scores (mean ±SD).

the diagnostic requirements. Sarcopenic obesity was not observed in any of the control participants.

DISCUSSION

This study is the first to confirm the presence of sarcopenic obesity among individuals with FSHD, as reflected by a mean ALMI and % FM of <7.26 kg/m^2 and >28% in afflicted males; furthermore, we are the first to show that individuals with FSHD exhibit the anatomic characteristics of sarcopenic obesity more often than age- and sex-matched controls. These observations are consistent with previous reports of significant alterations in body composition in people with FSHD, including widespread increases in measures of adiposity and reductions in LM [5, 6, 136].

Sarcopenic Obesity in Muscular Dystrophy

Sarcopenic obesity is commonly found in other forms of muscular dystrophy, but has yet to be observed in FSHD. In fact, previous research has confirmed the presence of sarcopenic obesity among individuals with Bethlem myopathy, Ullrich congenital muscular dystrophy, rigid spine syndrome, limb girdle MD type 2d, Duchenne MD, and Becker MD [42, 92, 93]. Consistent with our findings, alterations in body composition – including greater FM and lower LM— have been reported among individuals with FSHD [5]. In fact, Skalsky et al. noted a whole-body LM value that was 17% lower in people with FSHD, as compared to control groups, a value which nearly mirrored our own observations (whole-body LM 15% lower in FSHD group) [5]. Additionally, our findings of a % FM that was 46% greater among people with FSHD was similar to that reported by Skalsky et al., in which % FM was found to be 41% higher in the clinical group [5]. Interestingly, despite observations of a higher volume of FM, these authors noted a BMI

that was similar between groups [5], a finding that was consistent with our study. Also in line with our research, Skalsky et al. reported significantly lower LM in the arms and legs. Similarly, observations of a greater volume of FM in the legs, but not in the arms, was also documented among people with FSHD [5]. It is worthwhile to note that the DXA scanner utilized in the Skalsky et al. study compartmentalized the limbs into upper and lower portions (arm, forearm, thigh, leg), while the device used in our research provided more generalized values (arms, legs). Therefore, drawing exact comparisons between appendicular measures of LM and FM between the two studies is not possible. Finally, as Skalsky et al. did not assess ALM or ALMI in their study, we are not able to determine whether FSHD study participants met the diagnostic criteria for sarcopenic obesity, though as mean values of % FM in the FSHD group did not meet minimal threshold values (mean: 25.41±8.33%) [5], the presence of the condition within this group of FSHD participants appears unlikely.

While the presence of sarcopenic obesity was only observed among male FSHD participants in our research, other studies have documented the condition among both sexes, in alternative forms of muscular dystrophy. In fact, in work by Miscione et al., in which 8 participants (male: 3, female: 5) with either Bethlem myopathy or Ullrich congenital muscular dystrophy were studied, sarcopenic obesity was confirmed in two of three males and all five females. Although the sample size was low in Miscione et al, they had three additional females, with all five meeting the criteria for sarcopenic obesity. Neither of our two females met the criteria which could indicate that the presence of sarcopenic obesity in different forms of muscular dystrophy is not equivocal. Notably, in adults without muscular dystrophy, it has been shown that females may be less, more, or

as likely to develop sarcopenic obesity, as their male counterparts [137]. Among whites, which formed the majority of our study (85%), rates of sarcopenic obesity are similar between age-matched males and females [137]. However, sex-specific differences in FSHD phenotype presentation, in which females appear to be less affected than their male counterparts, have been reported [138, 139]. In our study, an elevated mean % FM of 44.1±1.0% in the two females with FSHD did result in the fulfillment of one of the two objective requirements to meet the criteria for sarcopenic obesity (% FM: >40%). However, we also observed a mean ALMI of 6.2±1.0 kg/m^2 in the females with FSHD, a value that exceeds the minimum threshold associated with presentation of the condition (ALMI: <5.45 kg/m^2). Therefore, although neither of the two females reached the diagnostic criteria for sarcopenic obesity, the small sample size in this study precludes any speculation regarding sex differences in sarcopenic obesity in adults with FSHD.

Clinical Relevance of Sarcopenic Obesity

Identifying the presence of sarcopenic obesity in adults with FSHD is of high significance, as it may indicate an increased propensity towards greater impairments in functional capacity, and greater risk of morbidity and mortality. According to the Concord Health and Aging Project, sarcopenic obesity is linked to an increased risk of frailty and instrumental activity of daily living disability, a measure that is characterized by an inability to perform tasks for independent living [16]. Furthermore, research by Baumgartner notes that older men (>60 years of age) with sarcopenic obesity are eight times more likely to develop three or more disabilities than age- and sex-matched controls [80]. This observation is even more striking among older females with sarcopenic obesity, in which the risk for multiple disabilities was increased by a factor of 11 [80]. In

addition, the relationship between impaired physical function and sarcopenic obesity appears to be stronger than an association with either obesity or sarcopenia alone [80], thereby highlighting the cumulative effect of these factors on functionality in an aging population. Overall, these reports are in line with our own findings, in which a trending relationship between markers of sarcopenic obesity and self-reported impairments in mobility and ambulation or activity limitation was observed. While it is difficult to know whether sarcopenic obesity is more influential than FSHD when it comes to the etiology of physical impairment, these observations suggest that, at a minimum, it likely compounds the physical disability that individuals with FSHD already experience [140].

While DXA scanning is widely believed to be the gold-standard in body composition assessment, BMI charts are frequently used to estimate % FM in community settings and among older adults [141, 142]. However, the relationship between % FM and BMI is believed to be significantly influenced by age and sex, and may not be the best indicator of total body fatness [143]. In our research, BMI was similar between FSHD and controls, but FM was significantly greater in people with FSHD. Furthermore, despite trending relationships between measures of sarcopenic obesity and physical function, an association between BMI and these same parameters was absent. Together, these findings cast doubt on the relevancy of using BMI as a valid tool for physical and functional assessment among people with FSHD. In final, DXA is a cost-effective and time efficient imaging modality that can be utilized as a measurement of disease severity and disease progression for future clinical trials.

Mechanisms Contributing to Sarcopenic Obesity

Though complex in nature, sarcopenic obesity is believed to be driven by a synergistic combination of biologic, hormonal, and behavioral influences. In fact, the atrophy and loss of type II muscle fibers – a phenomenon that is believed to be predominantly responsible for the presentation of sarcopenia [87]—is reportedly caused by an amalgamation of factors, including neurodegenerative processes within spinal α-motor neurons, dysregulation of anabolic hormone production (insulin, growth, and sex hormones), and inadequate nutritional intake [87]. Furthermore, sarcopenia is believed to be mediated by a deconditioned state [87], and the presence of a significantly reduced physical activity score in the FSHD cohort in our study suggests that deconditioning may have been a precipitating factor in the manifestation of sarcopenia. It is worthwhile to note that while individuals with FSHD have been reported to exhibit reductions in both type IIa and IIx muscle fibers [41], it is unclear whether this phenomenon is caused by the factors described above, or is purely an intrinsic result of the disease itself. As such, individuals with FSHD exhibit indirect evidence of DUX4 protein expression in muscle biopsies [144], which leads to an inability to properly replace diseased or damaged muscle tissue with new myofibers [145] which may lead to a pro-adipogenic state within muscle of adults with FSHD. In addition, physical inactivity has been widely cited as a contributor in the development of obesity [146]. Observations of physical inactivity have been noted in other forms of muscular dystrophy, whereby 44% of people with limb-girdle or Charcot-Marie-Tooth muscular dystrophy exhibit an inability to meet minimum threshold recommendations for daily exercise [147]. Thus, the physical inactivity demonstrated in our FSHD cohort likely not only contributed to sarcopenia, but also obesity. The lack of a correlation between either physical activity and % FM ($p=0.79$) or physical activity and

ALMI (p=0.15), among people with FSHD, however, suggests that the inherent influence of FSHD likely plays a significant role in the etiology of sarcopenic obesity.

Limitations

Limitations of this study should be considered when interpreting the data. The small sample size in female participants, likely contributed to an inability for the females on average to meet the diagnostic criteria for sarcopenic obesity. Previous research by Miscione et al. has confirmed the presence of sarcopenic obesity in both male and female dystrophic groups, but not specifically in FSHD [93]. Furthermore, as we did not control for clinical severity within the FSHD group, it is possible that our female FSHD participants exhibited a lesser-degree of disease than their male counterparts, whereby the presence of anatomic alterations in body composition were not yet manifest, a theory which has been supported by previous research [138, 139]. Since our study used a DXA scan and not MRI imaging to assess body composition, we were unable to assess whether differences in % FM between FSHD and control groups were driven by general increases in adiposity, or by intramuscular fat infiltration, a finding which has been previously reported [6], and which is believed to be a hallmark characteristic of FSHD. Finally, while all FSHD study members were Caucasian, 3 of 10 control participants (all men) were of differing races, a factor that may have a confounding effect on study outcomes. Though alterations in body composition between races have been widely reported [148], it appears that these differences may be driven by sex, whereby variances are noted primarily among female racial groups [149]. According to research by Gerace et al., measures of total body FM and fat-free mass are similar between Black and non-Hispanic white males [150], though whether the same is true between white and Hispanic or Asian males remains to be

elucidated. Finally, because of the case-control study design, we are unable to establish a causative relationship between FSHD and presence of sarcopenic obesity.

CONCLUSION AND NEXT STEPS

In this chapter, I have shown that men with FSHD meet the diagnostic criteria for sarcopenic obesity more often than age-matched controls. Furthermore, this chapter has highlighted the complex nature of sarcopenic obesity, which may contribute to a number of both acute and long-term implications. Identifying individuals that may be at an increased risk for sarcopenic obesity will lead to preventative rehabilitative strategies to reduce the prevalence of the condition, among individuals of all ages and health statuses. Similarly, by identifying sarcopenic obesity as a comorbidity of FSHD, the ways in which anatomic and physiologic alterations may contribute to impaired health and physical function in this genetic disease will be better understood. In fact, this area will be explored in greater detail in chapter 4, whereby I detail research investigating the influence of sarcopenic obesity on the development of exercise intolerance, among people with FSHD. Future research in this area should focus on strategies (i.e., exercise) to address the sarcopenic obesity-driven losses in functionality and improve quality of life among individuals with neuromuscular impairment.

Chapter 4: Exercise Intolerance in Facioscapulohumeral Muscular Dystrophy

The contents of this chapter are in review at the Medicine & Science in Sports and Exercise Journal.

OVERVIEW

This chapter is designed to determine whether adults with FSHD exhibit exercise intolerance, and if so, to identify potential disease-specific contributing mechanisms to exercise intolerance. For this purpose, eleven people with FSHD (47±13 years, 4 females) and eleven controls (46±13 years, 4 females) completed one visit, which included a volitional VO_{2peak} test. Breath-by-breath gas exchange, V_E and cardiovascular responses were measured at rest and during exercise. The test was composed of three-minute stages (speed: 65-70 revolutions per minute) with an incremental increase in intensity (FSHD: 20-watts/stage; control: 40 to 60-watts/stage). Body LM (LM (kg, %)) was collected via DXA scan.

VO_{2peak} was 32% lower (24.5±9.7 vs, 36.2 ±9.3 mL/kg/min; $p<0.01$) and wattage was 55% lower in FSHD (112.7±56.1 vs. 252.7±67.7 watts; $p<0.01$). When working at a relative submaximal intensity (40% of VO_{2peak}), wattage was 55% lower in FSHD (41.8±30.3 vs. 92.7±32.6 watts, $p=0.01$), though ratings of perceived exertion (RPE) (FSHD: 11±2 vs. control: 10±3, $p=0.61$), and dyspnea (FSHD: 3±1 vs. control: 3±2, $p=0.78$) were similar between groups. At an absolute intensity (60-watts), RPE was 63% higher (13±3 vs. 8±2, $p<0.01$) and dyspnea was 180% higher in FSHD (4±2 vs. 2±2, $p<0.01$). VO_{2peak} was most strongly correlated with O_2 pulse in controls ($p<0.01$, r=0.90) and %leg LM in FSHD ($p<0.01$; r=0.88). Among FSHD participants, VO_{2peak} was associated with self-reported functionality (FSHD-HI score; activity limitation: $p<0.01$,

r=-0.78), indicating a strong association between perceived and objective impairments. Overall, this chapter shows that disease-driven losses of LM contribute to exercise intolerance in FSHD, as evidenced by a lower VO_{2peak} and elevated symptoms of dyspnea and fatigue during submaximal exercise. Participation in resistance-based exercise may aid in preserving both LM and exercise tolerance in this population.

INTRODUCTION

Facioscapulohumeral muscular dystrophy (FSHD) is a dominantly-inherited myopathy, resulting in progressive, often asymmetrical muscular atrophy and weakness, most severely expressed in the face, shoulder girdle, and upper-arm region of affected individuals [1, 3]. Among people with FSHD, the loss of lean mass (LM) manifests physiologically in a reduced functional capacity [75], or "an individual's ability to perform work [75]." Therefore, adults with FSHD have the potential for exercise intolerance, a condition which has been characterized by exertional fatigue, labored breathing (dyspnea) during exercise, and an inability to meet age- and sex-predicted values of physical performance [17]. Exercise intolerance can be driven by a combination of individual factors, such as those which stem from neural, hemodynamic, and peripheral causes, and include low cardiac output (Q) and stroke volume [151, 152], impaired pulmonary function [152], alterations in the absolute and proportional volumes of adipose and muscle tissue [67-69], and skeletal muscle myopathies [153-155]. Further, exercise intolerance is a strong indicator of mortality in certain clinical groups [30, 59, 79], making its identification valuable in guiding medical care in a high-risk dystrophic population.

While exercise intolerance has already been demonstrated in the elderly [15] and among people with chronic disease [9, 80], its presence among people with FSHD has not yet

been defined. Notably, the well-defined alterations in LM [44], and the subsequent contribution of this marker on exercise tolerance [67-69], means that the phenomenon is likely present within this population. The purpose of this study, therefore, is to investigate whether exercise intolerance is more pronounced among people with FSHD, as compared with a control group; secondly, we also aim to determine whether markers of LM can predict exercise intolerance among people with FSHD. Based on the influence of body composition [44], we hypothesize that measures of exercise intolerance will be higher in the FSHD group, as compared to age- and sex-matched controls. Furthermore, we believe that disparate mechanisms will contribute to exercise intolerance between adults with FSHD and controls. These findings will serve as an important foundation in developing rehabilitative strategies designed to improve functional performance and quality-of-life in the FSHD population.

METHODS

Subjects

Eleven people with genetically confirmed FSHD (47±13 years) and eleven age- and sex-matched control participants (46±13 years) (n=22 combined; males: 14, females: 8) completed the study. Inclusion criteria included an age of ≥18 years, and no prior history of cardiovascular, pulmonary, orthopedic, or neuromuscular disorders other than FSHD; female participants were excluded if they were currently pregnant or breastfeeding [131, 132]. Physical activity level was calculated via the Minnesota Leisure Time Physical Activity Questionnaire [133], and reflected as an activity metabolic index score. The FSHD Health Index survey was used to compute the severity of disease burden, whereby a score of 100 reflects the highest disease, and 0 reflects no disease

burden [134, 135]. The study was approved by the University of Minnesota Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

Experimental Protocol

Participants completed one experimental session, during which a description of study design was provided, and written informed consent was obtained. During the experimental session, participants completed a volitional peak exercise protocol on an upright stationary bicycle. The exercise protocol included three-minutes of a baseline rest period, followed by three-minute graded stages at a speed of 65-70 revolutions per minute, and of incrementally increasing intensity (FSHD wattage: 20-watts/stage; control wattage: 40 to 60-watts/stage). As study participants completed protocols featuring differing workloads based on FSHD group, sex, and perceived fitness level, measures of exercise intolerance were compared 1) at an absolute workload of 60 watts (*n*=17, FSHD: 9, 2 female; Control: 8, 2 female), 2) at a relative workload corresponding with the stage at which they achieved 40% of their VO_{2peak} (*n*=22, FSHD: 11, 4 female; Control: 11, 4 female), and 3) at VO_{2peak}). The test was terminated when the participants exhibited either a plateau in VO_2 or could no longer maintain cadence speed.

Physiologic Monitoring and Data Collection

Breath-by-breath gas exchange and ventilation were collected throughout rest and during exercise with a Medgraphics metabolic cart (Ultima System CardiO_2, Medical Graphic, St. Paul, MN, USA). The gas analyzer was calibrated according to manufacture guidelines before each test, using calibration gases of 5% carbon dioxide, 12% oxygen, and balanced nitrogen. Gas volumes were measured through a Prevent® flow sensor using a 3-Liter calibration syringe and corrected for ambient conditions prior to each test.

The V_E equivalent to carbon dioxide production (VCO_2, V_E/VCO_2 slope) was calculated and used as a surrogate measure of ventilatory efficiency, whereby:

$$V_E/VCO_2 \text{ slope} = \frac{V_{EExercise} - V_{EBaseline}}{VCO_{2Exercise} - VCO_{2Baseline}}$$

Heart rate was measured via 12-lead ECG; blood pressure was measured manually through sphygmomanometry at the end of each exercise stage. Dyspnea and rating of perceived exertion (RPE; scale 6-20) were measured at the end of each stage during the exercise protocol. To account for the influence of LM on exercise intolerance, a total body DXA scan (Lunar iDXA, GE Healthcare, Chicago, IL, USA) was conducted. Female participants completed a urine human chorionic gonadotropin test (Clinical Guard, Atlanta, GA, USA) to determine they were not pregnant.

Statistical Analysis

Data is reported as group averages (mean ± standard deviation); distribution normality was assessed and parametric vs. non-parametric methods were used as appropriate. Differences in exercise intolerance between FSHD and control participants were identified via independent samples t-tests; in cases of nonparametric data, the Mann-Whitney U test was performed. Associations between continuous variables were identified with the Pearson product-moment correlation; a stepwise linear regression model was used to determine whether measures of body composition or cardiopulmonary function could predict exercise intolerance differentially between the groups (dependent variable: VO_{2peak} (mL/min), independent variables: leg LM (LLM (kg)), % leg LM (LLM), %WBFM, resting VO_2/HR ((mL/beat), a non-invasive estimate of stroke volume

[156]). Statistical analyses were performed using SPSS v26.0 (SPSS, Inc., Chicago, IL, USA); significance defined as an α-level of $p<0.05$ for all comparisons.

RESULTS

Subject Characteristics

FSHD and control participants were similar in age (47±13 vs. 46±13 years, $p=0.86$), height (1.78±0.08 vs. 1.72±0.09 m, $p=0.19$), weight (84.8±11.4 vs. 80.6±17.1 kg, $p=0.50$), and BMI (27.0±4.0 vs. 26.8±4.1 kg/m^2, $p=0.94$). In the FSHD group, one participant self-reported as Hispanic, the remaining 10 self-identified as non-Hispanic white (6 men, 4 women); racial self-identification by control study participants was as follows: Hispanic: 2, non-Hispanic white: 8 (4 men, 4 women), black: 1. An attenuated amount of daily physical activity was reported among people with FSHD, as compared to controls (activity metabolic index score; 41.8±65.1 vs. 252.6±146.1 kcal/day, $p<0.01$). Additional self-reported measures include the FSHD Health Index (HI) score, values of which are shown in **Table 4.1**.

FSHD Health Index (HI) Category	**Mean Score (n=11)**
Shoulder and arm function	36.7±24.2
Fatigue	31.2±21.2
Mobility and ambulation	29.0±21.2
Social performance	27.2±23.7
Core strength and function	23.5±17.3
Body image	22.4±22.7
Activity limitation	21.7±13.5
Social satisfaction	21.2±20.2
Emotional health	19.7±15.3
Pain	17.0±10.8
Hand and finger function	13.5±21.2
Communication	11.3±15.2
Gastrointestinal function	6.6±10.1
Cognitive function	4.8±10.6
Total FSHD-HI Score	23.9±13.8

Table 4.1: Self-reported measures of functionality in FSHD.

Body Composition

Lean mass. Measures of LM in FSHD are in **Table 4.2**. Absolute measures of LM, including whole-body LM (WBLM), trunk LM (TLM), LLM, arm LM (ARMLM), and the combined appendicular region (ALM; sum of fat- and bone-free tissue in the arms and legs [42]) were all similar between FSHD and control groups (p>0.05 for all). However, the % whole-body LM (%WBLM) among people with FSHD was found to be 13% lower than in controls (p=0.03), a finding which was furthermore seen among male (p=0.045) but not female (p=0.41) study participants. Similarly, the FSHD group exhibited a %LLM that was 16% lower than that observed in controls (p=0.02); this finding was replicated among male FSHD-control pairs (p=0.03), though not in females (p=0.34). Multiple measures of LM were found to be correlated with self-reported physical activity in the FSHD group (WBLM: r=0.60, p=0.049; LLM: r=0.62, p=0.04; %WBLM: r=0.66, p=0.03), though these relationships were not present among controls or when groups were combined.

Fat mass. Measures of FM in FSHD are in **Table 4.2**. People with FSHD were found to have an absolute volume of whole-body FM (WBFM) that was 42% higher than controls (p=0.03). Furthermore, in people with FSHD, the lower body did appear to be more affected, with the absolute volume of leg FM (LFM) reaching a level that was 46% higher than in controls (p<0.01); the relative proportion of leg FM (%LFM) was likewise different between groups, with FSHD participants exhibiting a value that was 37% higher than in controls (p=0.03). Furthermore, sex-driven differences in %LFM were observed, as values were significantly higher for male FSHD-control pairs (p=0.05), but not female counterparts (p=0.34). Absolute values of trunk FM (TFM) and arm FM (ARMFM) were similar between FSHD and control groups (p>0.05 for all).

	FSHD	Range (FSHD)	Control	Range (Control)	Significance (*p*-value)
Lean Mass (kg)					
Whole-body lean mass	47.9±7.9	40.5-67.6	58.2±12.6	33.9-78.7	0.19
% whole-body lean mass	58.8±10.2	46.3-82.1	67.6±7.0	52.7-75.8	0.03
% whole-body lean mass: Men	59.3±11.5	49.7-82.1	70.0±5.2	61.1-75.8	0.045
% whole-body lean mass: Women	57.9±9.0	46.3-67.5	63.5±8.7	52.7-71.0	0.41
Trunk lean mass	23.7±3.5	19.8-32.2	27.1±5.5	15.2-34.8	0.43
Leg lean mass	15.5±3.8	11.6-24.1	19.9±4.2	12.0-26.5	0.10
% leg lean mass	60.8±11.1	48.5-84.8	72.2±8.9	50.7-82.3	0.02
% leg lean mass: Men	62.5±13.0	48.5-84.8	77.0±4.4	68.6-82.3	0.03
% leg lean mass: Women	57.8±7.4	49.7-67.6	63.9±9.1	50.7-71.1	0.34
Arm lean mass	5.3±1.0	4.4-7.8	7.8±2.5	3.6-12.5	0.12
Appendicular lean mass	20.7±4.6	16.4-31.9	27.7±6.7	15.6-39.0	0.15
Fat Mass (kg)					
Whole-body fat mass	32.1±12.0	11.1-49.6	22.8±6.7	13.4-35.8	0.03
Trunk fat mass	17.4±9.1	4.7-31.5	12.8±4.9	5.1-21.8	0.12
Leg fat mass	10.1±2.9	4.3-14.9	6.6±1.6	4.9-11.7	<0.01
% leg fat mass	39.2±11.2	15.2-51.5	28.7±10.0	17.7-49.3	0.03
% leg fat mass: Men	37.4±13.1	15.2-51.5	24.7±8.4	17.7-43.0	0.05
% leg fat mass: Women	42.2±7.4	32.4-50.3	36.0±9.1	28.9-49.3	0.34
Arm	3.2±1.1	1.2-4.9	2.6±0.8	1.5-4.2	0.13

Table 4.2: Measures of body composition in FSHD (unless otherwise indicated, data is for combined analyses of men and women) ($p<0.05$).

	FSHD	Range (FSHD)	Control	Range (Control)	Significance (*p*-value)
Rest					
HR (bpm)	79±13	57-101	74±14	56-100	0.40
SBP (mmHg)	130±9	114-142	122±11	102-136	0.08
DBP (mmHg)	83±6	70-92	82±9	64-92	0.85
MAP (mmHg)	99±5	91-106	94±10	79-107	0.19
VO_2/HR (mL/beat)	3.1±0.6	2.4-4.5	3.7±1.3	1.4-6.1	0.20
V_E (L/min)	8.8±1.8	6.6-12.0	9.4±2.9	4.7-13.3	0.70
R_R (br/min)	14±2	10-18	10±4	6-16	0.01
V_T (mL/min)	656±125	478-822	1018±522	481-1805	0.10
Submax exercise (40% of VO_2peak)					
HR (bpm)	103±18	77-134	106±17	86-128	0.66
SBP (mmHg)	148±16	114-142	148±23	102-136	0.93
DBP (mmHg)	89±8	70-92	85±9	64-92	0.22
MAP (mmHg)	109±10	91-106	106±11	79-107	0.62
VO_2/HR (mL/beat)	7.5±2.3	4.7-11.9	11.0±4.5	4.1-18.0	0.047
V_E/VCO_2 slope	25.2±3.4	21.2-33.4	24.3±3.8	20.0-30.6	0.56
Submax exercise (60-watts)					
HR (bpm)	114±23	90-165	96±16	76-125	0.07
SBP (mmHg)	166±18	144-196	138±18	102-166	<0.01
DBP (mmHg)	92±11	70-104	86±6	76-94	0.17
MAP (mmHg)	116±12	99-135	105±7	96-116	0.03
VO_2/HR (mL/beat)	8.9±1.5	6.7-10.6	8.9±2.9	4.1-12.4	0.96
V_E/VCO_2 slope	27.6±8.1	20.8-48.2	25.8±3.7	21.7-30.6	1.0
Maximal exercise					
HR (bpm)	143±28	109-185	152±28	92-190	0.43
SBP (mmHg)	187±22	158-232	194±30	140-238	0.55
DBP (mmHg)	95±11	70-106	94±112	79-110	0.91
MAP (mmHg)	125±13	99-141	123±123	69-148	1.0
VO_2/HR (mL/beat)	11.1±3.3	7.3-16.5	17.5±6.6	6.6-30.3	0.02
V_E/VCO_2 slope	31.4±6.0	26.5-48.2	30.6±8.5	21.9-54.2	0.52

Table 4.3: Measures of cardiopulmonary function in FSHD (L, liters; min, minute; br, breaths; mL, milliliters; bpm, beats per minute; mmHg, millimeters of mercury; $p<0.05$).

Measures of Cardiopulmonary Function at Rest

Measures of cardiopulmonary function at rest are in **Table 4.3**. V_E was similar between people with FSHD and control participants (p=0.70) while in a resting state. However, the FSHD group was found to have a resting R_R which was 33% higher than in control participants (p=0.01), though differences in V_T between groups were not observed (p=0.10). No differences in HR, systolic blood pressure, diastolic blood pressure, mean arterial pressure, or O_2 pulse were noted between FSHD and control groups at rest (p>0.05 for all, **Table 4.3**). In combined analysis, a relationship between systolic blood pressure at rest and physical activity level was noted (r=-0.45, p=0.04), though this observation was not found in single-group analyses; similarly, associations between these variables and age were not present (p>0.05 for all).

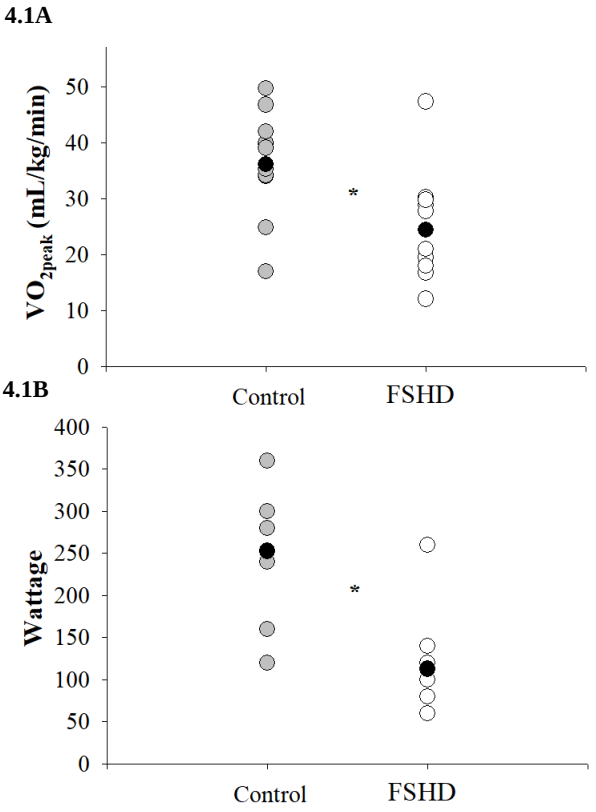


Figure 4.1: Peak exercise capacity in FSHD. 4.1A) VO_{2peak} was lower than that observed in controls; 4.1B) Peak wattage was lower among people with FSHD (VO_2, volume of oxygen consumption; p<0.01 for both).

Measures of Cardiopulmonary Function During Peak Exercise

VO_{2peak} was 32% lower (p<0.01, **Figure 4.1A**) and peak wattage was 55% lower in the FSHD group (p<0.01, **Figure 4.1B**). Additionally, people with FSHD were found

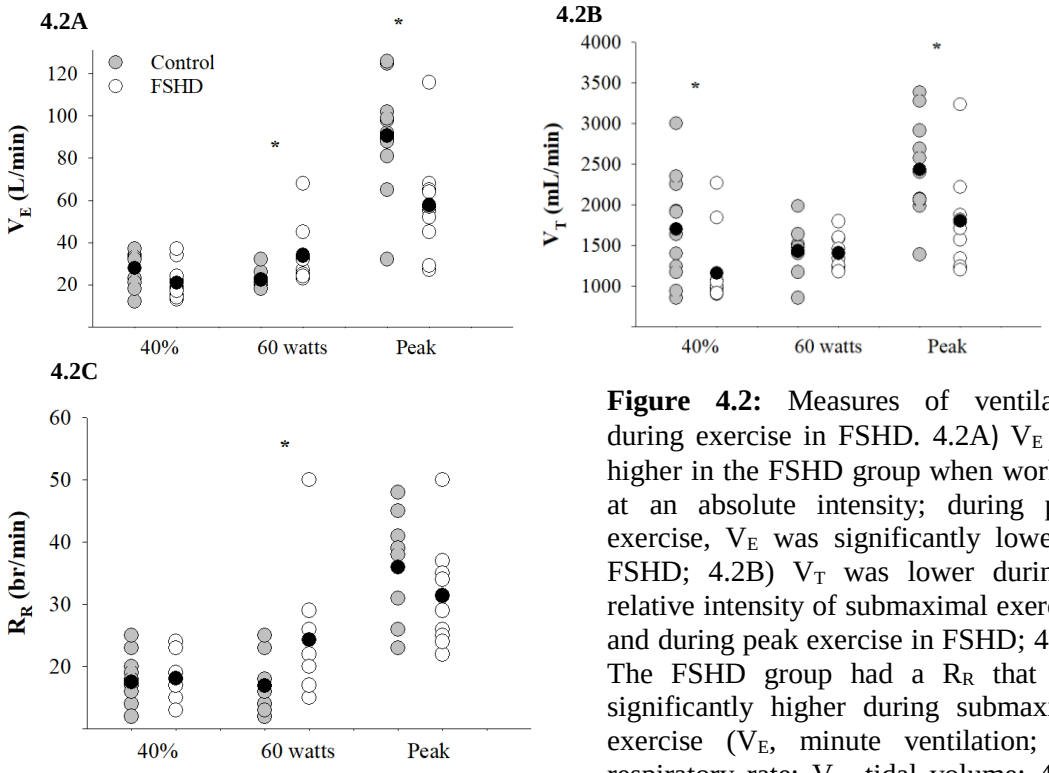


Figure 4.2: Measures of ventilation during exercise in FSHD. 4.2A) V_E was higher in the FSHD group when working at an absolute intensity; during peak exercise, V_E was significantly lower in FSHD; 4.2B) V_T was lower during a relative intensity of submaximal exercise, and during peak exercise in FSHD; 4.2C) The FSHD group had a R_R that was significantly higher during submaximal exercise (V_E, minute ventilation; R_R, respiratory rate; V_T, tidal volume; 40%, submaximal exercise at 40% of VO_2peak; 60watts, submaximal exercise at 60-watts; $p<0.05$).

to have a V_E that was 37% lower than controls during peak exercise ($p<0.01$, **Figure 4.2A**), a measure that appeared to be driven primarily by a V_T that was 26% lower, as compared to controls ($p=0.02$, **Figure 4.2B**). Though R_R was likewise 14% lower among people with FSHD, it was not different between groups ($p=0.18$, **Figure 4.2C**). Furthermore, HR, systolic blood pressure, diastolic blood pressure, mean arterial pressure, and V_E/VCO_2 slope were all similar between FSHD and control participants ($p>0.05$ for all, **Table 4.3**); O_2 pulse during peak exercise was 37% lower in the FSHD group ($p=0.02$, **Table 4.3**). RPE ($p=0.61$, **Figure 4.3A**) and dyspnea ($p=0.48$, **Figure 4.3B**) were similar at peak workloads between groups.

Associations and predictors of exercise intolerance. A regression analysis examining the mechanisms of exercise intolerance failed to find interactions between the FSHD and control group using resting O_2 pulse, or various measures of body composition (%WBFM, LLM, %LLM) in the model, thus suggesting that none of these variables could independently predict VO_{2peak} between the groups (p>0.05 for all). However, as correlations between VO_{2peak} and multiple c

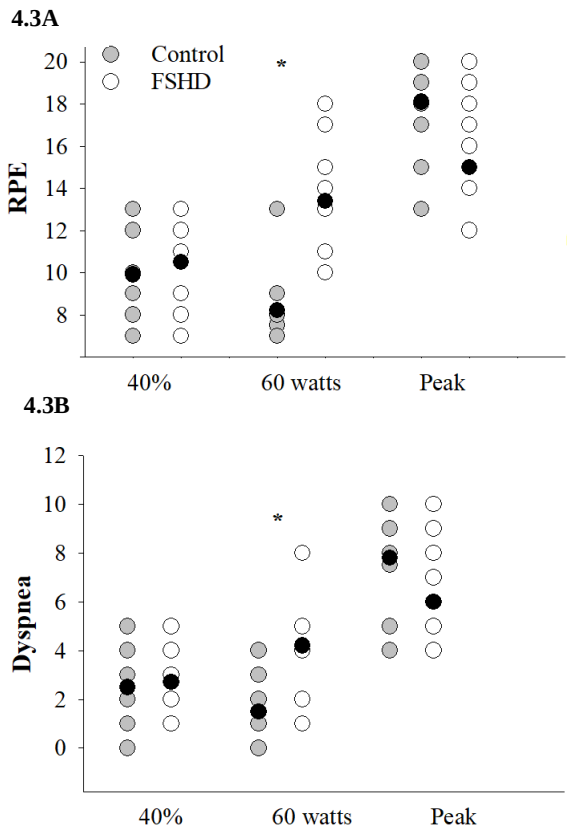


Figure 4.3: Self-reported exertion levels in FSHD. 4.3A) During absolute work, RPE was greater among people with FSHD; 4.3B) The FSHD group had greater self-reported levels of breathlessness when working at an absolute intensity (RPE, rating of perceived exertion; 40%, submaximal exercise at 40% of VO_{2peak}; 60watts, submaximal exercise at 60-watts; p<0.01 for both.

and compositional measures were noted in combined analysis (%LLM: p<0.01, r=0.90; LLM: p<0.01, r=0.84; O_2 pulse at VO_2peak: p<0.01, r=0.79; %WBFM: p<0.01, r=-0.78; resting O_2 pulse: p<0.01, r=0.76; HR at VO_2peak: p=0.03, r=0.46), it is likely that these factors did limit exercise performance to at least some degree for both groups. Moreover, when groups were assessed separately, differences in the strength of the correlations on VO_{2peak} were observed, signifying potential variations in the mechanisms of this

limitation between FSHD and control participants. In the control group, VO_{2peak} was most strongly related to resting O_2 pulse (p<0.01, r=0.90), followed by %LLM: p<0.01, r=0.89; LLM: p<0.01, r=0.79; O_2 pulse at VO_2peak: p=0.01, r=0.72; and %WBFM: p=0.01, r=-0.70; among people with FSHD, LLM% was most influential on VO_{2peak} (p<0.01, r=0.88), though a relationship between VO_2peak and other factors was likewise found (LLM: p<0.01, r=0.87; O_2 pulse at VO_2peak: p<0.01, r=0.77; %WBFM: p<0.01, r=-0.74). Interestingly, self-reported measures of functionality, as indicated via the FSHD-HI survey, were found to be negatively correlated with VO_2peak (activity limitation: p<0.01, r=-0.78; total FSHD-HI score: p=0.03, r=-0.65), thus suggesting that impairments in performance are pronounced enough to be subjectively noticeable by people with FSHD.

Measures of Cardiopulmonary Function During Submaximal Exercise

Relative analysis (40% of VO_2peak). When exercising at a relative intensity of 40% VO_{2peak}, wattage was 55% lower in people with FSHD compared with controls (41.8±30.3 vs. 92.7±32.6 watts, p=0.01). V_E (p=0.10, **Figure 4.2A**) and R_R (p=0.73, **Figure 4.2C**) were similar between groups, though V_T was observed to be 32% lower in people with FSHD than controls (p=0.03, **Figure 4.2B**). HR, systolic blood pressure, diastolic blood pressure, mean arterial pressure, and V_E/VCO_2 slope between people with FSHD and control participants were similar (p>0.05 for all, **Table 4.2**); however, the FSHD group did exhibit a 32% lower O_2 pulse than that of controls (p<0.05, **Table 4.2**). RPE (p=0.61, **Figure 4.3A**) and dyspnea (p=0.78, **Figure 4.3B**) were similar between FSHD and controls.

Absolute analysis (60 watts). During absolute submaximal exercise, people with FSHD demonstrated a VO_2 that was 21% higher than in controls (FSHD: 1017.2±141.2 vs. Control: 840.5±187.1 mL/min, p=0.04). Similarly, the FSHD group exhibited a V_E and R_R that was 55% and 41% higher, respectively, than in controls ($p<0.05$ for both, **Figures 4.2A and 4.2C**), though differences in V_T between groups at this exercise intensity were absent (p=0.86, **Figure 4.2B**). HR, diastolic blood pressure, O_2 pulse, and V_E/VCO_2 slope were similar between individuals with FSHD and control ($p>0.05$ for all, **Table 4.2**); conversely, systolic blood pressure and mean arterial pressure were 20% and 10% higher, respectively, among people with FSHD, as compared with the control group ($p<0.05$ for both, **Table 4.2**). RPE 63% higher ($p<0.01$, **Figure 4.3A**) and dyspnea was 180% higher in the FSHD group compared with controls ($p<0.01$, **Figure 4.3B**).

DISCUSSION

This study is the first to identify a greater exercise intolerance among people with FSHD, as reflected by a VO_{2peak} that was 32% lower than controls. Additionally, the presence of increased exercise intolerance in the FSHD group is further supported by self-reported RPE and dyspnea which were 63% and 180% higher, respectively, at an absolute, submaximal workload, than among control participants. Importantly, our study revealed disparate mechanisms of exercise limitation between study groups, whereby %LLM was strongly related with VO_{2peak} among people with FSHD, though in the control group, VO_{2peak} was shown to be most strongly correlated with resting O_2 pulse. Notably, resting O_2 pulse, an indicator of stroke volume, was not a limiting factor for the FSHD group during exercise, thus consistently demonstrating a sparing of cardiac function in this population [1]. Finally, we observed an attenuated cardiopulmonary

response at both the relative submaximal and peak exercise workload, while during the absolute intensity exercise, VO_2 and measures of cardiopulmonary function were elevated in the FSHD group. These findings suggest that during activities of daily living, such as stair-climbing or yard work, people with FSHD will likely require greater work (VO_2) and will feel as if the work is harder, while leaving them more breathless than individuals without FSHD. Conversely, during activity performed at a relative intensity, a combination of a lower absolute workload and a blunted hemodynamic and pulmonary response may manifest in cardiopulmonary limitations to exercise tolerance in FSHD.

Exercise Intolerance in FSHD

Previous work on exercise intolerance among people with FSHD has yielded results that support our findings. In research by Morse et al., people with FSHD were found to have a distance traveled during the assisted 6-minute cycle test that was 28% lower than that seen in controls [75]; this variable was used as a surrogate measure for VO_{2peak}, thereby matching our own mean difference in exercise capacity of 32%. However, ratings of dyspnea and fatigue were not measured by Morse et al., making it difficult to know whether exercise intolerance was truly present in his study.

Mechanisms of Exercise Intolerance

Exercise intolerance has been described as a syndrome which "coalesces as dysfunction across multiple physiologic systems [157]," including those at both the peripheral and central levels. Identifying and addressing unique mechanisms of exercise intolerance, such as those related to body composition and hemodynamic function among people with FSHD, is an important first step in the development of therapeutic interventions designed to treat the condition.

Peripheral mechanisms. A combination of peripheral factors, including alterations in muscle fiber type and the over-activation of group III/IV LM afferents, are believed to play a primary role in the development of exercise intolerance [25, 70, 71]. These contributors to exercise intolerance appear to be especially impacful for clinical populations, where a low volume of LM, a reduced rate of maximal force production, and associations between VO_{2peak} and measures of LM (calf LM: r=0.48; mid-arm LM: r=0.36) have been widely reported [67-69]. Our research coincides with these observations, as we report that among people with FSHD, %LLM and LLM were strongly associated with VO_{2peak}, suggesting that the presence of exercise intolerance among these participants was related primarily to losses in LM in the lower body. While a transition from a fast-glycolytic to slow-oxidative phenotype has been reported among people with FSHD [41], fiber typing was not performed in this study, and therefore, it is difficult to know the extent to which this phenomenon may have contributed to exercise intolerance among our participants.

Hemodynamic mechanisms. Prominent mechanisms of exercise intolerance are *Q*, a value which is driven by the combined influences of HR and stroke volume [62]. While measures of *Q* have not previously been studied among people with FSHD, other dystrophic groups, including those with Duchenne muscular dystrophy, have exhibited a submaximal *Q* that is 50% lower than that seen in controls, when assessed proportionately to resting values [158]. Importantly, this observation seems to be driven primarily by a stroke volume that is 20% lower than in controls, as differences in HR during physical activity between Duchenne muscular dystrophy and control groups do not appear to present [158]. These observations are in line with our own study, in which people with FSHD had a resting HR that did not differ from control counterparts;

additionally, HR was similar between FSHD and control counterparts at all intensities of physical activity, though it trended higher among people with FSHD at absolute, submaximal workloads. While we did not directly measure stroke volume in our study, a surrogate measure—O_2 pulse—was similar between groups at rest and during absolute, submaximal exercise workloads; notably, O_2 pulse was strongly associated with VO_{2peak} among controls, though not in people with FSHD. Furthermore, people with FSHD were found to have an O_2 pulse that was 32% lower than in controls when exercising at 40% of VO_2peak and at peak exercise, suggesting that when working at the same relative intensity, stroke volume is reduced. Based on these observations, we believe that while *Q* may play a role in the development of exercise intolerance during activity that is performed at a relative intensity among control participants, its contribution to the phenomenon at all exercise workloads is surpassed by that of LM in the FSHD population.

Additional pulmonary mechanisms of exercise intolerance include ventilatory dysfunction, particularly in people with chronic disease. In work by Morse et al., the low exercise capacity demonstrated by people with FSHD was accompanied by a corresponding low V_E during the later stages of a relative, maximal intensity exercise test [75]. In our study, ventilatory efficiency (V_E/VCO_2 slope) was similar between FSHD and control groups at all exercise intensities, though variations in V_E function between groups were found, which manifested differently, at various intensities and workloads. In fact, during the relative submaximal exercise, people with FSHD were found to have a V_E response that trended to be lower than in controls; this observation was mirrored during relative exercise at a maximal intensity, whereby V_E was 37% lower in the FSHD group.

Notably, this observed hypoventilation at relative workloads is likely a result of shallow breathing, as V_T was lower in the FSHD group at both intensities of relative exercise, though R_R was not affected during either exercise period. In contrast to this finding, we noted that V_E was 55% higher among people with FSHD at an absolute workload of 60-watts, and furthermore, that this difference was likely driven in large part by a correspondingly high R_R, as V_T did not differ between groups at this exercise intensity. Together, these findings suggest that the V_E response is blunted among people with FSHD when working at a relative intensity, and exaggerated at an absolute workload, indicating that mechanisms of exercise intolerance are likely intensity dependent.

Blood pressure has also been listed as a potential contributor to the development of exercise intolerance. In our study, all measures of arterial pressure, including systolic blood pressure, diastolic blood pressure, and mean arterial pressure, were similar between FSHD and control groups at rest, and during both submaximal and maximal relative workloads. When working at an absolute intensity, however, people with FSHD were found to have a systolic blood pressure and mean arterial pressure that was 20% and 10% higher, respectively, than in the control group. This suggests that the elevated cardiovascular response in FSHD was a consequence of lower LLM, thus making the same absolute volume of work proportionately harder. This theory is supported by the elevated VO_2 and ventilatory response during absolute work in the FSHD group.

Limitations

Limitations of our research include a small number of study participants, particularly of females. Additionally, nine of 11 FSHD-control pairs in our study were matched by race, though two non-Hispanic white males with FSHD were partnered with

controls who self-identified as belonging to a dissimilar racial group (black: 1, Hispanic: 1). While differences in VO_2max between races have been documented, these observations have primarily been made among Hispanic and non-Hispanic black females [159], whereas no significant differences in VO_2max among males of differing races have been observed [159]. Therefore, we believe that the divergent ethnic and racial backgrounds among two male FSHD-control pairs had minimal influence on primary markers of exercise intolerance. Finally, we did not control for medication use in our study, and in one instance, blood pressure-lowering medications (calcium-channel blockers) were used by a female control participant, but not her FSHD partner. When examining individual values for this participant, we found that MAP at rest and VO_{2peak} was 11 and 12 mmHg higher, respectively, as compared to in the control group overall. Thus, her MAP was elevated vs reduced when compared to the control group.

Medication class	FSHD	Control
Angiotensin-converting enzyme (ACE) inhibitors	1	1
Antacids	1	0
Anticholinergics/antimuscarinics	1	0
Antifungals	1	0
Atypical antipsychotics	0	1
Calcium-channel blockers	0	1
Corticosteroids	1	0
HMG-CoA reductase inhibitors	1	1
Lincomycin antibiotics	0	1
Norepinephrine-dopamine reuptake inhibitors	1	0
NSAIDs	2	0
Proton pump inhibitors	1	1
Selective serotonin and norepinephrine reuptake inhibitors (SNRIs)	1	0
Selective serotonin receptor agonists (SSRAs)	1	0
Selective serotonin reuptake inhibitors (SSRIs)	1	1
Supplements	8	2
Tricyclic antidepressants	1	0
Vasodilators	1	0
Total medications	23	9

Table 4.4: Medication use among study participants.

CONCLUSION AND NEXT STEPS

The findings of this chapter are important, as they show that people with FSHD are more likely to suffer from exercise intolerance than age- and sex-matched controls, especially when performing work at absolute and peak exercise level. Additionally, the work in this chapter has shown that the mechanisms contributing to activity limitation between groups are vastly different. While resting O_2 pulse is a primary influence on VO_{2peak} among control groups, it does not appear to limit exercise capacity in FSHD; likewise, VO_{2peak} is not driven by other resting or peak aspects of hemodynamic function (HR, systolic blood pressure, diastolic blood pressure, mean arterial pressure) in this clinical group. Instead, markers of LM are found to be strongly correlated withVO_{2peak} in

FSHD, primarily as a result of muscle atrophy in the lower body. Together, these results demonstrate that targeted therapeutic interventions, such as those which specifically address unique changes in LM, are especially important in preventing exercise intolerance in the FSHD population. As such, I believe that future research in this area should focus on the development of specific guidelines for resistance training among people with FSHD, thereby making impactful strikes forward in the functional treatment of FSHD. Specific suggestions for the ways in which this research may be designed are outlined in **Chapter 6**.

Chapter 5: Resting Metabolic Rate in Adults with Facioscapulohumeral Muscular Dystrophy

The contents of this chapter are in press with Applied Physiology, Nutrition and Metabolism (February 2021).

OVERVIEW

The purpose of this chapter is to investigate whether RMR is altered in adults with FSHD. To answer this question, we recruited eleven people with FSHD (51±12yrs, 2 females) and eleven controls (48±14yrs, 2 females), all of whom underwent 30-minutes of indirect calorimetry and DXA scanning. RMR was calculated from resting VO_2/VCO_2, and regional/whole-body FM and LM were collected from the DXA scan.

Absolute RMR was 15% lower among people with FSHD (p=0.04), though when adjusted for regional/local LM, no differences in RMR were observed (p>0.05). Absolute RMR was correlated with total LM for all participants combined (p<0.01, r=0.70, males only: p<0.01, r=0.81) and when analyzed separately (FSHD males: p=0.001, r=0.92 and control males: p=0.004, r=0.85). Whole-body LM was 16% lower in the FSHD group; similarly, leg, arm and appendicular LM were lower among people with FSHD (p<0.05 for all), though trunk LM was not (p=0.15). Whole-body FM was 45% higher in FSHD, with greater leg FM (p=0.01), but not trunk or arm FM (p>0.05 for both). Together, the results of this chapter show that when RMR is expressed relative to LM, no differences in RMR are expected. The implications of these findings are important, as they suggest that the low levels of LM observed in people with FSHD likely contribute to a reduction in RMR within this population.

INTRODUCTION

FSHD is a complex and slowly progressive genetic disease, which is believed to strongly affect the volume and function of LM [3]. In fact, FSHD causes atrophy of LM, which contributes to many of the characteristic features of the condition, including a loss in muscular strength [128], decrease in functional capacity [75], and an increase in the likelihood of disability [16, 80]. In addition to its influence on physical function, LM is also known to be a primary contributor to RMR [160], or the amount of energy required by the body to sustain life when in a resting state [94]. Furthermore, fat-free mass -of which LM is a primary component– is theorized to contribute to 70-80% of the inter-individual variability in RMR [20].

An observed low volume of LM among people with muscular dystrophy has subsequently led to speculation that measures of metabolic function – including RMR – may be reduced within this group [21]. Paradoxically, however, previous research investigating the influence of various forms of muscular dystrophy on metabolic rate have yielded contradictory results. For example, individuals with limb-girdle muscular dystrophy have been shown to have a basal metabolic rate that is similar to control groups [23], a finding that is likewise noted among people with Becker's muscular dystrophy [21]. While observations of a lower RMR among people with Duchenne muscular dystrophy has been documented [22], opposing evidence of profound hyper-metabolic properties in this same population, as defined by a basal metabolic rate that was 20-30% higher than that seen in age- and sex-matched counterparts, has also been reported [23]. In addition, after adjusting for LM, animal models of Duchenne muscular dystrophy have also demonstrated elevated RMR [161, 162], though the presentation of the

mdx phenotype is weaker in mice than in humans, indicating that the interpretation of these results are equivocal. Finally, in a mouse model of FSHD, observations of periodic hyper-metabolism, as characterized by alternating phases of high and precipitously low volumes of VO_2 and VCO_2, have also been documented [125]. The discrepant findings in the aforementioned studies could be due to differences in muscular dystrophy phenotypes, lack of control group and/or small sample sizes. Consequently, there is no clear depiction on how RMR is influenced by muscular dystrophy.

Additionally, literature demonstrating if RMR is influenced among people with FSHD is lacking. Furthermore, if disease-related loss in LM contributes to RMR in people with FSHD remains unclear. The purpose of this study, therefore, is to investigate if RMR is altered in adults with FSHD. We hypothesize that RMR will be attenuated in the FSHD population and primarily determined by the lower LM volume, as compared with controls. Should this hypothesis be confirmed, it may serve as important foundational knowledge in the development of targeted nutritional and exercise strategies for people with FSHD.

METHODS

Subjects

Eleven individuals with genetically-confirmed FSHD (51±12 years) and eleven age-, sex- and BMI-matched control participants (48±14 years) (n=22 combined; males: 18, females: 4) completed the study. Inclusion criteria included an age of ≥18 years, and no prior history of cardiovascular, pulmonary, orthopedic, or neuromuscular disorders other than FSHD; female participants were excluded if they were currently pregnant or

breastfeeding [131, 132]. Menstrual cycle was not controlled for in the four females. Activity metabolic index score, a measure of physical activity level, was calculated via the Minnesota Leisure Time Physical Activity Questionnaire [133]. The study was approved by the University of Minnesota Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

Experimental Protocol

Participants completed one experimental session, during which a description of study design was provided, and written informed consent was obtained. During the experimental protocol, study participants rested for 30-minutes, and RMR was calculated via resting VO_2 and VCO_2 measurement with indirect calorimetry [163] (Ultima CardiO_2, MCG Diagnostics, St. Paul, MN, USA). The gas analyzer was calibrated according to manufacture guidelines before each test, using calibration gases of 5% carbon dioxide, 12% oxygen, and balanced nitrogen. Gas volumes were measured through a Prevent® flow sensor using a 3-Liter calibration syringe and corrected for ambient conditions prior to each test. To measure LM, a DXA scan (Lunar iDXA, GE Healthcare, Chicago, IL, USA) was conducted after measurements of RMR. Female participants completed a urine human chorionic gonadotropin test (Clinical Guard, Atlanta, GA, USA) to determine they were not pregnant.

Data Collection Techniques

For accurate measures of RMR, participants were asked to fast and abstain from caffeine and physical activity for at least five hours [163]. Each individual was fitted with a preVent ® facemask (MGC Diagnostics). During indirect calorimetry, they rested quietly

in a supine position, in a dimly-lit and thermally-neutral testing room early in the morning, for a period of 30-minutes. Data was collected on a continuous basis; five-minute averages were calculated during the 30-minute experimental period. Coefficient of variation was calculated (standard deviation/mean) for measures of VO_2 and VCO_2 during each five-minute timeframe (overall range of VO_2: 18.2-26.4%; VCO_2: 20.9-28.2%). Steady-state metabolic conditions were identified during the 10-15min interval, in which the variation of both VO_2 and VCO_2 were at their lowest levels (18.2% and 20.9%, respectively) [164, 165]. Five-minute average values from the 10-15min interval were subsequently used to calculate RMR, with an abbreviated version of the modified Weir equation:

RMR (kcal/day) = *[VO_2 (L/min) x 3.941) + (VCO_2 (L/min) x 1.11) * 1440]* [163]

Body composition was obtained from the DXA scan enCORE v16 (GE Healthcare, Chicago, IL, USA) and assessed using a three-compartment model (bone mineral content, FM, LM); data from the DXA scan was subsequently used as markers of regional and local LM and FM. To account for the contribution of LM on metabolism [166], RMR was normalized to regional and local measures of LM (kg). BMI was calculated from manual measurements of height (m) and weight (kg).

Statistical Analysis

We based our a priori sample size calculation on RMR data from a similar study in individuals with Becker's muscular dystrophy [21]. Based on their means and standard deviations, RMR in controls was 1913.6±203.2 and 1676± 246.3; $p<0.05$ in ambulatory adults with muscular dystrophy. A large effect size (d=1.05) was obtained and thus for such a result, 11 individuals in each group were needed to have sufficient power of 80%.

Data is reported as group averages (mean ± standard deviation); distribution normality was assessed and parametric vs. non-parametric methods were used as appropriate. Differences in RMR between FSHD and control participants were identified via independent samples t-tests; in cases of nonparametric data, the Mann-Whitney U test was performed. A univariate ANCOVA was used to investigate the influence of confounding variables; Spearman's rank correlation coefficient identified relationships between continuous variables. Due to large differences in standard deviation between groups, effect size was calculated using Glass's *delta (Δ)* [167], whereby:

$$\Delta = \frac{\text{Mean}_1 - \text{Mean}_2}{\sigma_{control}}$$

Differences between slopes in the linear regression for RMR and LM were tested between the FSHD and control groups. A power analysis was conducted using preliminary data with a β-level of 80%, and statistical analyses were performed using SPSS v25.0 (SPSS, Inc., Chicago, IL, USA); significance defined as an α-level of $p < 0.05$ for all comparisons.

RESULTS

Subject Characteristics

FSHD and control participants were similar in age (51±12 vs. 48±14 years, $p = 0.62$), height (1.80±0.07 vs. 1.74±0.08 m, $p = 0.08$), weight (85.3±12.2 vs. 83.6±14.0 kg, $p = 0.76$) and BMI (26.3±4.2 vs 27.4±4.2 kg/m^2, $p = 0.52$). In the FSHD group, one individual self-identified as Hispanic, while the remainder self-reported as non-Hispanic white (8 men, 2 women); racial self-identification in the control group was as follows: Hispanic: 2, non-Hispanic white: 7 (5 men, 2 women), black: 1, Asian: 1.

	FSHD	Range (FSHD)	*t*-value	95% confidence interval	Control	Range (Control)	*t*-value	95% confidence interval	Significance (*p*-value)
Measures of Lean Mass (kg)									
Whole-body lean mass	47.5±5.7	40.5-57.4	27.5	43.7-51.4	57.0±10.6	45.9-78.7	17.7	49.8-64.2	0.02
Trunk lean mass	23.5±2.0	20.7-26.8	39.8	22.2-24.8	26.7±4.7	20.7-34.8	18.6	23.5-29.9	0.15
Leg lean mass	15.0±3.3	10.8-20.5	14.9	12.8-17.2	19.2±3.8	14.0-26.5	16.8	16.6-21.7	0.01
Arm lean mass	5.5±1.0	4.4-7.4	17.3	4.8-6.2	7.6±2.0	5.1-12.5	12.4	6.3-9.0	<0.01
Appendicular lean mass	20.5±4.2	15.3-24.8	16.1	17.6-23.3	26.8±5.7	20.6-39.0	15.7	23.0-30.6	<0.01
Measures of Fat Mass (kg)									
Whole-body fat mass	33.9±9.6	21.8-49.6	11.7	27.4-40.3	23.3±7.8	11.2-35.8	9.9	18.0-28.5	0.01
Trunk fat mass	18.8±7.9	5.1-31.5	7.9	13.4-24.1	13.4±5.5	5.9-22.1	8.1	9.7-17.1	0.08
Leg fat mass	10.5±2.4	7.2-14.9	14.6	8.9-12.1	6.4±1.9	3.1-9.8	11.1	5.1-7.6	<0.01
Arm fat mass	3.2±0.8	2.4-4.9	12.5	2.6-3.8	2.6±0.9	1.4-4.2	10.0	1.9-3.1	0.09

Table 5.1: Measures of body composition in FSHD. Individuals with FHSD have an overall lower volume of LM in the whole-body; LM in the legs, arms, and appendicular region are also lower in the FSHD group. The overall volume of whole-body FM is greater among people with FSHD, as is FM in the legs. kg, kilograms; $p<0.05$.

Measures of Lean Mass

The absolute volume of whole-body LM was 16% lower in FSHD than in controls (p = 0.02) **(Table 5.1).** In addition, the absolute volume of leg LM was 22% lower in people with FSHD (p = 0.01); similarly, arm LM was 29% lower (p < 0.01) and ALM was 24% lower in the FSHD group (p < 0.01) **(Table 5.1)**. Differences in the volume of trunk LM between FSHD and control groups were not statistically significant (23.5±2.0 vs. 26.7±4.8 kg, p = 0.15) **(Table 5.1)**. Correlations between measures of LM and age were not found, either in a combined analysis, or when FSHD and control groups were assessed separately (p > 0.05 for all).

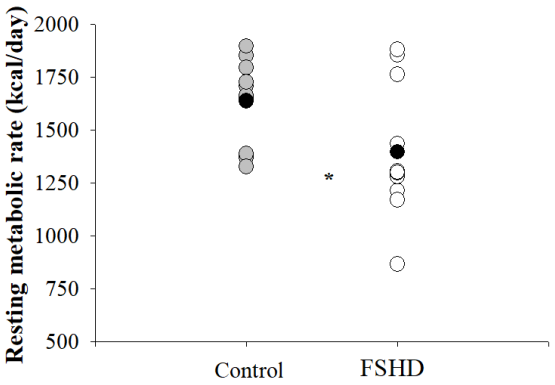


Figure 5.1: Resting metabolic rate (RMR) in FSHD. RMR is significantly lower among people with FSHD, as compared to in control participants (p=0.04). kcal, kilocalories; p<0.05).

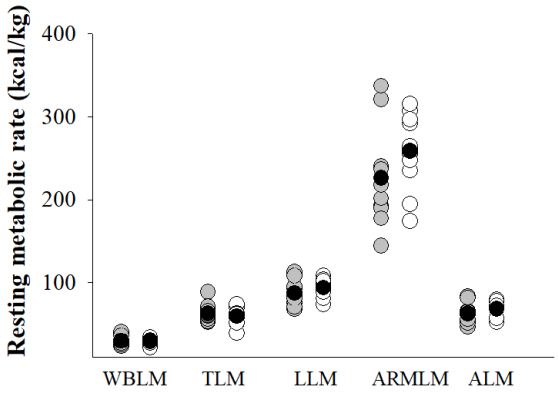


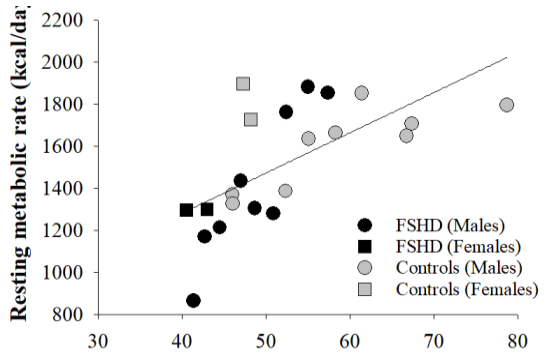
Figure 5.2: Resting metabolic rate (RMR), relative to lean mass (LM), in FSHD. When normalized to regional and local measures of LM, differences in RMR between FSHD and control groups were not observed. kcal, kilocalories; kg, kilograms; p<0.05).

Measures of Fat Mass

In the FSHD group, the absolute volume of whole-body FM was 45% higher than in controls (p = 0.01); similarly, the absolute volume of leg FM was 64% higher in people with FSHD (p < 0.01) **(Table 5.1)**. While the volume of FM in the trunk and arms was 40% and 23% higher, respectively, in the FSHD

group, statistical differences in these measures were not observed ($p > 0.05$ for both) **(Table 5.1)**. Measures of FM were not associated with age in the FSHD nor the control group, nor when participants were analyzed in combination ($p > 0.05$ for all).

5.3A



5.3B

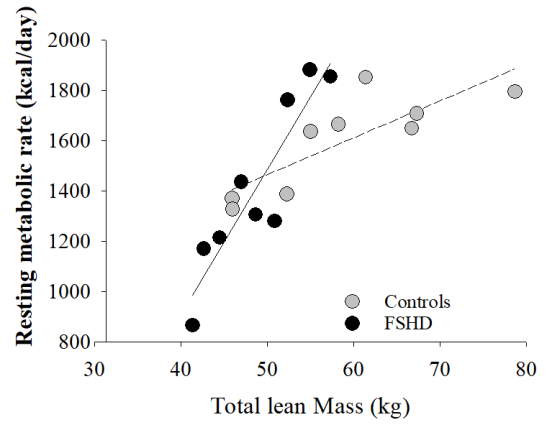


Figure 5.3: Relationship between resting metabolic rate (RMR) and whole-body lean mass (LM) in combined controls and FSHD including females (A) and separate analysis by group excluding females (B). RMR was positively correlated with whole-body LM in both groups when combined ($p<0.01$, r=0.81) and separated with the exclusion of females (FSHD: p<0.01, r=0.92; Control: p=0.004, r= 0.85)). There was a significant difference between the slopes between FSHD and controls (FSHD: 57.7, control: 14.6, t = 4.2, df = 14, p<0.01) indicating a stronger relationship between FSHD and LM in males with FSHD. kcal, kilocalories; kg, kilograms; $p<0.05$.

Self-Reported Measures of Functionality

An attenuated amount of physical activity completed each day was observed among people with FSHD, as compared with controls (activity metabolic index score; 25.1 ± 32.1 vs. 181.6 ± 148.2 kcal/day; $p < 0.01$). Relationships between physical activity and measures of LM [whole-body LM: r (20) = 0.47, p = 0.03; trunk LM: r (20) = 0.47, p = 0.03; ALM: r *(20)* = 0.46, p = 0.03] were noted among FSHD and control participants.

Energy Expenditure

People with FSHD were found to have an absolute RMR that was 15% lower than in the control group (*Glass's Δ* = 1.23, 95% CI [7.6, 472.9], p = 0.04) **(Figure 5.1)**. To determine whether RMR was also influenced by physical activity, we co-varied for physical activity levels. This

reduced the significance of the difference between the groups ($p = 0.09$), indicating that physical activity is likely to account for variation in RMR. Importantly, when RMR was adjusted to regional and local measures of LM **(Figure 5.2**), there were no differences between FSHD and controls [$RMR_{\text{whole-body LM}}$: *Glass's Δ* = 1.03, 95% CI [-3.9, 4.2], p = 0.94; $RMR_{\text{trunk LM}}$: *Glass's Δ* = 0.32, CI [-6.0-12.8], p = 0.46; $RMR_{\text{leg LM}}$: *Glass's Δ* = 0.45, CI [-17.4, 4.7], p = 0.25; $RMR_{\text{arm LM}}$: *Glass's Δ* = 0.56, CI [-78.7, 13.5], p = 0.16 and RMR_{ALM}: *Glass's Δ* = 0.50, CI [-14.6, 3.1], p = 0.18].

Associations were found between RMR, weight ($r\ (20)$ = 0.50, p = 0.02) and BMI ($r\ (20)$ = 0.54, p= 0.01), though not age (p = 0.93). In addition, RMR trended to correlate with physical activity levels ($r\ (20)$ = 0.40, p = 0.07). Furthermore, as expected, RMR was correlated with multiple measures of the absolute volume of LM when groups were combined [whole-body LM: $r\ (20)$ = 0.70, $p < 0.01$, **Figure 5.3A**; trunk LM: $r\ (20)$ = 0.65, $p < 0.01$); leg LM: $r\ (20)$ = 0.82, $p < 0.01$; arm LM: $r\ (20)$ = 0.59, p = 0.01; ALM: $r\ (20)$ = 0.77, $p < 0.01$]. Notably, when the relationship between RMR and whole-body LM in both groups were analyzed separately, the controls were only significant when the two females were removed from the data (r (7) = 0.85, $p < 0.01$). This is likely because the two control female participants had higher RMR values than expected. Thus, when removing the females from each group, RMR was strongly correlated to whole-body LM when combining groups (r (16) = 0.81, p = 0.004) and when separated (FSHD: r (7) = 0.92, $p < 0.01$; control: r (7), = 0.85, p = 0.004, **Figure 5.3B**). The slope for the line of regression for FSHD was steeper than the slope for the line of regression for controls (FSHD: β = 57.7, control: β = 14.6, t (14) = 4.2, $p < 0.01$), indicating a stronger relationship between RMR and whole-body LM in the FSHD group.

DISCUSSION

This study is the first to identify alterations in RMR among people with FSHD which was 15% lower than in control participants. Importantly, this finding appears to be related primarily to differences in the absolute volume of LM, as normalized energy expenditure—or the number of kilocalories expended per kg of LM—was similar between FSHD and control populations. In addition, our study indicates that levels of physical activity confounded the differences in RMR between people with FSHD and controls, with a trend for an association between RMR and physical activity. Collectively, these findings suggest that although RMR is lower in people with FSHD, it is primarily explained by the disease-related reduction in lower LM.

Our observations of a reduced absolute RMR in the FSHD population mirrors research by Gonzalez-Bermejo et al., in which absolute RMR among 20 males (age: 25±4 years) with Duchenne muscular dystrophy (DMD) was 39% lower than in controls [122]. As in our work, this finding disappeared after the correction of RMR by the total volume of LM [122]. These observations are further supported from work by Shimizu-Fujiwara et al., in which 77 people with Duchenne muscular dystrophy, aged 10-37 years, were shown to have an RMR that was significantly lower than in control counterparts. Body composition was not assessed, thus, it is difficult to know the degree to which RMR was affected by the volume of LM [123]. Finally, Saure et al. noted a lower RMR among obese males with Duchenne muscular dystrophy, as compared to control counterparts with multifactorial obesity [22]. As both FSHD and control groups in our study fell into the BMI category of "overweight," this finding is especially interesting, as it suggests that

differences in absolute RMR between dystrophic and control populations may hold true across a range of body weights and compositions.

While we believe that our findings are well-supported by the observations described above, contradictory findings have also been reported. In fact, Okada et al. note that people with Duchenne muscular dystrophy have an absolute basal metabolic rate that exceeds that of healthy age- and sex-matched controls by 20-30% [23], however it is notable that in their work, they did not actually compare to a control group, but rather simply to established normative values. This methodology was likewise replicated among people with limb-girdle muscular dystrophy [23], thereby casting doubt on the claim that basal metabolic rate is similar between this group and controls. Furthermore, while Jacques et al. have reported an RMR that does not differ between people with Becker's muscular dystrophy (n=21) and controls (n=12) [21], a potentially high-degree of variability, combined with a small sample size could have contributed to the lack of significant differences. Importantly, as in our work, people with Becker's muscular dystrophy were found to have a physical activity level that was lower than that observed in controls, though as this group did not co-vary for physical activity or note any correlations with RMR, it is difficult to assess the extent to which it influences or is associated with RMR in the aforementioned study. A low sample size may also explain why Vaisman et al. did not find differences in RMR between young males with Emery-Dreifuss muscular dystrophy (n=6) and controls (n=4), despite observations of a caloric expenditure that was greater in the dystrophic group, following normalization to LM [124]. Methodologic concerns aside, another explanation for these inconsistent findings may be

related to wide-ranging inter-individual variability and/or altered physiologic function within dystrophic subgroups.

Contributions to Resting Metabolic Rate

Body composition. Body composition, or the relative proportion of FM and fat-free mass in the body, has been widely shown to have a strong influence on RMR across the lifespan [99]. Importantly, the impact of fat-free mass on RMR appears to be especially profound, with estimations of the influence of this parameter reaching levels of as high as 70-80% [20]. In our study, we observed positive correlations between multiple measures of RMR and LM, including whole-body LM, which was found to explain 49% of the variance in RMR between FSHD and control groups. We observed that the two females in the control group had relatively high RMR and when only including the males in the analysis (n=18), 65% of the variance in RMR was explained by LM. Further, when groups were separated, the association between RMR and LM was significantly stronger in the FSHD group, with 84% of the variance in RMR explained by LM and 72% of the variance in RMR explained by LM in the controls. This observation provides strong support for LM as a major physiologic "driver" of RMR among people with FSHD, and further underscores the importance of prioritizing interventions to retain LM within this population.

Physical activity. Although RMR is classically measured at rest, an exercise-induced "carry-over effect" following participation in physical activity (thermic effect of exercise) [118], is believed to exert a strong influence on RMR [114]. Furthermore, while the influence of thermic effect of exercise is often considered only in the immediate, post-exercise period, it appears that regular exercise participation can also have a chronic

effect on RMR, even in the absence of acute exercise. According to research by Tremblay et al., individuals who self-identify as engaging in regular physical activity have an RMR that is 11% higher than their sedentary counterparts [119]; perhaps even more strikingly, these observations appear to hold true, even following adjustments for differences in LM [168]. Furthermore, cardiovascular fitness, a measure that is improved through regular participation in structured physical activity, appears to play an important role in the determination of resting energy metabolism, as a relationship between RMR and VO_{2peak} has been previously reported [114]. These observations are important, as our study found self-reported physical activity levels that were significantly lower among people with FSHD, as compared with controls. Furthermore, after co-varying for physical activity, a difference in RMR between FSHD and control participants was not observed, though a trending correlation between RMR and self-reported physical activity among our sample of FSHD and control participants was found. Overall, these observations suggest that a decreased rate of physical activity participation may influence RMR among people with FSHD and suggests the possible benefit of participation in a structured exercise program within this group.

Age. Aging has been associated with a progressive decline in RMR, at a rate of approximately 1-2% per decade, after the age of 20 years [102]. While age-related declines in metabolically-active LM are theorized to be a primary contributor to this phenomenon [105], research by Krems et al., in which RMR was significantly lower in older vs. younger adults, despite adjustments for LM, suggests that the mechanism is not fully explained by changes in body composition alone [104]. In our study, participants were matched for age and age was not associated with measures of LM, FM, or RMR; furthermore, we did not

find age to have a confounding effect on RMR. Taken together, it appears differences in metabolic function between participant groups were likely driven by a combination of factors outside of the aging process.

Sex. Sex appears to have a strong influence on metabolic rate, as males have been shown to have an absolute RMR that is as much as 33% higher than age-matched female counterparts [110]. As with age, this sex-effect appears to be driven primarily by alterations in body composition between males and females, as research by Buchholz et al. notes that the adjustment of RMR for volume of LM resulted in a non-significant difference between sexes of only 4% ($p=0.22$) [110]. Of 22 total participants, our study included only 4 females (FSHD: 2; Control: 2), and therefore, we are unable to draw any conclusions as to whether RMR is different between men and women with FSHD. Previous research has demonstrated sex-driven differences in body composition between male and female FSHD study participants [44], suggesting that should sex-related alterations in RMR among people with FSHD arise, they are likely driven by differences in the ratio of LM-to-FM between sexes within this group. Unexpectedly, the females appeared to weaken the relationship between RMR and LM. Although previous research indicates that these relationships should exist for both males and females [169], the small number of females in our study suggest that the relationship may be stronger in males. Further research is warranted to investigate these relationships in females with and without FSHD.

Limitations

Limitations should be considered when interpreting the data in this study. The small number of study participants, particularly of females, makes it difficult to determine the extent to which sex differences in LM, FM, and other biologic or

environmental determinants influence RMR among people with FSHD. In fact, while sex hormones (estradiol (E_2), progesterone, luteinizing hormone, follicle stimulating hormone) are believed to have an effect on energy metabolism, the extent to which they each contribute to this phenomenon is unclear [103]. Our study did not control for menstrual cycle phase, nor did we identify whether females had yet gone through menopause. However, with a small number of female study participants (FSHD: 2, 38-51 years; control: 2, 31-50 years), it is plausible that any sex hormone-driven variability on RMR overall would be masked by contributions from males, as well as by a combination of other biologic and environmental factors and could contribute to the reduced slope between RMR and LM with inclusion of women.

In our study, three non-Hispanic white FSHD participants (all male) were paired with sex-matched controls of differing races, thereby resulting in the potential for race-related alterations in RMR. Black individuals have been repeatedly shown to have an RMR that is lower than in non-Hispanic white counterparts [115], though this observation has been made primarily in female, and not male, study participants [116]. Likewise, after adjusting for alternations in body composition, differences in RMR between Asians and non-Hispanic whites do not appear to be present [117]. Together, these findings suggest that despite imperfect racial and ethnicity pairing between FSHD and control participants, differences in RMR as related to race are unlikely to contribute to the findings of our study.

Finally, other potential confounding factors that may have contributed to our findings include differences in fluid volume status between study participants, which has been shown to have an impact on the volume of hydrated tissues (i.e., LM) [170]. Though

water intake was not restricted, we did limit this factor as much as possible by requiring study participants to avoid all other beverages during the preparatory fasting period.

CONCLUSION AND NEXT STEPS

This chapter has shown that adults with FSHD have a lower RMR compared with control groups, though it is similar when adjusted for LM. Furthermore, the findings of this section reveal that the low RMR among people with FSHD, particularly males, is strongly driven by a disease-related reduction in LM. RMR is highly correlated with body weight, and therefore, identifying alterations in this measure in a potentially vulnerable, high-risk clinical group is an important translational finding. Historically, RMR prediction equations, which may be used when guiding weight gain, loss, or maintenance, have been based primarily on individual demographic characteristics (i.e., height, weight, age) [24]. However, observations of a measured RMR that is lower than that seen in controls, despite a similar height and weight, suggests that these prediction equations likely *overestimate* kilocalorie needs in the FSHD population. As people with FSHD already appear to have a greater propensity towards adiposity [5, 6, 44, 136], potential recommendations for unnecessarily high caloric intake within this group may further exacerbate the presence of excess body fat stores, and impede attempts at weight loss/maintenance. Consequently, these observations highlight the need for personalized nutritional guidance for people with degenerative neuromuscular disease, both to optimize physiologic function, and reduce the risk of obesity-related morbidity/mortality. In addition, the evidence that this chapter has provided on the influence of and contributions by physical activity on RMR among people with FSHD suggests that engagement in a regular exercise routine may aid in the regulation of body weight, via

both the preservation of LM, as well as through the optimization of an exercise-induced "carry-over effect;" in concluding chapters, I will further discuss potential strategies by which this goal may be achieved. Future research in this area should be focused on identifying more specific macronutrient needs within the FSHD population and determining whether modifications to traditional dietary guidelines may aid in slowing anatomical disease progression. Finally, we encourage further studies designed to determine the influence of exercise on body composition, physical function, and RMR in people with FSHD.

Chapter 6: General Discussion

The studies in this thesis highlight the "real-life" repercussions of genetic mutation among people with FSHD, and more fully outline the implications of an altered body composition on anatomy, functionality, and physiology within this group. **Chapter 3** reports on specific anatomic differences in the volume of LM and FM between people with FSHD and controls, and addresses whether, in combination, these alterations may contribute to a concurrent clinical diagnosis of sarcopenic obesity. Absolute and relative measures of body composition were collected via DXA scanning; these values were compared to sex-specific markers of sarcopenic obesity in the FSHD group and among controls. In addition to notable differences in LM and FM between groups, I found that when assessed in combination, people with FSHD met minimum threshold values for sarcopenic obesity; moreover, when examined individually, the condition was shown to be highly present among males with FSHD, though not among females in the clinical group or in controls of either sex. Together, this suggests that males with FSHD are at high risk for the development of an impactful comorbidity, which has been linked to high rates of disability, further morbidity, and mortality.

In **Chapter 4**, I explored the ways in which people with FSHD respond to exercise, and studied the whether an altered body composition may have pathologic effects on functionality within this group. To better understand the implications of FSHD on exercise tolerance during various aspects of day-to-day life, I examined physiological responses to relative and absolute intensities of exercise, in both FSHD and control groups. This work yielded multiple important findings, to include observations of a greater intolerance to exercise among people with FSHD. Additionally, my hypothesis

that exercise intolerance would be driven by disparate mechanisms was confirmed, as among people with FSHD, activity limitation was shown to be related primarily to peripheral factors, whereas central mechanisms were more influential on exercise intolerance among controls. Finally, I found that while people with FSHD had a cardiopulmonary response that differed from controls, this variation presented in alternative ways during relative and absolute exercise. These observations show that regardless of the intensity at which people with FSHD are working, they will likely be subject to noticeable differences in performance, including greater symptoms of breathlessness and exertion, and may experience impairments in their ability to complete activities of daily living.

The physiologic implications of an altered body composition in FSHD are outlined in **Chapter 5**. In this section, I discuss the ways in which measures of metabolic function, including RMR, differ between people with FSHD and controls, and further reinforce the contributions of LM on these measures. RMR was measured during a 30-minute period of indirect calorimetry, and a three-compartment model of body composition (LM, FM, bone mineral content) was collected via DXA scanning; to account for the influence of body composition on metabolism, RMR was additionally normalized to regional and local measures of LM. RMR was shown to be lower among people with FSHD, however, the normalization of energy expenditure revealed that this was primarily a result of low LM within this group. Furthermore, energy expenditure was confounded by physical activity level, thereby suggesting that the low absolute RMR among people with FSHD was partially explained by a corresponding low volume of self-reported exercise participation. Overall, these observations are impactful, as they

highlight the influence of body composition on metabolic function within the FSHD population, and show that the preservation LM, such as that which may occur via engagement in an exercise regime, is of vital importance.

Limitations

My work includes limitations, which should be kept in mind when designing future studies in this area. First, this research included a small number of participants, potentially resulting in an "underpowered" study and an inability to identify some differences between groups. Moreover, as the number of female FSHD participants was especially small, I was unable to draw any conclusions as to whether differences in outcomes were driven by sex. While the small sample size increased the feasibility of the work, it may be that I unwittingly enrolled a fairly homogenous group of FSHD participants who do not fully reflect the spectrum of the disease; furthermore, as minimally-affected FSHD-females formed only a small proportion of the clinical group, it is likely their influence was overwhelmed by the greater quantity of more profoundly-affected FSHD males. As such, future translational research on the tangible implications of body composition in FSHD should focus on increasing enrollment, with an enhanced emphasis on FSHD females. Tightening inclusion/exclusion criteria, such as medication use, hormonal status, hydration level, and disease severity, would also aid in making the interpretation of data in this work more complete.

In addition to the factors described above, differences in race and ethnicity may impact the conclusions that are drawn from this work. In fact, race has been shown to be an influential driver of several measured variables in this research (RMR, VO_2peak), though it appears that this relationship occurs primarily among females; disparities in

racial pairing in this study occurred only among male FSHD-control partners. In the future, both males and females with FSHD should be matched to controls who are of the same self-reported racial group, thereby providing greater clarity on the anatomic, functional, and physiologic influence of body composition in this clinical population.

Future Directions

Future research with a larger sample size and a more balanced proportion of females is needed to fully understand the anatomic, functional, and physiologic implications of an altered body composition among people with FSHD. This research would further: 1) identify sex-driven differences in clinical markers, exercise performance, and metabolic function among males and females with FSHD, and 2) allow for sub-analyses among people with differing clinical severity levels, thus aligning biologic/biomechanical markers of disease with practical clinical outcomes, and assisting in the clarification of "disease trajectory" for afflicted individuals. To achieve these aims, researchers should thoughtfully select a clinical grading scale, which would allow for appropriate classification of FSHD participants into groups, based on the severity of their disease; using objective means to distinguish between people with FSHD1 and FSHD2 would likewise provide value for future research in this area.

As an altered body composition appears to be profoundly impactful among people with FSHD, identifying ways in which to stop or slow the FSHD-driven changes in the volume of LM and FM among people with this condition is crucial. Importantly, research has previously shown that through participation in an exercise program, people with FSHD can achieve increases in muscular strength, a marker which is believed to be reflective of LM volume; moreover, engagement in this type of activity has not been

found to degrade or compromise LM stores, thereby highlighting its safety and appropriateness for this clinical group [77]. What is lacking in existing research, however, is clarification on whether people with FSHD respond differently to exercise training, as compared to controls, and as such, whether exercise recommendations designed to achieve a desirable body composition may differ between FSHD and control populations. While currently absent, I believe that the creation of a complete set of exercise guidelines for people with FSHD would showcase the safety and benefits of regular physical activity, thereby increasing exercise participation and providing a tangible way for people with FSHD to take a more active role in the management of their disease.

Along with exercise, diet has been shown to play an influential role in the regulation of body weight and composition [171], though the exact extent to which it contributes to the preservation of LM among people with FSHD is not clear. Furthermore, the dietary needs of people with FSHD have not been fully studied, making it difficult to know whether macro- and micronutrient requirements differ between FSHD and general populations. As such, future research in this area should focus on establishing dietary guidelines for people with FSHD, and, identifying whether people with FSHD need a greater intake of dietary protein to maintain LM stores, as compared to controls. Additionally, to ensure a more structured and targeted approach to interventional therapy, future research should focus on discerning whether diet or exercise –alone or in combination—is better able to advantageously influence body composition within this clinical group.

Final Considerations

Among people with FSHD, disadvantageous changes in body composition contribute to severe physical and functional impairment. As such, this dissertation has identified the anatomic, functional, and physiology repercussions of genetic dysregulation in FSHD, thereby laying the groundwork for the creation of tailored therapeutic interventions within this group. I fully believe that by continuing to engage in and promote research in this area, substantial improvements in the quality of life among people with FSHD can be achieved.

REFERENCES

1. Statland JM, Tawil R. Facioscapulohumeral Muscular Dystrophy. *Continuum (Minneap Minn)*. Dec 2016;22(6, Muscle and Neuromuscular Junction Disorders):1916-1931. doi:10.1212/CON.0000000000000399
2. Gabellini D, Green MR, Tupler R. Inappropriate gene activation in FSHD: a repressor complex binds a chromosomal repeat deleted in dystrophic muscle. *Cell*. Aug 2002;110(3):339-48. doi:10.1016/s0092-8674(02)00826-7
3. Wang LH, Tawil R. Facioscapulohumeral Dystrophy. *Curr Neurol Neurosci Rep*. 07 2016;16(7):66. doi:10.1007/s11910-016-0667-0
4. Marra M, Heskamp L, Mul K, et al. Specific muscle strength is reduced in facioscapulohumeral dystrophy: An MRI based musculoskeletal analysis. *Neuromuscular Disorders*. 2018;28(3):238-245.
5. Skalsky AJ, Abresch RT, Han JJ, Shin CS, McDonald CM. The relationship between regional body composition and quantitative strength in facioscapulohumeral muscular dystrophy (FSHD). *Neuromuscul Disord*. Nov 2008;18(11):873-80. doi:10.1016/j.nmd.2008.07.005
6. Janssen B, Voet. N, Nabuurs C, et al. Distinct Disease Phases in Muscles of Facioscapulohumeral Dystorphy Patients Identified by MR Detected Fat Infiltration. *PloS one*. 2014;9(1):e85416.
7. Batsis JA, Villareal DT. Sarcopenic obesity in older adults: aetiology, epidemiology and treatment strategies. *Nat Rev Endocrinol*. 09 2018;14(9):513-537. doi:10.1038/s41574-018-0062-9
8. (WHO) WHO. *Obesity: Preventing and Managing the Global Epidemic*. 2000.
9. Baek SJ, Nam GE, Han KD, et al. Sarcopenia and sarcopenic obesity and their association with dyslipidemia in Korean elderly men: the 2008-2010 Korea National Health and Nutrition Examination Survey. *J Endocrinol Invest*. Mar 2014;37(3):247-60. doi:10.1007/s40618-013-0011-3
10. Lim HS, Park YH, Suh K, et al. Association between Sarcopenia, Sarcopenic Obesity, and Chronic Disease in Korean Elderly. *J Bone Metab*. Aug 2018;25(3):187-193. doi:10.11005/jbm.2018.25.3.187
11. Park SH, Park JH, Song PS, et al. Sarcopenic obesity as an independent risk factor of hypertension. *J Am Soc Hypertens*. 2013 Nov-Dec 2013;7(6):420-5. doi:10.1016/j.jash.2013.06.002
12. Srikanthan P, Hevener AL, Karlamangla AS. Sarcopenia exacerbates obesity-associated insulin resistance and dysglycemia: findings from the National Health and Nutrition Examination Survey III. *PLoS One*. May 2010;5(5):e10805. doi:10.1371/journal.pone.0010805
13. Moon JH, Kong MH, Kim HJ. Implication of Sarcopenia and Sarcopenic Obesity on Lung Function in Healthy Elderly: Using Korean National Health and Nutrition Examination Survey. *J Korean Med Sci*. Nov 2015;30(11):1682-8. doi:10.3346/jkms.2015.30.11.1682
14. Lee SE, Park JH, Kim KA, Kang YS, Choi HS. Association Between Sarcopenic Obesity and Pulmonary Function in Korean Elderly: Results from the Korean National Health and Nutrition Examination Survey. *Calcif Tissue Int*. Oct 2019;doi:10.1007/s00223-019-00623-z

15. Baumgartner RN, Wayne SJ, Waters DL, Janssen I, Gallagher D, Morley JE. Sarcopenic obesity predicts instrumental activities of daily living disability in the elderly. *Obes Res*. Dec 2004;12(12):1995-2004. doi:10.1038/oby.2004.250
16. Hirani V, Naganathan V, Blyth F, et al. Longitudinal associations between body composition, sarcopenic obesity and outcomes of frailty, disability, institutionalisation and mortality in community-dwelling older men: The Concord Health and Ageing in Men Project. *Age Ageing*. 05 2017;46(3):413-420. doi:10.1093/ageing/afw214
17. Owens S, Gutin B. Exercise intolerance. *Pediatr Rev*. Jan 2000;21(1):6-9. doi:10.1542/pir.21-1-6
18. Kitzman DW, Groban L. Exercise intolerance. *Cardiol Clin*. Aug 2011;29(3):461-77. doi:10.1016/j.ccl.2011.06.002
19. Cunningham JJ. Body composition as a determinant of energy expenditure: a synthetic review and a proposed general prediction equation. *Am J Clin Nutr*. Dec 1991;54(6):963-9. doi:10.1093/ajcn/54.6.963
20. Sparti A, DeLany JP, de la Bretonne JA, Sander GE, Bray GA. Relationship between resting metabolic rate and the composition of the fat-free mass. *Metabolism*. Oct 1997;46(10):1225-30. doi:10.1016/s0026-0495(97)90222-5
21. Jacques MF, Orme P, Smith J, Morse CI. Resting Energy Expenditure in Adults with Becker's Muscular Dystrophy. *PLoS One*. 2017;12(1):e0169848. doi:10.1371/journal.pone.0169848
22. Saure C, Caminiti C, Weglinski J, de Castro Perez F, Monges S. Energy expenditure, body composition, and prevalence of metabolic disorders in patients with Duchenne muscular dystrophy. *Diabetes Metab Syndr*. 2018 Apr - Jun 2018;12(2):81-85. doi:10.1016/j.dsx.2017.08.006
23. Okada K, Manabe S, Sakamoto S, Ohnaka M, Niiyama Y. Protein and energy metabolism in patients with progressive muscular dystrophy. *J Nutr Sci Vitaminol (Tokyo)*. Apr 1992;38(2):141-54. doi:10.3177/jnsv.38.141
24. Flack KD, Siders WA, Johnson L, Roemmich JN. Cross-Validation of Resting Metabolic Rate Prediction Equations. *J Acad Nutr Diet*. 09 2016;116(9):1413-1422. doi:10.1016/j.jand.2016.03.018
25. Keller-Ross ML, Johnson BD, Carter RE, et al. Improved Ventilatory Efficiency with Locomotor Muscle Afferent Inhibition is Strongly Associated with Leg Composition in Heart Failure. *Int J Cardiol*. Jan 2016;202:159-66. doi:10.1016/j.ijcard.2015.08.212
26. Keller-Ross ML, Chantigian DP, Evanoff N, Bantle AE, Dengel DR, Chow LS. VE/VCO2 slope in lean and overweight women and its relationship to lean leg mass. *Int J Cardiol Heart Vasc*. Dec 2018;21:107-110. doi:10.1016/j.ijcha.2018.10.009
27. Lalande S, Cross TJ, Keller-Ross ML, Morris NR, Johnson BD, Taylor BJ. Exercise Intolerance in Heart Failure: Central Role for the Pulmonary System. *Exerc Sport Sci Rev*. Jan 2020;48(1):11-19. doi:10.1249/JES.0000000000000208
28. Leosco D, Rengo G, Iaccarino G, et al. Exercise promotes angiogenesis and improves beta-adrenergic receptor signalling in the post-ischaemic failing rat heart. *Cardiovasc Res*. May 2008;78(2):385-94. doi:10.1093/cvr/cvm109
29. Upadhya B, Haykowsky MJ, Eggebeen J, Kitzman DW. Sarcopenic obesity and the pathogenesis of exercise intolerance in heart failure with preserved ejection fraction. *Curr Heart Fail Rep*. Jun 2015;12(3):205-14. doi:10.1007/s11897-015-0257-5

30. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med*. Mar 2002;346(11):793-801. doi:10.1056/NEJMoa011858
31. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc*. Sep 2000;32(9 Suppl):S498-504. doi:10.1097/00005768-200009001-00009
32. Bosnakovski D, Xu Z, Gang EJ, et al. An isogenetic myoblast expression screen identifies DUX4-mediated FSHD-associated molecular pathologies. *EMBO J*. Oct 2008;27(20):2766-79. doi:10.1038/emboj.2008.201
33. Wijmenga C, Hewitt JE, Sandkuijl LA, et al. Chromosome 4q DNA rearrangements associated with facioscapulohumeral muscular dystrophy. *Nat Genet*. Sep 1992;2(1):26-30. doi:10.1038/ng0992-26
34. van Deutekom JC, Wijmenga C, van Tienhoven EA, et al. FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit. *Hum Mol Genet*. Dec 1993;2(12):2037-42. doi:10.1093/hmg/2.12.2037
35. Lemmers RJ, de Kievit P, Sandkuijl L, et al. Facioscapulohumeral muscular dystrophy is uniquely associated with one of the two variants of the 4q subtelomere. *Nat Genet*. Oct 2002;32(2):235-6. doi:10.1038/ng999
36. van Overveld PG, Lemmers RJ, Sandkuijl LA, et al. Hypomethylation of D4Z4 in 4q-linked and non-4q-linked facioscapulohumeral muscular dystrophy. *Nat Genet*. Dec 2003;35(4):315-7. doi:10.1038/ng1262
37. Lemmers RJ, Tawil R, Petek LM, et al. Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. *Nat Genet*. Dec 2012;44(12):1370-4. doi:10.1038/ng.2454
38. Lemmers RJ, van der Vliet PJ, Klooster R, et al. A unifying genetic model for facioscapulohumeral muscular dystrophy. *Science*. Sep 2010;329(5999):1650-3. doi:10.1126/science.1189044
39. Tapscott SJ. The circuitry of a master switch: Myod and the regulation of skeletal muscle gene transcription. *Development*. Jun 2005;132(12):2685-95. doi:10.1242/dev.01874
40. Fanzani A, Conraads VM, Penna F, Martinet W. Molecular and cellular mechanisms of skeletal muscle atrophy: an update. *J Cachexia Sarcopenia Muscle*. Sep 2012;3(3):163-79. doi:10.1007/s13539-012-0074-6
41. Celegato B, Capitanio D, Pescatori M, et al. Parallel protein and transcript profiles of FSHD patient muscles correlate to the D4Z4 arrangement and reveal a common impairment of slow to fast fibre differention and a general deregulation of MyoD-dependent genes. *Proteomics*. 2006;6(19):5303-5321.
42. Merlini L, Vagheggini A, Cocchi D. Sarcopenia and sarcopenic obesity in patients with muscular dystrophy. *Front Aging Neurosci*. 2014;6:274. doi:10.3389/fnagi.2014.00274
43. Kim HK, Merrow AC, Shiraj S, Wong BL, Horn PS, Laor T. Analysis of fatty infiltration and inflammation of the pelvic and thigh muscles in boys with Duchenne muscular dystrophy (DMD): grading of disease involvement on MR imaging and correlation with clinical assessments. *Pediatr Radiol*. Oct 2013;43(10):1327-35. doi:10.1007/s00247-013-2696-z

44. Vera KA, McConville M, Kyba M, Keller-Ross ML. Sarcopenic Obesity in Facioscapulohumeral Muscular Dystrophy. *Front Physiol*. 2020;11:1008. doi:10.3389/fphys.2020.01008
45. Bachasson D, Temesi J, Bankole C, et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: benefits and limits of femoral nerve magnetic stimulation. *Clin Neurophysiol*. Feb 2014;125(2):396-405. doi:10.1016/j.clinph.2013.08.001
46. Lassche S, Stienen GJ, Irving TC, et al. Sarcomeric dysfunction contributes to muscle weakness in facioscapulohumeral muscular dystrophy. *Neurology*. Feb 2013;80(8):733-7. doi:10.1212/WNL.0b013e318282513b
47. Goldstein JA, McNally EM. Mechanisms of muscle weakness in muscular dystrophy. *J Gen Physiol*. Jul 2010;136(1):29-34. doi:10.1085/jgp.201010436
48. Association AOT. Functional Capacity Evaluation. Accessed 06/04/2019, 2019.
49. Morse C, Bostock E, Twiss H, Kapp L, Orme P, Jacques M. The cardiorespiratory response and physiological determinants of the assisted 6-minute handbike cycle test in adult males with muscular dystrophy. *Muscle & Nerve*. 2018;58(3):427-433.
50. Hamrick MW, McGee-Lawrence ME, Frechette DM. Fatty Infiltration of Skeletal Muscle: Mechanisms and Comparisons with Bone Marrow Adiposity. *Front Endocrinol (Lausanne)*. 2016;7:69. doi:10.3389/fendo.2016.00069
51. van Dijk GP, van der Kooi E, Behin A, et al. High prevalence of incomplete right bundle branch block in facioscapulohumeral muscular dystrophy without cardiac symptoms. *Funct Neurol*. 2014 Jul-Sep 2014;29(3):159-65.
52. Laforêt P, de Toma C, Eymard B, et al. Cardiac involvement in genetically confirmed facioscapulohumeral muscular dystrophy. *Neurology*. Nov 1998;51(5):1454-6. doi:10.1212/wnl.51.5.1454
53. Trevisan CP, Pastorello E, Armani M, et al. Facioscapulohumeral muscular dystrophy and occurrence of heart arrhythmia. *Eur Neurol*. 2006;56(1):1-5. doi:10.1159/000094248
54. Unknown. *Heart rate variability. Stands of measurement, physiological interpretation, and clinical use.*
55. Della Marca G, Frusciante R, Scatena M, et al. Heart rate variability in facioscapulohumeral muscular dystrophy. *Funct Neurol*. 2010 Oct-Dec 2010;25(4):211-6.
56. Stübgen JP, Schultz C. Lung and respiratory muscle function in facioscapulohumeral muscular dystrophy. *Muscle Nerve*. Jun 2009;39(6):729-34. doi:10.1002/mus.21261
57. Scully MA, Eichinger KJ, Donlin-Smith CM, Tawil R, Statland JM. Restrictive lung involvement in facioscapulohumeral muscular dystrophy. *Muscle Nerve*. Nov 2014;50(5):739-43. doi:10.1002/mus.24218
58. Kasugai A. Using exercise tolerance to predict mortality in hemodialysis patients. *Journal of the Medical Society of Toho University*. 2002;50
59. Ness KK, Plana JC, Joshi VM, et al. Exercise Intolerance, Mortality, and Organ System Impairment in Adult Survivors of Childhood Cancer. *J Clin Oncol*. Jan 2020;38(1):29-42. doi:10.1200/JCO.19.01661
60. Green S, Askew C. VO_2peak is an acceptable estimate of cardiovascular fitness but not VO_2max. *J of Appl Physiol*. 2018;125(1):229-232.

61. Levine BD. .VO2max: what do we know, and what do we still need to know? *J Physiol*. Jan 2008;586(1):25-34. doi:10.1113/jphysiol.2007.147629
62. Ehrman J, Gordon P, Visich P, Keteyian S. *Clinical Exercise Physiology*. Human Kinetics; 2013.
63. Kitzman DW, Higginbotham MB, Cobb FR, Sheikh KH, Sullivan MJ. Exercise intolerance in patients with heart failure and preserved left ventricular systolic function: failure of the Frank-Starling mechanism. *J Am Coll Cardiol*. Apr 1991;17(5):1065-72. doi:10.1016/0735-1097(91)90832-t
64. Borlaug BA, Melenovsky V, Russell SD, et al. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. *Circulation*. Nov 2006;114(20):2138-47. doi:10.1161/CIRCULATIONAHA.106.632745
65. Guccione A, Wong R, Avers D. *Geriatric Physical Therapy*. 3rd ed. Elsevier; 2011.
66. Myers J, Salleh A, Buchanan N, et al. Ventilatory mechanisms of exercise intolerance in chronic heart failure. *Am Heart J*. Sep 1992;124(3):710-9. doi:10.1016/0002-8703(92)90282-z
67. Anker SD, Swan JW, Volterrani M, et al. The influence of muscle mass, strength, fatigability and blood flow on exercise capacity in cachectic and non-cachectic patients with chronic heart failure. *Eur Heart J*. Feb 1997;18(2):259-69. doi:10.1093/oxfordjournals.eurheartj.a015229
68. Cicoira M, Zanolla L, Franceschini L, et al. Skeletal muscle mass independently predicts peak oxygen consumption and ventilatory response during exercise in noncachectic patients with chronic heart failure. *J Am Coll Cardiol*. Jun 2001;37(8):2080-5. doi:10.1016/s0735-1097(01)01306-7
69. Minotti JR, Christoph I, Oka R, Weiner MW, Wells L, Massie BM. Impaired skeletal muscle function in patients with congestive heart failure. Relationship to systemic exercise performance. *J Clin Invest*. Dec 1991;88(6):2077-82. doi:10.1172/JCI115537
70. Lipkin DP, Jones DA, Round JM, Poole-Wilson PA. Abnormalities of skeletal muscle in patients with chronic heart failure. *Int J Cardiol*. Feb 1988;18(2):187-95. doi:10.1016/0167-5273(88)90164-7
71. Sullivan MJ, Green HJ, Cobb FR. Skeletal muscle biochemistry and histology in ambulatory patients with long-term heart failure. *Circulation*. Feb 1990;81(2):518-27. doi:10.1161/01.cir.81.2.518
72. Turki A, Hayot M, Carnac G, et al. Functional muscle impairment in facioscapulohumeral muscular dystrophy is correlated with oxidative stress and mitochondrial dysfunction. *Free Radic Biol Med*. Sep 2012;53(5):1068-79. doi:10.1016/j.freeradbiomed.2012.06.041
73. Smith JC, Abdala AL, Rybak IA, Paton JFR. Structural and functional architecture of respiratory networks in the mammalian brainstem. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2009;364(1529):2577-2587.
74. Barnabei MS, Martindale JM, Townsend D, Metzger JM. Exercise and muscular dystrophy: implications and analysis of effects on musculoskeletal and cardiovascular systems. *Compr Physiol*. Jul 2011;1(3):1353-63. doi:10.1002/cphy.c100062

75. Morse CI, Bostock EL, Twiss HM, Kapp LH, Orme P, Jacques MF. The cardiorespiratory response and physiological determinants of the assisted 6-minute handbike cycle test in adult males with muscular dystrophy. *Muscle Nerve*. 09 2018;58(3):427-433. doi:10.1002/mus.26146
76. Jakeways N, McKeever T, Lewis S, Weiss S, Britton J. Relationship between FEV_1 reduction and respiratory symptoms in the general population. *Eur Respir J*. 2003;21:658-663.
77. Bankolé LC, Millet GY, Temesi J, et al. Safety and efficacy of a 6-month home-based exercise program in patients with facioscapulohumeral muscular dystrophy: A randomized controlled trial. *Medicine (Baltimore)*. Aug 2016;95(31):e4497. doi:10.1097/MD.0000000000004497
78. Cruse MA. The impact of change in exercise tolerance on activities of daily living and quality of life in COPD: a patient's perspective. *COPD*. Sep 2007;4(3):279-81. doi:10.1080/15412550701480182
79. Kasugai A. Using exercise tolerance to predict mortality in hemodialysis patients. *Journal of the Medical Society of Toho University*. 2002;50:73-80.
80. Baumgartner RN. Body composition in healthy aging. *Ann N Y Acad Sci*. May 2000;904:437-48. doi:10.1111/j.1749-6632.2000.tb06498.x
81. Lee S, Kim TN, Kim SH. Sarcopenic obesity is more closely associated with knee osteoarthritis than is nonsarcopenic obesity: a cross-sectional study. *Arthritis Rheum*. Dec 2012;64(12):3947-54. doi:10.1002/art.37696
82. Androga L, Sharma D, Amodu A, Abramowitz MK. Sarcopenia, obesity, and mortality in US adults with and without chronic kidney disease. *Kidney Int Rep*. Mar 2017;2(2):201-211. doi:10.1016/j.ekir.2016.10.008
83. Hamer M, Batty GD, Kivimaki M. Sarcopenic obesity and risk of new onset depressive symptoms in older adults: English Longitudinal Study of Ageing. *Int J Obes (Lond)*. Dec 2015;39(12):1717-20. doi:10.1038/ijo.2015.124
84. Stephen WC, Janssen I. Sarcopenic-obesity and cardiovascular disease risk in the elderly. *J Nutr Health Aging*. May 2009;13(5):460-6. doi:10.1007/s12603-009-0084-z
85. Atkins JL, Whincup PH, Morris RW, Lennon LT, Papacosta O, Wannamethee SG. Sarcopenic obesity and risk of cardiovascular disease and mortality: a population-based cohort study of older men. *J Am Geriatr Soc*. Feb 2014;62(2):253-60. doi:10.1111/jgs.12652
86. Fong Y, Moldawer LL, Marano M, et al. Cachectin/TNF or IL-1 alpha induces cachexia with redistribution of body proteins. *Am J Physiol*. Mar 1989;256(3 Pt 2):R659-65. doi:10.1152/ajpregu.1989.256.3.R659
87. Walrand S, Guillet C, Salles J, Cano N, Boirie Y. Physiopathological mechanism of sarcopenia. *Clin Geriatr Med*. Aug 2011;27(3):365-85. doi:10.1016/j.cger.2011.03.005
88. Short KR, Nair KS. The effect of age on protein metabolism. *Curr Opin Clin Nutr Metab Care*. Jan 2000;3(1):39-44. doi:10.1097/00075197-200001000-00007
89. Narici MV, Maffulli N. Sarcopenia: characteristics, mechanisms and functional significance. *Br Med Bull*. 2010;95:139-59. doi:10.1093/bmb/ldq008
90. Oussaada SM, van Galen KA, Cooiman MI, et al. The pathogenesis of obesity. *Metabolism*. 03 2019;92:26-36. doi:10.1016/j.metabol.2018.12.012

91. Goodpaster BH, Park SW, Harris TB, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci*. Oct 2006;61(10):1059-64. doi:10.1093/gerona/61.10.1059
92. Rodríguez MA, Del Rio Barquero LM, Ortez CI, et al. Differences in Adipose Tissue and Lean Mass Distribution in Patients with Collagen VI Related Myopathies Are Associated with Disease Severity and Physical Ability. *Front Aging Neurosci*. 2017;9:268. doi:10.3389/fnagi.2017.00268
93. Miscione MT, Bruno F, Ripamonti C, et al. Body composition, muscle strength, and physical function of patients with Bethlem myopathy and Ullrich congenital muscular dystrophy. *ScientificWorldJournal*. 2013;2013:152684. doi:10.1155/2013/152684
94. Schofield KL, Thorpe H, Sims ST. Resting metabolic rate prediction equations and the validity to assess energy deficiency in the athlete population. *Exp Physiol*. 04 2019;104(4):469-475. doi:10.1113/EP087512
95. Trexler ET, Smith-Ryan AE, Norton LE. Metabolic adaptation to weight loss: implications for the athlete. *J Int Soc Sports Nutr*. Feb 2014;11(1):7. doi:10.1186/1550-2783-11-7
96. Zampino M, AlGhatrif M, Kuo PL, Simonsick EM, Ferrucci L. Longitudinal Changes in Resting Metabolic Rates with Aging Are Accelerated by Diseases. *Nutrients*. Oct 2020;12(10)doi:10.3390/nu12103061
97. Anker SD, Ponikowski P, Varney S, et al. Wasting as independent risk factor for mortality in chronic heart failure. *Lancet*. Apr 1997;349(9058):1050-3. doi:10.1016/S0140-6736(96)07015-8
98. Connolly J, Romano T, Patruno M. Selections from current literature: effects of dieting and exercise on resting metabolic rate and implications for weight management. *Fam Pract*. Apr 1999;16(2):196-201. doi:10.1093/fampra/16.2.196
99. Carpenter WH, Poehlman ET, O'Connell M, Goran MI. Influence of body composition and resting metabolic rate on variation in total energy expenditure: a meta-analysis. *Am J Clin Nutr*. 1995;61:4-10.
100. Svendsen OL, Hassager C, Christiansen C. Impact of regional and total body composition and hormones on resting energy expenditure in overweight postmenopausal women. *Metabolism*. 1993;42(12):1588-1591.
101. Nelson KM, Weinsier RL, Long CL, Schutz Y. Prediction of resting energy expenditure from fat-free mass and fat mass. *Am J Clin Nutr*. 1992;56:848-856.
102. Elia M, Ritz P, Stubbs RJ. Total energy expenditure in the elderly. *Eur J Clin Nutr*. Jun 2000;54 Suppl 3:S92-103. doi:10.1038/sj.ejcn.1601030
103. Poehlman ET, Goran MI, Gardner AW, et al. Determinants of decline in resting metabolic rate in aging females. *Am J Physiol*. Mar 1993;264(3 Pt 1):E450-5. doi:10.1152/ajpendo.1993.264.3.E450
104. Krems C, Lhrmann PM, Neuhuser-Berthold M. Physical activity in young and elderly subjects. *J Sports Med Phys Fitness*. Mar 2004;44(1):71-6.
105. St-Onge MP, Gallagher D. Body composition changes with aging: the cause or the result of alterations in metabolic rate and macronutrient oxidation? *Nutrition*. Feb 2010;26(2):152-5. doi:10.1016/j.nut.2009.07.004

106. He Q, Heshka S, Albu J, et al. Smaller organ mass with greater age, except for heart. *J Appl Physiol (1985)*. Jun 2009;106(6):1780-4. doi:10.1152/japplphysiol.90454.2008
107. Cree MG, Newcomer BR, Katsanos CS, et al. Intramuscular and liver triglycerides are increased in the elderly. *J Clin Endocrinol Metab*. Aug 2004;89(8):3864-71. doi:10.1210/jc.2003-031986
108. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *J Appl Physiol (1985)*. Jul 2000;89(1):104-10. doi:10.1152/jappl.2000.89.1.104
109. Zane AC, Reiter DA, Shardell M, et al. Muscle strength mediates the relationship between mitochondrial energetics and walking performance. *Aging Cell*. 06 2017;16(3):461-468. doi:10.1111/acel.12568
110. Buchholz AC, Rafii M, Pencharz PB. Is resting metabolic rate different between men and women? *British Journal of Nutrition*. 2001;86:641-646.
111. Arciero PJ, Goran MI, Poehlman ET. Resting metabolic rate is lower in women than in men. *J Appl Physiol (1985)*. Dec 1993;75(6):2514-20. doi:10.1152/jappl.1993.75.6.2514
112. Ferraro R, Lillioja S, Fontvieille AM, Rising R, Bogardus C, Ravussin E. Lower sedentary metabolic rate in women compared with men. *J Clin Invest*. Sep 1992;90(3):780-4. doi:10.1172/JCI115951
113. Melanson EL, Gavin KM, Shea KL, et al. Regulation of energy expenditure by estradiol in premenopausal women. *J Appl Physiol (1985)*. Nov 2015;119(9):975-81. doi:10.1152/japplphysiol.00473.2015
114. Serra M, Ryan AS. Resting Energy Expenditure: Determinants and Practical Applications for Athletes. *Sports Medicine*. 2016;
115. Gannon B, DiPietro L, Poehlman ET. Do African Americans have lower energy expenditure than Caucasians? *Int J Obes Relat Metab Disord*. Jan 2000;24(1):4-13. doi:10.1038/sj.ijo.0801115
116. Weyer C, Snitker S, Bogardus C, Ravussin E. Energy metabolism in African Americans: potential risk factors for obesity. *Am J Clin Nutr*. Jul 1999;70(1):13-20. doi:10.1093/ajcn/70.1.13
117. Wouters-Adriaens MP, Westerterp KR. Low resting energy expenditure in Asians can be attributed to body composition. *Obesity (Silver Spring)*. Oct 2008;16(10):2212-6. doi:10.1038/oby.2008.343
118. Binns A, Gray M, Di Brezzo R. Thermic effect of food, exercise, and total energy expenditure in active females. *J Sci Med Sport*. Mar 2015;18(2):204-8. doi:10.1016/j.jsams.2014.01.008
119. Tremblay A, Fontaine E, Poehlman ET, Mitchell D, Perron L, Bouchard C. The effect of exercise-training on resting metabolic rate in lean and moderately obese individuals. *Int J Obes*. 1986;10(6):511-7.
120. Thompson J, Manore MM, Skinner JS. Resting metabolic rate and thermic effect of a meal in low- and adequate-energy intake male endurance athletes. *Int J Sport Nutr*. Jun 1993;3(2):194-206. doi:10.1123/ijsn.3.2.194
121. Thompson J, Manore MM. Predicted and measured resting metabolic rate of male and female endurance athletes. *J Am Diet Assoc*. Jan 1996;96(1):30-4. doi:10.1016/S0002-8223(96)00010-7

122. Gonzalez-Bermejo J, Lofaso F, Falaize L, et al. Resting energy expenditure in Duchenne patients using home mechanical ventilation. *Eur Respir J*. Apr 2005;25(4):682-7. doi:10.1183/09031936.05.00031304
123. Shimizu-Fujiwara M, Komaki H, Nakagawa E, et al. Decreased resting energy expenditure in patients with Duchenne muscular dystrophy. *Brain Dev*. Mar 2012;34(3):206-12. doi:10.1016/j.braindev.2011.05.005
124. Vaisman N, Katzenellenbogen S, Nevo Y. Increased resting energy expenditure in subjects with Emery-Dreifuss muscular dystrophy. *Neuromuscul Disord*. Feb 2004;14(2):142-6. doi:10.1016/j.nmd.2003.10.012
125. Dandapat A, Perrin BJ, Cabelka C, et al. High Frequency Hearing Loss and Hyperactivity in DUX4 Transgenic Mice. *PLoS One*. 2016;11(3):e0151467. doi:10.1371/journal.pone.0151467
126. Deenen JC, Arnts H, van der Maarel SM, et al. Population-based incidence and prevalence of facioscapulohumeral dystrophy. *Neurology*. Sep 2014;83(12):1056-9. doi:10.1212/WNL.0000000000000797
127. McNally EM, Kaltman JR, Benson DW, et al. Contemporary cardiac issues in Duchenne muscular dystrophy. Working Group of the National Heart, Lung, and Blood Institute in collaboration with Parent Project Muscular Dystrophy. *Circulation*. May 2015;131(18):1590-8. doi:10.1161/CIRCULATIONAHA.114.015151
128. Statland JM, Tawil R. Risk of functional impairment in Facioscapulohumeral muscular dystrophy. *Muscle Nerve*. Apr 2014;49(4):520-7. doi:10.1002/mus.23949
129. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing*. Jul 2010;39(4):412-23. doi:10.1093/ageing/afq034
130. Prado CM, Wells JC, Smith SR, Stephan BC, Siervo M. Sarcopenic obesity: A Critical appraisal of the current evidence. *Clin Nutr*. Oct 2012;31(5):583-601. doi:10.1016/j.clnu.2012.06.010
131. Lof M, Olausson H, Bostrom K, Janerot-Sjöberg B, Sohlstrom A, Forsum E. Changes in basal metabolic rate during pregnancy in relation to changes in body weight and composition, cardiac output, insulin-like growth factor I, and thyroid hormones and in relation to fetal growth. *Am J Clin Nutr*. Mar 2005;81(3):678-85. doi:10.1093/ajcn/81.3.678
132. Dewey KG. Energy and protein requirements during lactation. *Annu Rev Nutr*. 1997;17:19-36. doi:10.1146/annurev.nutr.17.1.19
133. Richardson MT, Leon AS, Jacobs DR, Ainsworth BE, Serfass R. Comprehensive evaluation of the Minnesota Leisure Time Physical Activity Questionnaire. *J Clin Epidemiol*. Mar 1994;47(3):271-81. doi:10.1016/0895-4356(94)90008-6
134. Johnson NE, Quinn C, Eastwood E, Tawil R, Heatwole CR. Patient-identified disease burden in facioscapulohumeral muscular dystrophy. *Muscle Nerve*. Dec 2012;46(6):951-3. doi:10.1002/mus.23529
135. Hamel J, Johnson N, Tawil R, et al. Patient-Reported Symptoms in Facioscapulohumeral Muscular Dystrophy (PRISM-FSHD). *Neurology*. Sep 2019;93(12):e1180-e1192. doi:10.1212/WNL.0000000000008123
136. Orrell RW. *Handbook of Clinical Neurology*. 1 ed. vol 101. Elsevier; 2011.

137. Du K, Goates S, Arensberg M, Pereira S, Gaillard T. Prevalence of Sarcopenia and Sarcopenic Obesity Vary with Race/Ethnicity and Advancing Age. *Divers Equal Health Care*. 2018;15(4):175-183.
138. Zatz M, Marie SK, Cerqueira A, Vainzof M, Pavanello RC, Passos-Bueno MR. The facioscapulohumeral muscular dystrophy (FSHD1) gene affects males more severely and more frequently than females. *Am J Med Genet*. May 1998;77(2):155-61.
139. Tonini MM, Passos-Bueno MR, Cerqueira A, Matioli SR, Pavanello R, Zatz M. Asymptomatic carriers and gender differences in facioscapulohumeral muscular dystrophy (FSHD). *Neuromuscul Disord*. Jan 2004;14(1):33-8.
140. Kilmer DD, Abresch RT, McCrory MA, et al. Profiles of neuromuscular diseases. Facioscapulohumeral muscular dystrophy. *Am J Phys Med Rehabil*. 1995 Sep-Oct 1995;74(5 Suppl):S131-9. doi:10.1097/00002060-199509001-00007
141. Baumgartner RN, Heymsfield SB, Roche AF. Human body composition and the epidemiology of chronic disease. *Obes Res*. Jan 1995;3(1):73-95. doi:10.1002/j.1550-8528.1995.tb00124.x
142. Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys, 1960 to 1991. *JAMA*. Jul 1994;272(3):205-11. doi:10.1001/jama.272.3.205
143. Gallagher D, Visser M, Sepúlveda D, Pierson RN, Harris T, Heymsfield SB. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol*. Feb 1996;143(3):228-39. doi:10.1093/oxfordjournals.aje.a008733
144. Yao Z, Snider L, Balog J, et al. DUX4-induced gene expression is the major molecular signature in FSHD skeletal muscle. *Hum Mol Genet*. Oct 2014;23(20):5342-52. doi:10.1093/hmg/ddu251
145. Bosnakovski D, Gearhart MD, Toso EA, Ener ET, Choi SH, Kyba M. Low level DUX4 expression disrupts myogenesis through deregulation of myogenic gene expression. *Sci Rep*. 11 2018;8(1):16957. doi:10.1038/s41598-018-35150-8
146. Myers A, Gibbons C, Finlayson G, Blundell J. Associations among sedentary and active behaviours, body fat and appetite dysregulation: investigating the myth of physical inactivity and obesity. *Br J Sports Med*. Nov 2017;51(21):1540-1544. doi:10.1136/bjsports-2015-095640
147. Andries A, van Walsem MR, Frich JC. Self-reported physical activity in people with limb-girdle muscular dystrophy and Charcot-Marie-Tooth disease in Norway. *BMC Musculoskelet Disord*. Apr 2020;21(1):235. doi:10.1186/s12891-020-03246-w
148. Heymsfield SB, Peterson CM, Thomas DM, Heo M, Schuna JM. Why are there race/ethnic differences in adult body mass index-adiposity relationships? A quantitative critical review. *Obes Rev*. Mar 2016;17(3):262-75. doi:10.1111/obr.12358
149. Grumstrup-Scott J, Marriott BM. *Body composition and physical performance: applications for the military services*. National Academies Press; 1992.
150. Gerace L, Aliprantis A, Russell M, et al. Skeletal differences between black and white men and their relevance to body composition estimates. *Am J Hum Biol*. 1994;6(2):255-262. doi:10.1002/ajhb.1310060215
151. Smith JR, Joyner MJ, Curry TB, et al. Locomotor muscle group III/IV afferents constrain stroke volume and contribute to exercise intolerance in human heart failure. *J Physiol*. 12 2020;598(23):5379-5390. doi:10.1113/JP280333

152. Rocha A, Arbex FF, Sperandio PA, et al. Exercise intolerance in comorbid COPD and heart failure: the role of impaired aerobic function. *Eur Respir J*. 04 2019;53(4)doi:10.1183/13993003.02386-2018
153. Haykowsky MJ, Brubaker PH, Morgan TM, Kritchevsky S, Eggebeen J, Kitzman DW. Impaired aerobic capacity and physical functional performance in older heart failure patients with preserved ejection fraction: role of lean body mass. *J Gerontol A Biol Sci Med Sci*. Aug 2013;68(8):968-75. doi:10.1093/gerona/glt011
154. Kitzman DW, Nicklas B, Kraus WE, et al. Skeletal muscle abnormalities and exercise intolerance in older patients with heart failure and preserved ejection fraction. *Am J Physiol Heart Circ Physiol*. May 2014;306(9):H1364-70. doi:10.1152/ajpheart.00004.2014
155. Molina AJ, Bharadwaj MS, Van Horn C, et al. Skeletal Muscle Mitochondrial Content, Oxidative Capacity, and Mfn2 Expression Are Reduced in Older Patients With Heart Failure and Preserved Ejection Fraction and Are Related to Exercise Intolerance. *JACC Heart Fail*. 08 2016;4(8):636-45. doi:10.1016/j.jchf.2016.03.011
156. Crisafulli A, Piras F, Chiappori P, et al. Estimating stroke volume from oxygen pulse during exercise. *Physiol Meas*. Oct 2007;28(10):1201-12. doi:10.1088/0967-3334/28/10/006
157. Craig JC, Colburn TD, Caldwell JT, et al. Central and peripheral factors mechanistically linked to exercise intolerance in heart failure with reduced ejection fraction. *Am J Physiol Heart Circ Physiol*. 08 2019;317(2):H434-H444. doi:10.1152/ajpheart.00164.2019
158. Power LC, Gusso S, Hornung TS, et al. Exercise Cardiac Magnetic Resonance Imaging in Boys With Duchenne Muscular Dystrophy Without Cardiac Disease. *Pediatr Neurol*. Apr 2021;117:35-43. doi:10.1016/j.pediatrneurol.2020.12.011
159. Wang CY, Haskell WL, Farrell SW, et al. Cardiorespiratory fitness levels among US adults 20-49 years of age: findings from the 1999-2004 National Health and Nutrition Examination Survey. *Am J Epidemiol*. Feb 2010;171(4):426-35. doi:10.1093/aje/kwp412
160. Johnstone AM, Murison SD, Duncan JS, Rance KA, Speakman JR. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *Am J Clin Nutr*. Nov 2005;82(5):941-8. doi:10.1093/ajcn/82.5.941
161. Radley-Crabb HG, Marini JC, Sosa HA, Castillo LI, Grounds MD, Fiorotto ML. Dystropathology increases energy expenditure and protein turnover in the mdx mouse model of duchenne muscular dystrophy. *PLoS One*. 2014;9(2):e89277. doi:10.1371/journal.pone.0089277
162. Strakova J, Kamdar F, Kulhanek D, et al. Integrative effects of dystrophin loss on metabolic function of the mdx mouse. *Sci Rep*. 09 2018;8(1):13624. doi:10.1038/s41598-018-31753-3
163. Haugen HA, Chan LN, Li F. Indirect calorimetry: a practical guide for clinicians. *Nutr Clin Pract*. Aug 2007;22(4):377-88. doi:10.1177/0115426507022004377
164. Compher C, Frankenfield D, Keim N, Roth-Yousey L, Group EAW. Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. *J Am Diet Assoc*. Jun 2006;106(6):881-903. doi:10.1016/j.jada.2006.02.009

165. Gupta RD, Ramachandran R, Venkatesan P, Anoop S, Joseph M, Thomas N. Indirect Calorimetry: From Bench to Bedside. *Indian J Endocrinol Metab*. 2017 Jul-Aug 2017;21(4):594-599. doi:10.4103/ijem.IJEM_484_16
166. Byrne NM, Weinsier RL, Hunter GR, et al. Influence of distribution of lean body mass on resting metabolic rate after weight loss and weight regain: comparison of responses in white and black women. *Am J Clin Nutr*. Jun 2003;77(6):1368-73. doi:10.1093/ajcn/77.6.1368
167. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol*. Nov 2013;4:863. doi:10.3389/fpsyg.2013.00863
168. Ballor DL, Poehlman ET. Resting metabolic rate and coronary-heart-disease risk factors in aerobically and resistance-trained women. *Am J Clin Nutr*. Dec 1992;56(6):968-74. doi:10.1093/ajcn/56.6.968
169. Cunningham JJ. Body composition and resting metabolic rate: the myth of feminine metabolism. *Am J Clin Nutr*. Oct 1982;36(4):721-6. doi:10.1093/ajcn/36.4.721
170. Toomey CM, McCormack WG, Jakeman P. The effect of hydration status on the measurement of lean tissue mass by dual-energy X-ray absorptiometry. *Eur J Appl Physiol*. Mar 2017;117(3):567-574. doi:10.1007/s00421-017-3552-x
171. Drenowatz C, Shook RP, Hand GA, Hébert JR, Blair SN. The independent association between diet quality and body composition. *Sci Rep*. May 2014;4:4928. doi:10.1038/srep04928

APPENDIX A: MEDICAL HISTORY AND SCREENING FORM

General Information

Participant:

Name _____

Address _____

Contact phone numbers _____

Birth date _____

Marital Status:

□ Single □ Married □ Divorced □ Widowed

Sex:

□ Male □ Female

Education:

□ High School □ College (2-4 years)

□ Graduate School □ Degree _____

Present Medical History

Check those questions to which you answer yes (leave the others blank).

- ☐ Has a doctor ever said your blood pressure was too high?
- ☐ Do you ever have pain in your chest or heart?
- ☐ Are you often bothered by a thumping of the heart?
- ☐ Does your heart often race?
- ☐ Do you ever notice extra heartbeats or skipped beats?
- ☐ Has a doctor ever said that you have or have had heart trouble, an abnormal electrocardiogram (ECG or EKG), heart attack or coronary?
- ☐ Do you suffer from frequent cramps in your legs?
- ☐ Do you often have difficulty breathing?
- ☐ Do you get out of breath long before anyone else?

Comments: _____

_____

_____

_____

Do you now have or have you recently experienced:

- ☐ Chronic, recurrent or morning cough?

- ☐ Episode of coughing up blood?
- ☐ Increased anxiety or depression?
- ☐ Problems with recurrent fatigue, trouble sleeping or increased irritability?
- ☐ Migraine or recurrent headaches?
- ☐ Swollen or painful knees or ankles?
- ☐ Swollen, stiff or painful joints?
- ☐ Foot problems?
- ☐ Back problems?
- ☐ Stomach or intestinal problems, such as recurrent heartburn, ulcers, constipation or diarrhea?
- ☐ Significant vision or hearing problems?
- ☐ A fever, which can cause dehydration and rapid heart beat?
- ☐ A deep vein thrombosis (blood clot)?
- ☐ A hernia that is causing symptoms?

Comments: _____
_____
_____
_____

Women only answer the following. Do you have:

- ☐ Menstrual period problems?
- ☐ Significant childbirth - related problems?
- ☐ Urine loss when you cough, sneeze or laugh?

Comments: _____
_____
_____
_____

Men and women answer the following:

List any prescription medications you are now taking: _____
_____
_____

List any self-prescribed medications, dietary supplements, or vitamins you are now taking:
_____
_____
_____

Date of last complete physical examination: _____

☐ Normal ☐ Abnormal ☐ Never ☐ Can't remember

List any other medical or diagnostic test you have had in the past two years: _____
_____
_____

List hospitalizations, including dates of and reasons for hospitalization: _____
_____
_____

List any drug allergies: _____
_____
_____

Past Medical History

Check those questions to which your answer is yes (leave others blank).

- ☐ Heart attack if so, how many years ago? _____
- ☐ Heart murmur
- ☐ Diseases of the arteries
- ☐ Arthritis of legs or arms
- ☐ Diabetes or abnormal blood-sugar tests
- ☐ Phlebitis (inflammation of a vein)
- ☐ Dizziness or fainting spells
- ☐ Epilepsy or seizures
- ☐ Stroke
- ☐ Infectious mononucleosis
- ☐ Nervous or emotional problems
- ☐ Anemia
- ☐ Thyroid problems
- ☐ Asthma
- ☐ Other lung disease

Comments: _____
_____
_____
_____

In case of emergency contact information

Name:_____ Relationship:_____

Number: _____

APPENDIX B

Modified Minnesota Leisure Time Physical Activity Questionnaire

Instructions: To the best of your knowledge and as most accurately as you can, please fill out the physical activity questionnaire. Please indicate yes to activities that you have performed, the month of the activity, the average number of times per month and times per occasion. There are additional spaces under other if there are activities that you do that are not listed here. If you exercise regularly the section titled other is where you may indicate these activities.

ACTIVITY	Did you perform this activity?		Month of Activity												Average time per session		Times per month
	No	Yes	J	F	M	A	M	J	J	A	S	O	N	D	Hrs	Min	
Section A: Walking and Miscellaneous																	
-Walking for pleasure																	
-Walking to work																	
-Walking during work breaks																	
-Using stairs when elevator is available																	
-Biking to work and/or for pleasure																	
Section B: Conditioning Exercise																	
-Jog/Walk combination																	
Section C: Other																	

APPENDIX C

The Facioscapulohumeral Muscular Dystrophy Health Index						
Directions: Please check the box that best applies to you for each item.						
1. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Limitations with mobility or walking						
b.) Problems with hands or fingers						
c.) Emotional issues						
d.) Difficulty thinking						
e.) Decreased satisfaction in social situations						

f.) Decreased performance in social situations						
g.) Inability to do activities						
h.) Fatigue						
i.) Pain						
j.) Problems eating						
k.) Communication difficulties						
l.) Problems with shoulders or arms						
m.) Back, chest, or abdomen weakness						
n.) Impaired body image due to disease						

2. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Difficulty lifting objects						
b.) Difficulty reaching objects overhead						
c.) Difficulty brushing or washing hair						
d.) Arm weakness						
e.) Shoulder weakness						
f.) Reduced arm and shoulder range of motion						
g.) Difficulty putting away dishes overhead						

h.) Difficulty carrying a load						
i.) Difficulty reaching items on the back of a shelf or fridge						
j.) Difficulty lifting kids						
3. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Impaired walking						
b.) Difficulty with stairs						
c.) Falls						
d.) Leg weakness						

e.) Difficulty with balance						
f.) Difficulty walking long distances						
g.) Inability to run						
h.) Difficulty rising from a seated position						
i.) Difficulty getting up from a lying position						
j.) Difficulty walking up hills or inclines						
k.) Difficulty walking on rough or uneven ground						
l.) Difficulty standing						
m.) Slow walking						
n.) Tripping						

o.) Ankle weakness						
4. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Impaired endurance						
b.) Prolonged recovery time after exercise						
c.) Decreased leg endurance (stamina)						
d.) Tired muscles						
e.) Decreased activity level						
f.) Excessive sleep requirements						

g.) Breathing difficulties						
5. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Memory deficits						
b.) Problems concentrating						
6. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Difficulty playing sports						

b.) Increased time to complete activity						
c.) Difficulty getting in and out of vehicles						
d.) Difficulty doing yard work						
e.) Difficulty cleaning a home						
f.) Trouble getting out of a tub or shower						
g.) Impaired dancing						
h.) Impaired ability to exercise						
i.) Difficulty bathing or taking a shower						
j.) Difficulty changing a light bulb						
k.) Impaired sexual function						

l.) Difficultly holding a milk gallon						
m.) Difficulty getting off of a toilet						
n.) Trouble going up step ladders						
o.) Difficulty scrubbing surfaces						
p.) Difficulty putting on shoes						
q.) Difficulty using a hammer or other hand tool						
7. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Weak trunk (core) muscles						

b.) Back weakness						
c.) Difficulty bending down						
d.) Difficulty rolling over in bed						
e.) Difficulty sitting up from laying						
f.) Weak chest muscles						
g.) Difficulty getting out of bed due to weakness						
h.) Neck weakness						
i.) Abdominal muscle weakness						

8. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Problems swallowing						
b.) Difficulty using a straw						
9. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Reliance on family members						
b.) Limitations physically on what you can do						
c.) Limited activities due to weakness						

d.) Inability to keep pace with others while walking						
e.) Reliance on friends						
f.) Impaired social interactions						
g.) The avoidance of social situations						
10. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Impaired body image due to how you walk						
b.) Impaired image due to shoulder winging						

11. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Difficulty picking things up with your fingers						
b.) Hand weakness						
c.) Dropping objects						
d.) Difficulty opening jars or bottles						

12. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely

a.) Lack of ability to participate in fun activities						
b.) Not comfortable in social environment						
c.) Family stress						
13. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Back pain						
b.) Pain all over						
c.) Shoulder pain						
d.) Leg pain						
e.) Neck pain						

f.) Muscle pain after exertion						
g.) Eye irritation						
h.) Limited activity from pain						
i.) Muscle cramping						
j.) Hip pain						
k.) Arm pain						
14. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Depression						

b.) Frustration						
c.) Anxiety						
d.) Fear of falling						
e.) Anger						
f.) Sadness						
g.) Stress						
h.) Fear of choking						
i.) Feeling of being overwhelmed						

15. How much does the following impact your life now?	I don't experience this	I experience this but it does not affect my life	It affects my life a little	It affects my life moderately	It affects my life very much	It affects my life severely
a.) Impaired facial expression						
b.) Inability to smile						
c.) Hearing difficulties						
d.) Inability to raise hands						
END OF SURVEY						

APPENDIX D

UNIVERSITY OF MINNESOTA

Twin Cities Campus ***Division of Physical Therapy*** *Manda L Keller-Ross, PhD, DPT, PT*

Medical School *Mayo Mail Code 388*

420 Delaware Street S.E.

Minneapolis, MN 55455

Office: 612-625-3175

Dear Sir or Madam,

The Cardiovascular Research & Rehabilitation Laboratory within the Program of Physical Therapy and Rehabilitation Sciences at the University of Minnesota is conducting a research study to understand if the resting metabolic rate and cardiovascular response to exercise are affected by the genetic mutation that causes facioscapulohumeral muscular dystrophy.

We are seeking volunteers who have been diagnosed with facioscapulohumeral muscular dystrophy to participate in our study. Volunteers must be over the age of 18 and not be pregnant or nursing. This study requires 1-2 visits to the lab, and will last a total of approximately three hours. Monetary compensation is available for eligible participants.

If you are interested in hearing more about this study, please contact the study coordinator. Contact information is included in this letter as well as on the attached flier. If you are interested in learning more about our laboratory and our research please visit our website at:

https://www.rehabmedicine.umn.edu/research/research-labs/cardiovascular-research-and-rehabilitation-lab

We appreciate your consideration and look forward to talking with you further.

Sincerely,

Manda L Keller-Ross

Contact Information:

Study Coordinator: Kathryn Vera; 612-624-6534/crrl@umn.edu