INDIVIDUAL VARIABILITY IN MRNA EXPRESSION RESPONSES TO RESISTANCE EXERCISE

by

Jacob Bonafigia

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Abstract

Several studies have observed variability in the individual mRNA expression responses to acute exercise. However, because observed responses contain a degree of measurement error and within-subject variability, it is unknown whether variability in the mRNA expression responses to acute exercise exists. The purpose of this thesis was to determine whether variability in the individual mRNA expression responses to acute exercise exists. Utilizing freely available microarray data, variability in observed responses to acute resistance exercise (RE) and CON were compared to determine the variability in mRNA expression responses to RE. The previously published microarray study (1) collected skeletal muscle biopsies before (PRE) and 24 hours after (POST) RE/CON and genome-wide mRNA expression responses were measured using Affymetrix microarray chips. Standard deviation (SD) in the observed responses to CON were subtracted from RE to determine an effect size of variability in the responses (ESIR) to RE. Although 15849 transcripts had an incalculable ESIR (i.e. SD in observed responses to CON > RE), 29933 transcripts had an ESIR > 0 and 1486 mRNA had a large ESIR (i.e. ESIR > 0.6). WebGestalt’s gene set enrichment analysis revealed that the 1486 mRNA with a large ESIR were involved in several pathways proposed to play roles in regulating the adaptive responses to resistance training (RT). The 1486 mRNA with a large ESIR were also compared to a group of 2756 mRNA (2) previously found to have acute expression responses that correlate with skeletal muscle strength and size adaptations following RT. 109 mRNA with large ESIR were also among the mRNA previously found to correlate with both skeletal muscle strength and size adaptations to RT. Together, these results suggest that individual variability in the mRNA expression responses to RE exist, and highlight 109 mRNA that potentially play key roles in predicting skeletal muscle adaptations to RT.
Co-Authorship

Dr. Kier J. Menzies and Dr. Brendon J. Gurd contributed to conceptualizing the study design and the experimental approach, as well as assisting in data retrieval and analysis. The manuscript presented in this thesis is the work of Jacob Bonafigla in collaboration with Dr. Brendon J. Gurd.
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# List of Abbreviations

CaMKII – calcium dependent protein kinase II  
cDNA – complimentary DNA  
cmRNA – complimentary mRNA  
CREB – cAMP response element binding protein  
CON – no-exercise control  
DNA – deoxyribonucleic acid  
e\text{EX}/e\text{CON}/e\text{RE} – measurement error in responses to exercise/control/resistance exercise  
e\text{PRE}/e\text{POST} – measurement error at PRE/POST  
ES\text{IR} – effect size of individual responses  
FOX – forkhead box protein  
GEO – genome expression omnibus  
IGF-1 – insulin-like growth factor 1  
KEGG – Kyoto encyclopedia of genes and genomes  
MAFbx – muscle atrophy F-box  
MAPK – mitogen-activated protein kinase  
MPS – muscle protein synthesis  
mRNA – messenger ribonucleic acid  
mTOR – mechanistic target of rapamycin  
MyoD – myogenic differentiation  
MyoG – myogenin  
PGC-1α – peroxisome proliferator activated receptor gamma coactivator-1 alpha  
PKB/Akt – protein kinase B  
PRE – before exercise  
POST – after exercise  
RT-qPCR – reverse transcription quantitative real-time polymerase chain reaction  
RE – resistance exercise  
RNA – ribonucleic acid  
RT – resistance training  
SD – standard deviation  
SD(\text{BSL}\text{EX}) – standard deviation of baseline measurements in exercise group  
SD\text{IR} – standard deviation of individual responses  
SD(OR\text{EX})/SD(OR\text{CON})/SD(OR\text{RE}) – standard deviation of observed responses to exercise/control/resistance exercise  
SD(PRE\text{RE}) – standard deviation of PRE measures in resistance exercise group  
SNP – single nucleotide polymorphism  
VO\text{2}\text{peak} – peak oxygen consumption  
V(OR\text{EX})/V(OR\text{RE}) – variability in the observed responses to exercise/resistance exercise  
V(TR\text{EX})/V(TR\text{RE}) – variability in the true responses to exercise/resistance exercise  
W – within-subject variability  
W\text{EX}/W\text{CON}/W\text{RE} – within-subject variability in responses to exercise/control/resistance exercise
Chapter 1

Introduction

1.1 Individual variability in responses to exercise

Exercise is widely recognized as a “lifestyle-based medicine” that improves many parameters of musculoskeletal, metabolic, and whole-body health (3). Although the benefits of exercise are consistently observed at the group level, it is becoming increasingly evident that a considerable degree of heterogeneity exists in the individual responses to exercise (4–9). For instance, despite completing the same dose of exercise training, individual responses in a given physiological variable can increase (4–9), decrease (10–13), or remain unchanged (10,14–18). This apparent individual variability warrants a modification to the “one size fits all” approach for exercise prescription and supports a move toward recognizing exercise as an individualized medicine (19). In order to understand individual responses to exercise and move toward personalizing prescriptions, research designed to elucidate the mechanisms that underlie the individual variability in responses to exercise is needed.

1.2 Individual variability in skeletal muscle mRNA expression responses to acute exercise

Whole-body health and performance adaptations to exercise training are largely driven by changes in skeletal muscle structure and function (3,20–22). During an acute/single bout of exercise, skeletal muscles experience several homeostatic perturbations that result in transcribing DNA to form mRNA, increasing mRNA
expression and eventually produce active proteins (3,20–22). Repeated bouts of acute exercise (i.e., training) alters skeletal muscle protein content (3,20–22). Because proteins regulate changes in skeletal muscle structure and function, alterations in protein content underlie adaptations to exercise training (3,20–22). Therefore, alterations in mRNA expression in response to acute exercise represent the induction of chronic skeletal muscle adaptations (3,21–23).

Because chronic skeletal muscle adaptations are initiated by alterations in mRNA expression, it is possible that individual variability in responses to exercise training are a result of individual variability in mRNA expression responses to acute exercise (24). A limited number of studies have presented individual mRNA expression responses to acute exercise (25–29) and a recent reanalysis from some of our previous work (30–33) revealed considerable individual variability in the observed peroxisome proliferator activated receptor gamma coactivator-1 alpha (PGC-1α) mRNA expression responses to a given aerobic exercise stimulus (Figure 1). While this reanalysis supports the potential that individual variability in mRNA expression responses to acute exercise exists, these findings are limited by the fact that only one mRNA was measured; an important consideration given the thousands of mRNA that regulate skeletal adaptations to exercise (2). Therefore, much more work is needed to confirm whether individual variability in mRNA expression responses to acute exercise exists.
Variability in observed responses to exercise does not necessarily reflect individual variability in exercise responses

Studies examining individual responses to exercise have used variability in the observed responses as evidence for individual variability. However, because observed responses to exercise contain a degree of measurement error (e.g. technical error) and/or within-subject variability (e.g. changes between measurements in an individual’s biology and/or behavior [e.g. diet, sleep, exercise habits, etc.]), it is unclear whether variability in the observed responses reflects variability in the responses to exercise (i.e. independent of measurement error and within-subject variability; refs. 34–37). Atkinson and colleagues (35,36) assert that variability in the responses to exercise can be ascertained by comparing variability in the observed responses to a no-exercise control group (CON).
Specifically, variability in the responses to exercise exists if the variability in observed responses to exercise exceeds the variability in observed responses to CON (35,36). Although this approach has been used for peak oxygen consumption (VO₂peak) responses to training (36), no study has determined whether variability in the mRNA expression responses to acute exercise exists.

1.4 Thesis focus and experimental approach

The focus of this thesis was to examine the variability in the mRNA expression responses to acute exercise. Although mRNA expression is commonly measured using reverse transcription quantitative real time polymerase chain reaction analysis (RT-qPCR), this technique is limited in the number of mRNA that can be examined. Because microarrays can measure mRNA expression of the entire human genome, we used freely available microarray data from the genome expression omnibus (GEO) database to investigate the variability in the mRNA expression responses to acute exercise. Following a thorough search on GEO, it was determined that only the recent study by Murton et al. (1) used a microarray to measure mRNA expression responses to acute exercise and CON. Because Murton et al. (1) employed an acute resistance exercise (RE) protocol, the focus of this thesis was to examine variability in the mRNA expression responses to RE.

1.5 Thesis objective

To determine whether individual variability in the mRNA expression responses to acute exercise exists by utilizing freely available data from a previously published
Chapter 2

Literature Review

2.1 General introduction

Resistance exercise (RE) involves repeated forceful skeletal muscle contractions. Repeated bouts of RE (i.e. resistance training; RT) improves many aspects of skeletal muscle function and morphology, as well as many parameters of whole-body health including bone mineral density, insulin sensitivity, and basal metabolic rate (3). Unlike aerobic training, RT increases skeletal muscle strength and size; both of which are associated with improvements in exercise performance, musculoskeletal health, and all-cause mortality (3,38,39). Given its clear ability to improve overall health, RT has been incorporated into the Canadian Physical Activity Guidelines (40).

In response to RT, human skeletal muscle undergoes several molecular, morphological, and functional adaptations (3,20,21,38,39). Importantly, the basis of training-induced (i.e. chronic) skeletal muscle adaptation is the initiation of protein synthesis in response to acute exercise (22). Following transcription (formation of mRNA from DNA) and translation (formation of proteins from mRNA), newly-synthesized proteins undergo several post-translational modifications to become activated (e.g. covalent modifications, protein folding, etc.; ref. 23). Newly synthesized and activated proteins underlie skeletal muscle adaptations to RT (38). Because transcription is the first
stage of protein synthesis, acute increases in skeletal muscle mRNA expression precede elevations in protein content (41,42). Therefore, measuring alterations in mRNA expression following acute exercise can provide important mechanistic information about chronic adaptations that occur following training. Although several laboratory techniques can be used to measure mRNA expression, microarrays are an attractive method given their ability to capture genome-wide changes in mRNA expression.

While RT consistently induces skeletal muscle adaptations at the group level (38,39), some evidence suggests that there is individual variability in the response to RT (43–47). Because transcriptional responses represent the initiation of chronic adaptation (22), it is possible that individual variability in the responses to RT is a result of variance in mRNA expression responses following each bout of RE (24). Given that skeletal muscle adaptations to RT are associated with local and whole-body health (3,38,39), exploring individual differences in acute mRNA expression following RE may represent an important step toward individualizing exercise as personalized medicine (19).

2.2 Overview of literature review

The following literature review will begin by discussing the acute responses, particularly changes in mRNA expression, to RE. This will be followed by an introduction of microarrays including a brief overview of their methodology, a discussion of their role in exercise science, and a review of current findings arising from microarray studies in human skeletal muscle. The final section introduces the concept of individual variability in the responses to exercise with specific examples from RT/RE studies and ends by discussing individual responses to exercise from a statistical perspective.
2.3 Skeletal muscle gene expression responses to acute RE

2.3.1 Section introduction

This section will begin by discussing the importance and implications of mRNA expression responses to acute RE. To better understand the studies to date that have investigated mRNA expression responses to acute RE, this section then overviews the processes that occur during exercise that lead to transcriptional alterations in skeletal muscle. Lastly, this section ends with a brief discussion justifying the need for microarray studies to examine mRNA expression responses to RE.

2.3.2 Human skeletal mRNA expression responses to RE: Importance and implications

Skeletal muscle is a remarkably plastic tissue that undergoes a number of molecular and morphological adaptations in response to contractile activity (48). Contracting skeletal muscles during acute exercise experience several homeostatic perturbations (e.g. changes in mechanical stress, calcium flux, cellular energetics) that subsequently activate signaling pathways leading to transcriptional, translational, and post-translational events (3,20–22). Because these acute molecular events precede functional/morphological changes in muscle, they purportedly represent the initial step in the induction of chronic skeletal muscle adaptation (3,20–22). Therefore, examining skeletal muscle responses to RE may elucidate the molecular mechanisms that regulate chronic adaptations to RT.

While a number of studies have extensively characterized the impact of aerobic exercise on altering skeletal muscle mRNA expression (3), the acute transcriptional
events that regulate adaptations to RT are less clear (39). Instead of investigating acute transcriptional responses to RE, a greater emphasis has been placed on examining changes in signaling pathways that promote increases in skeletal muscle translational capacity (3,39). The mechanistic target of rapamycin (mTOR) signaling pathway, a pathway that has received a considerable amount of research attention (3,39), is activated in response to RE-induced intracellular mechanical stress (49) and exercise-mediated binding of extracellular growth factors to skeletal muscle membranes (50). The mTOR signaling pathway activates several ribosomal proteins that form the translation-initiation complex (51); the rate limiting step of protein translation (3). Therefore, RE-induced activation of the mTOR signaling pathway contributes to increased muscle protein synthesis (MPS), and thus skeletal muscle hypertrophy (3). An increase in MPS and hypertrophy, in particular synthesis of myofibrillar contractile proteins, represent hallmark molecular events that underlie chronic skeletal muscle adaptations to RT (3,20,38).

In addition to measuring alterations in activation (e.g. phosphorylation) of the mTOR signaling pathway, the induction of protein synthesis in response to RE can be determined by examining changes in skeletal muscle mRNA expression. For example, the activation of satellite cells, which are key regulators of MPS, can be gauged by measuring mRNA expression of several regulatory factors (3,20). Additionally, quantifying mRNA expression of activators/inhibitors of the mTOR signaling pathway can determine the initiation of the mTOR-dependent hypertrophic response to RE (3,20).
Therefore, measuring mRNA expression responses to RE can provide important information regarding the mechanisms that regulate skeletal muscle adaptations to RT.

2.3.3 Human skeletal muscle mRNA expression responses to RE: How do they occur?

Contraction-mediated homeostatic perturbations (e.g. changes in calcium flux, mechanical stress, oxygen pressure) result in changes in skeletal muscle mRNA expression (3). A number of proteins, including protein kinases, sense these perturbations and respond by activating signaling pathways in an attempt to restore cellular homeostasis (3,23). Specifically, these signaling pathways activate transcription factors; molecules that translocate to the nucleus and induce/inhibit transcription of their downstream targets thereby regulating skeletal muscle mRNA expression (23). In summary, RE changes skeletal muscle mRNA expression by activating transcription factors via homeostatic-responsive signaling pathways (23,52–54).

Activation of several signaling pathways, and subsequently transcription factors, alter expression of the mRNA involved in regulating the hypertrophic response to RE (e.g. myogeneic differentiation [MyoD] and myogenin [MyoG]). For example, calcium-dependent protein kinase II (CaMKII) phosphorylates, and thus activates, several transcription factors in response to contraction-mediated increases in calcium flux (3). CaMKII phosphorylates cAMP response element binding protein (CREB), which increases transcription of MyoD and MyoG (55). In response to changes in mechanical stress, protein kinase B (PKB/Akt) signaling also alters MyoD and MyoG transcription (49). Additionally, Akt signaling inhibits transcription of several factors involved in
protein degradation by translocating forkhead box proteins (FOXs; a family of transcription factors that stimulates protein degradation) out of the nucleus (56).

Collectively, these examples provide a glimpse of the myriad of signaling pathways/transcription factors that are activated in response to skeletal muscle contractions that ultimately alter mRNA expression of mRNA regulating the hypertrophic response to RE.

2.3.4 Human skeletal muscle mRNA expression responses to RE: A need for microarray studies

Although several studies have shown that RE alters mRNA expression in human skeletal muscle (26,57–66), the use of RT-qPCR limits the number of mRNA that can be examined. Despite these studies demonstrating changes in expression of mRNA that regulate the hypertrophic response to RT (e.g. MyoD and MyoG; refs. 57,61,67–70), it is unknown whether expression of other mRNA that were not selected for RT-qPCR analysis are significantly altered in response to RE. Given that thousands of mRNA are significantly altered in response to RE (2), pre-selecting a small number of mRNA to be measured with RT-qPCR precludes the ability to comprehensively determine skeletal muscle mRNA expression responses to RE. There is a need for studies to utilize genome-wide approaches (e.g. microarrays) to comprehensively determine the impact of RE on mRNA expression responses in human skeletal muscle.
2.4 Microarray technology: Introduction, methodology, and applications in exercise science

2.4.1 Section Introduction
Because microarrays are capable of capturing changes in mRNA expression of the entire human genome, they have become a powerful tool for measuring transcriptional responses to acute exercise. This section introduces microarrays’ early scientific applications to provide a background on how microarrays transitioned into exercise science. A brief overview of the methodology of microarrays, and a discussion of findings from microarray studies examining changes in mRNA expression in response to RE in human skeletal muscle. To better understand the discussions in section 2.5, this section ends by highlighting the limited number of microarray studies that provide evidence of individual variability of mRNA expression responses to acute RE.

2.4.2 Introduction of microarrays and their scientific applications
Following Watson and Crick’s explanation of DNA’s double helix structure, and the subsequent discovery that DNA’s strands can be separated and renatured by adjusting temperature, David Gillespie and Sol Spiegelman developed one of the first gene expression measurement techniques (71). Since these seminal discoveries, the evolution of microarray technologies has immensely advanced the field of genetic research. Unlike RT-qPCR where expression of only a small number of mRNA can be measured, microarrays can quantify expression of over 800 000 mRNA transcripts (72), and thus contain the capacity to measure the entire human genome (71). Therefore, microarrays
enable the potential of elucidating novel, and non-\textit{a priori} hypothesized, transcriptional discoveries (73).

Coinciding with an increase in development, accessibility, and scientific interest in microarray technologies, several review articles were published at the turn of the 21\textsuperscript{st} century that discussed ongoing and future scientific applications of microarrays (74). Microarrays can be used to compare genomic sequences between individuals exhibiting different phenotypes (e.g. healthy vs. diseased) (73,75). This exploratory approach (i.e. examining mRNA expression of the entire genome rather than measuring a select few genes) has led to the discovery of genetic variants and single nucleotide polymorphisms (SNPs; variation in a single base pair; e.g. a thymine-adenine pair is present instead of a cytosine-guanine) that are associated with a given phenotype/disease. Therefore, determining whether an individual contains these genetic variants/SNPs assists in diagnosing a large number of health conditions ranging from cognitive disorders to cancer (76). Experimental biologists, however, utilize microarrays to examine mRNA expression responses following exposure to a given stimulus such as drug administration (77), macronutrient supplementation (78), and exercise (discussed in more detail in section 2.4.4). While many previous studies have utilized microarrays for phenotypic comparisons/experimental interventions, there remains a number of unknowns in genetic research that future studies can address using microarrays.

\textbf{2.4.3 A brief overview of microarray methodology}
Although the methodological principles are generally consistent between different microarray manufacturers, the information in this following section pertains to the Affymetrix GeneChip® manual (72).

The surface of array plates, referred to as chips, are typically made of glass and can contain over 800,000 segmented areas called probe cells. Each probe cell, also referred to as features (71), contains hundreds of probes (customized sequences of the four base pairs: thymine/uracil, adenine, cytosine, and guanine) designed to bind a specific transcript (i.e., a specific sequence of nucleotide bases that belongs to a specific mRNA). Probes are fixed to the surface of chips via localized inkjet printing of base pairs or photolithography where ultraviolet fuses base pairs to the chip (71,79). Because each probe within a probe cell is designed to target a specific mRNA sequence, each probe cell can only provide information regarding the expression of a single mRNA. However, as entire sequences for mRNA can be thousands of base pairs long, expression of a single mRNA can be measured across multiple probe cells using probes that target different transcripts/sections along an mRNA’s sequence. Figure 2 illustrates the organization of microarray chips and the potential of targeting the same mRNA using different probes.
Figure 2. Exemplary schematic of a microarray chip that measures the same mRNA (IGF-1) twice using different probes targeting different transcripts of IGF-1.
There are four unique steps to measuring mRNA expression using microarrays:
1) biotinylating complimentary mRNA (cmRNA), 2) target hybridization, 3) washing and staining, and 4) scanning (72). Similar to preparation for RT-qPCR, RNA needs to be extracted from samples, which can be done using commercially available kits or following the acid guanidinium thiocyanate-phenol-chloroform method (80). After reverse-transcribing extracted RNA into complimentary DNA (cDNA), the sample is biotinylated (biotin is mixed with and binds to the cDNA sample) in order to permit future binding of fluorescent molecules. cDNA is then transcribed to cmRNA in preparation for hybridization where the sample is exposed to the chip and cmRNA fragments will bind/hybridize to probes. Following several washes to remove non-hybridized fragments, a fluorescent stain is applied to the chip that binds to biotinylated and hybridized cmRNA. The chip is exposed to a computer-programmed laser scanner where expression of mRNA is quantified by the magnitude of fluorescence at a given probe cell. Although the majority of cmRNA molecules will not hybridize (72), the more that a specific mRNA is expressed the greater its hybridization and thus fluorescence at that probe cell. In some experimental protocols, control and experimental samples are mixed together before hybridization because they will fluoresce different colours. Microarray analysis software can discern which colour is fluoresced more at a given probe cell to determine whether expression of that mRNA has increased or decreased with the experimental intervention.

The technical variability and measurement error of microarrays can result in “false positives” (type I errors) and/or “false negatives” (type II errors); a particular
problem for non-abundantly expressed mRNA (77). These errors underscore the importance of measuring expression of a single mRNA with multiple probe sets (77) and validating detected mRNA expression with RT-qPCR or northern blots (75). Once validated, experimenters can determine the expression of a single mRNA or input their data into genomic pathway software to analyze expression of a large group of mRNA involved in the same signaling/functional pathway (i.e. a group of mRNA that encode proteins that carry out similar cellular functions). Because signaling/functional pathways consist of hundreds of mRNA, pathway analysis provides greater confidence compared to analyzing expression of one/a few mRNA in determining whether a cellular functional change (e.g. an increase in mitochondrial biogenesis) has occurred in response to an experimental intervention and/or is associated with a given phenotype.

2.4.4 The applications of microarrays in exercise science

In a review published shortly after the completion of the Human Genome Project (a large international project where 99.99% of the nucleotide sequence of human DNA was determined) Fehrenbach et al. (81) outlined two major applications of microarrays in the field of exercise science: 1) sequencing exercise-related phenotypes, and 2) profiling gene expression responses to exercise.

Major application 1: Sequencing exercise-related phenotypes

Similar to elucidating genetic sequences that explain diseases, Rankinen et al. (82) reviewed early work attempting to identify genetic polymorphisms that explain exercise/health-related phenotypes. Although a limited number of preliminary
investigations successfully demonstrated a link between genetic polymorphisms and an exercise/health-related phenotype (82,83), Rankinen and colleagues have annually reviewed compelling studies that have identified a number of SNPs that are associated with cardiorespiratory fitness, muscular strength and power, anthropometric measures, and many other phenotypes (84–94). Importantly, because the strength of these associations are typically weak-moderate (82–94), genetic variants are not perfect predictors of whether an individual will exhibit a given exercise/health-related phenotype. Nonetheless, as genetic variants that partially explain the variance in clinically-relevant responses to exercise continue to be unveiled, future studies should determine whether this information can be applied into clinical settings (19). For instance, it is unknown whether an individual’s response to a given exercise prescription can be predicted by measuring the SNPs that relate to differences in clinically-relevant responses such as VO$_2$peak (95–97) and muscle strength (98).

**Major application 2: Profiling mRNA expression responses to exercise**

Because microarrays have the ability to capture changes in mRNA expression of every gene involved in a given functional pathway, they are superior to RT-qPCR (pre-selecting a small number of genes to be examined) in the ability to comment with confidence about whether exercise has induced transcriptional events that initiate a given physiological adaptation (e.g. skeletal muscle hypertrophy; ref. 73). Although microarrays were first used to examine exercise-induced transcriptional responses in human blood (81), human skeletal muscle has since been extensively studied. The first microarray analysis on human skeletal muscle revealed that expression of 158 mRNA
were significantly altered following nine months of aerobic training (99). The following section (2.4.5) discusses current findings from RE microarray experiments in human skeletal muscle.

2.4.5 Findings from RE microarray experiments in human skeletal muscle

Although some microarray studies have examined responses to exercise training (2,100), Murton et al. (1) argue that microarrays are better used to measure mRNA expression following acute exercise because microarrays capture the large number of transient transcriptional events that initiate skeletal muscle adaptations (3,21–23,48). To my knowledge, Kostek et al. (101) were the first to conduct a microarray study that examined the transcriptional responses to RE. Inconsistent with demonstrations of mRNA expression either transiently increasing (67,68) or peaking (63) within 4-8 hours post-acute RE, Kostek et al. (101) found that more genes were differentially expressed at 24 than three hours post exercise. However, because subsequent genome-wide studies revealed that substantially more genes (>1100) are altered 4-hours post-exercise (2) compared to 24-hours post (0 - ~350; refs 1,102), it is possible that Kostek et al’s (101) administration of a drink containing sucrose and essential amino acids altered mRNA expression at 24-hours but not three-hours post exercise. Nevertheless, Kostek et al.’s study (101) led to many future experiments that used microarrays to capture the transcriptional responses to RE.

Despite many studies aimed at using microarrays to characterize the impact of RE on mRNA expression, varying issues with study designs have limited the validity of findings from some of these studies. Some experimenters have separated the collection of
post-exercise and baseline (103–106) or control (107,108) samples by one or several weeks, and have thus risked introducing a large degree of biological variability between measures. Other studies that compare mRNA expression post-bilateral exercise between a trained and untrained arm/leg (i.e. use untrained limb as control instead of a baseline sample) neglect the potential impact of exercise training on basal mRNA expression (109,110). For example, because exercise training increases basal expression of some mRNA (2), it is possible that the increase in mRNA expression following acute exercise is attenuated in the trained limb compared to the untrained limb. Therefore, study designs that involve collecting a baseline biopsy immediately before exercise and taking post-exercise samples in the same limb permit investigating the transcriptional responses to acute exercise; however, few microarray studies have followed such a design (1,2,101,102,111,112).

Studies with appropriate study designs that used microarrays to determine the impact of RE on human skeletal muscle mRNA expression have yielded similar results to experiments using RT-qPCR. For example, microarrays have captured RE-induced changes in mRNA expression of the hypertrophic regulatory factors mentioned in section 2.3.3 (1,2,101,102). In addition to corroborating results from RT-qPCR studies, researchers using microarrays have identified close to 2000 mRNA that are differentially expressed in response to RE (1,2,101,102). While many of these ~2000 mRNA are known to be involved in functional pathways (e.g. IGF-1 signaling, satellite cell activation, etc.), future work is needed to identify the role of the remaining mRNA that are differentially expressed in response to RE.
RE microarray studies and individual variability in mRNA expression responses

While no microarray study has deliberately investigated whether there is individual variability in mRNA expression responses to RE, Raue et al. (2) assessed individual responses via correlations between changes in mRNA expression and chronic adaptations to RT. Confirming the contention that acute responses in mRNA expression reflect an early phase of the initiation of chronic skeletal muscle adaptations (3,21–23,48), Raue et al. (2) demonstrated that acute responses in 2756 mRNA correlated with training-induced changes in skeletal muscle strength and/or size. Therefore, it appears that the variability in acute mRNA expression responses explains, at least in part, the variance in chronic skeletal muscle adaptations (e.g. individuals with a large acute mRNA expression response in one of the 2756 mRNA will likely have large increases in skeletal muscle strength and/or size following training). Further evidence of individual variability in acute mRNA expression responses to RE shows that participants ranged from having zero to ~3000 differentially expressed transcripts 24 hours following bilateral knee extension (1). Interestingly, whether an individual exhibited muscle damage during exercise, as measured via heat shock protein content, appeared to determine the number of transcripts differentially expressed following acute RE (1). Although these results collectively demonstrate variability in the observed mRNA expression responses to acute RE, it is possible that this observed variability reflects a large degree of measurement error and/or within-subject variability instead of variability in the responses to exercise. For example, because Raue et al. (2) analyzed observed
responses (which contain a degree of measurement error/within-subject variability), it is unknown whether the correlations between chronic adaptations and the 2756 mRNA are physiologically meaningful (i.e. correlations exist because variability in mRNA expression responses to RE relate to chronic adaptations) or spurious (i.e. correlations exist because measurement error and/or within-subject variability in the observed mRNA expression responses to RE spuriously related to chronic adaptations). Therefore, future work is needed to confirm whether RE introduces variability in the mRNA expression responses independent of measurement error and/or within-subject variability.

2.5 Individual variability in skeletal muscle mRNA expression responses to acute RE

2.5.1 Section Introduction

This section begins with an introduction of the early evidence of individual variability in responses to exercise training. Following repeated demonstrations of variability in training responses, studies began investigating the variability in responses to acute exercise. Although a limited number of studies have presented individual mRNA expression responses, no study has convincingly demonstrated individual variability in the mRNA expression responses. This section ends by discussing statistical perspectives that question whether variability in the responses to exercise actually exists.

2.5.2 Individual variability in responses to exercise training

Although the conventional approach to reporting results involves providing group means ± standard deviation (113), an increasing body of literature in exercise science has
accepted the contemporary notion of presenting individual responses (19,114). Although many early studies graphically presented individual data, Bouchard et al. (4) were among the first to analyze individual responses to exercise and observed considerable heterogeneity in VO$_2$peak changes following endurance training. Despite completing an identical exercise training program, participants in the HERITAGE study demonstrated changes in VO$_2$peak that ranged from ~ -100 to ~ +1000 mL/min/kg (4,115). Since these seminal findings, many studies have observed a considerable degree of variability in responses to endurance (5–10,43,116), high intensity interval (10,14,18,117), and resistance training (43–47).

With respect to skeletal muscle adaptations to exercise training, several studies have graphically presented individual responses in a number skeletal muscle measures (118–122); however, few have discussed and/or conducted analysis of individual variability (44,45,123,124). Recent contentions argue that individual differences in chronic skeletal muscle adaptations can explain, at least in part, the variance in performance/clinically-relevant variables to exercise training (24,113,125). Consistent with this contention, several studies have revealed positive correlations between muscle strength/size gains and skeletal muscle molecular/morphological changes following RT (21,45,126). Although several factors may contribute to the observed variability in exercise responses (113), the precise mechanisms that are responsible for the individual differences in skeletal muscle adaptations to exercise training are unclear. It is possible that variability in acute mRNA expression, which represents the initiation of chronic
adaptation (3,21–23,48), contributes to the variability in skeletal muscle adaptations to exercise training.

2.5.3 Limited evidence of individual variability in skeletal muscle mRNA expression responses to RE

Discovering the mechanism(s) that dictate whether an individual will adapt to training may elucidate an approach for individualizing exercise prescription (19). Because acute-exercise skeletal muscle responses precede and thus initiate chronic adaptations (22,41), Timmons (24) proposed the possibility that the magnitude of an individual’s acute response determines the extent of their subsequent training-induced adaptations. Given the variability in adaptations to RT (43–47), Timmons’ (24) contention suggests that mRNA expression responses to RE should also be variable.

Although few RE studies have reported results at the individual level, there appears to be a range of mRNA expression responses to RE (25–27). For example, although Hameed et al. (27) failed to observe an increase in insulin-like growth factor-1 (IGF-1) mRNA expression at the group level following acute RE, three out of eight participants demonstrated a ~1-fold increase compared to baseline. However, much more work is needed to confirm that variability in mRNA expression responses to RE exists. Specifically, it is currently unknown whether variability in mRNA expression responses to RE is a result of variability in the true exercise responses per se.

2.5.4 A statistical perspective on individual variability in responses to exercise
A degree of measurement error, due to technical error/noise in equipment, exists in the measurement of all physiological variables and thus impedes the ability to ascertain true values (127). Because measurement error exists in pre- and post-exercise measurements, it contributes to variability in the observed responses (post − pre) to exercise (34,37). In addition to measurement error, within-subject variability (i.e. biological variability due to changes in diet, sleep, habitual physical activity, etc.; ref. 113) contributes to variability in the observed responses to exercise (34,37). Therefore, variability in the responses to exercise (i.e. “between subject variability of true pre-post differences” or the “subject-by-training interaction”; ref. 35) cannot be directly measured because of measurement error and/or within-subject variability (34,37). Collectively, variability in the observed responses to exercise is a result of measurement error, within-subject variability, and the variability in the responses to exercise (34,37), as mathematically illustrated in equation (1) below:

\[ V(OR_{EX}) = e_{PRE} + e_{POST} + W + V(R_{EX}) \]  

where the variability (V) of the observed responses to exercise (OR) is the sum of measurement error (e) in pre- and post-exercise measures, within-subject variability between measures (W), and the variability in the exercise responses (R_{EX}). Per equation (1), variability in the observed responses may not reflect the variability in the responses to exercise, but rather a high degree of measurement error and/or within-subject variability (35,36).
Several biostatisticians (35–37) contest that the variability in the responses to exercise cannot be ascertained without the comparison to a non-exercise control group (CON). With the exception of not partaking in exercise (acute or training), CON participants should be treated, measured, and tested identically as participants allocated to the exercise group (EX). Therefore, assuming that measurement error and within-subject variability unequivocally impacts the variability in the observed responses between CON and EX groups, variability in the responses to exercise can be quantified by subtracting variability in the observed responses in CON from variability in the observed responses in EX (35–37); mathematically illustrated in equation (2) below:

\[ V(OR_{EX}) - V(OR_{CON}) = (e_{EX} + W_{EX} + V(R_{EX}) - (e_{CON} + W_{CON}) = V(R_{EX}) \]

where \( e_{CON} \) and \( W_{CON} \) represent the measurement error and within-subject variability in pre-post measures in the CON group, respectively. Using the aforementioned statistical arguments, Atkinson and colleagues (35,36) have generated an equation that calculates the variability (i.e. standard deviation; SD) of the individual responses to exercise (SD_{IR}; see equation (3) below). Importantly, following the calculation of SD_{IR}, an effect size (ES) can be calculated that describes the magnitude of variability in the individual responses to exercise (ES_{IR}; see equation (4) below).

\[ SD_{IR} = \sqrt{SD(OR_{EX})^2 - SD(OR_{CON})^2} \]
\[
ES_{IR} = \frac{SD_{IR}}{SD(\text{BSL}_{EX})}
\]

where SD(\text{BSL}_{EX}) represents the variability (SD) of the measures at baseline in the EX group. The resulting ES_{IR} reflects a small, moderate, large, very large, or extremely large effect of exercise on introducing variability in the responses to exercise if the value is greater than 0.1, 0.3, 0.6, 1.0, and 2.0, respectively (35–37).

While no acute exercise study has followed these statistical arguments, Williamson et al. (36) calculated SD_{IR} for responses to exercise training. Remarkably, the re-analysis of a seminal study by Prud’homme et al. (128) revealed that the variability in the observed VO_{2peak} responses was greater in CON than EX, suggesting that the variability in the responses to exercise training was less than the variability attributable to measurement error and within-subject variability (36). Although this finding suggests that exercise training does not contribute to the variability in the observed VO_{2peak} responses, whether acute exercise introduces variability in the responses beyond what can be explained by measurement error and within-subject variability is unknown. The significant relationships between mRNA expression responses and adaptations to training (2,31) suggest that the observed variability in these transcriptional responses represent variability in the responses to acute exercise. However, no study has confirmed whether the variability in observed mRNA expression responses to RE exceeds the variability in observed responses to CON. Therefore, it is currently unknown whether variability in observed mRNA expression responses to RE represents variability in the mRNA expression responses to RE. Utilizing microarrays to analyze variability in the observed
mRNA expression responses to acute RE vs. CON can determine whether variability in the responses to exercise exists, and allows comparing the magnitude of variability in responses between every mRNA in the human genome.

2.6 Knowledge gaps in the literature, research objectives and hypotheses

2.6.1 Gaps in the literature

1) No study to date has utilized a microarray to examine individual variability in mRNA expression responses of the entire human genome following RE.

2) No study has compared the variability in observed mRNA expression responses to RE vs. CON to determine whether variability in the mRNA expression responses to RE exists.

2.6.2 Research Objectives

1) To determine whether variability in the mRNA expression responses to RE exists by comparing the variability in the observed responses to RE and CON using freely available data from a previously published microarray study (1).

2) To calculate an ESIR for mRNA expression responses to RE for every mRNA in the human genome.

3) To determine if mRNA with a large ESIR are also among a group of mRNA previously found (2) to have acute responses that correlate with skeletal muscle strength and size adaptations to RT.

2.6.3 Hypotheses
1) Variability in the observed mRNA expression responses to RE will exceed CON for many mRNA, thus suggesting that variability in the responses to RE exists (i.e. independent of measurement error and/or within-subject variability).
3.1 Introduction

There appears to be heterogeneity in the individual mRNA expression responses to RE in human skeletal muscle (25–27). For example, mRNA expression of IGF-1, a regulator of exercise-induced skeletal muscle hypertrophy (20), increases, decreases, or remains unchanged in response to RE (27). Similarly, despite participants completing the same RE protocol, mRNA expression responses of mRNA that regulate skeletal muscle cell growth ranged from approximately -330% to +550% (25). However, because these studies did not include a CON group, it is unknown whether the observed variability in mRNA expression responses reflects individual differences in responsiveness to RE or a large degree of measurement error and/or within-subject variability.

Atkinson and colleagues (35,36) recently argued that comparing the variability in the observed responses to exercise and CON allows the variability in the responses to exercise (i.e. individual differences in responsiveness) to be determined. Because participants in CON are treated identically to those in the exercise group, variability in the observed responses to CON are a result of measurement error and/or within-subject variability; both of which contribute to the observed variability in responses to exercise (35,36). Therefore, if variability in the observed responses to exercise exceeds the variability in the observed responses to CON, then Atkinson and colleagues (35,36) assert that individual variability in the responses to exercise exists (i.e. independent of
measurement error and/or within subject variability). Despite several acute exercise studies including a CON group (1,129,130), no study has compared the variability in observed mRNA expression responses to RE and CON to determine whether variability in the mRNA expression responses to RE exists.

Although a recent study by Raue et al. (2) did not directly examine individual responses, these researchers revealed significant correlations between mRNA expression responses to RE and skeletal muscle size and strength adaptations to RT. Despite Raue et al. (2) not including a CON group, because the variability mRNA expression responses to RE were physiologically meaningful (i.e. explained, at least in part, the variance in adaptations to RT), it is likely that the variability in the observed responses reflects variability, independent of measurement error/within-subject variability, in the mRNA expression responses to RE. These results suggest that individual variability in the responses to RE exists; however, this variability has yet to be confirmed by the demonstration that variability in the observed responses to RE exceeds variability in the observed responses to CON.

Therefore, the purpose of this study was to test the hypothesis that variability in the mRNA expression responses to RE exists by determining whether variability in the observed mRNA expression responses to RE exceeds variability in the observed responses to CON. Specifically, this study sought to comprehensively determine if RE introduces variability in the mRNA expression responses by using freely available microarray data (1). Because only Murton et al. (1) have conducted an acute exercise microarray study with a CON group, the present study investigated the individual
variability in changes in mRNA expression 24 hours following acute RE using the data from Murton et al. available through the genome expression omnibus (GEO) database. A secondary purpose of the present study was to determine whether the mRNA with large variability in the responses to RE were also a part of a subset of mRNA with acute responses that correlate with chronic adaptations to RT, as demonstrated by Raue et al. (2). Determining whether variability in the mRNA expression responses to RE exists may provide mechanistic information that underlies the variability in the adaptations to RT that ultimately improve musculoskeletal and whole-body health (3).

3.2 Methods

Experimental Protocol

Following a search on GEO microarray data repository (NCBI, U. S. A.), it was determined that only Murton et al. (1) completed a microarray study with an acute exercise and CON group. Murton et al.’s (1) freely available data was downloaded into Microsoft Excel for subsequent analysis. Additionally, data from Raue et al. (2) was obtained in a similar manner to determine whether mRNA with highly variable responses to RE were also among the group of mRNA that Raue et al. (2) found had acute responses that correlated with chronic adaptations to RT. The original study by Raue et al. (2) included 28 (16 young [8 males/8 females, age: 20-30 years] and 12 old [6 males/6 females, age: > 80 years) untrained healthy (i.e. disease free), non-smoking participants.

Murton et al. (1): Participants, experimental design and microarray analysis
Detailed methodologies are available in the original manuscript published by Murton et al. (1); however, a brief summary of relevant experimental details is provided below. Sixteen recreationally active, non-smoking, and healthy men (age: 22 ± 2 years) arrived at the laboratory in the morning after an overnight fast for four consecutive days. On day 1, a baseline skeletal muscle biopsy (PRE) was taken from the vastus lateralis (lateral quadricep muscle) of the dominant leg. The participants were then randomly assigned to RE (n = 8) or CON (n = 8). Participants in RE completed five bouts of 30 maximal isokinetic knee extensions with one minute of rest separating each bout. On day 2, a second muscle biopsy (POST) was taken from the same leg ~24 hours after CON/RE. RE was repeated on day 3 and a third biopsy was performed on day 4; however, only the samples from day 1 and 2 were included in the present analysis. Figure 3 illustrates the experimental design for day 1 and 2 from Murton et al (1). This experimental design allowed the comparison of variability in the observed mRNA expression responses 24 hours following RE and CON.
Figure 3. Schematic of experimental design from Murton et al. (1). Std., standardized; CON, no-exercise control; RE, resistance exercise.
RNA was extracted from muscle samples using a commercially available kit (TRIzol; Molecular Research Center, Inc., U.S.A.). Following spectrophotometric RNA quantification, samples were prepared for microarray analysis according to Affymetrix’s instructions (72) using Human Genome U133 Plus 2.0 Array chips (Affymetrix, U.S.A.). mRNA expression of myostatin and muscle atrophy F-box (MAFbx) were measured via RT-qPCR, which validated the microarray data as correlations of mRNA expression between methods revealed significant ($p < 0.0001$) and very strong relationships ($r > 0.8$).

**Analysis of variability in mRNA expression responses**

The variability (i.e. standard deviation [SD]) in the observed responses (OR) to RE [$SD(OR_{RE})$] was compared to the SD in the OR to CON [$SD(OR_{CON})$]. Because the random error in mRNA expression responses (i.e. measurement error [$e$] and/or within-subject variability [$W$]) are presumably identical between RE and CON (35,36), subtracting $SD(OR_{CON})$ from $SD(OR_{EX})$ calculates the variability in the responses to RE [$V(RE)$] (35,36); see equation (2) below:

$$SD(OR_{RE}) - SD(OR_{CON}) = (e_{RE} + W_{RE} + V(RE)) - (e_{CON} + W_{CON})$$

$$= V(RE)$$

(2)

Therefore, the SD of the true individual responses ($SD_{IR}$) to RE was calculated using the following equation:

$$SD_{IR} = \sqrt{SD(OR_{RE})^2 - SD(OR_{CON})^2}$$

(3)
The magnitude/effect size of variability in the individual responses to RE (ES\textsubscript{IR}) was calculated using the following equation:

\[
ES_{IR} = \frac{SD_{IR}}{SD(PRE_{RE})}
\]  

(4)

where SD(PRE\textsubscript{RE}) is the SD of PRE measures in the RE group. The ES\textsubscript{IR} was interpreted as small, moderate, large, very large, or extremely large if the ES\textsubscript{IR} value exceeded 0.1, 0.3, 0.6, 1.0, and 2.0, respectively (35–37). SD\textsubscript{IR} and ES\textsubscript{IR} was calculated separately for each transcript included on the microarray chip. To determine the functional pathways with mRNA that are largely variable in the responses to RE WebGestalt’s gene-set enrichment analysis (131), which categorizes mRNA into their known signaling funciona pathways, was performed on mRNA with large effect sizes (i.e. an ES\textsubscript{IR} > 0.6). The mRNA with an ES\textsubscript{IR} > 0.6 were also compared to the 2756 mRNA presented in Raue et al. (2) with acute response that correlate with changes in knee extension one-repetition maximum (1-RM) and quadriceps cross sectional area (CSA) following RT. The functional pathway of each mRNA that had an ES\textsubscript{IR} > 0.6 and were previously found to correlate with chronic changes in both 1-RM and CSA was determined through individual searches on Kyoto Encyclopedia of Genes and Genomes (KEGG, Japan) because the number of these mRNA was too small for WebGestalt analysis. KEGG was chosen because it was the database used in the gene-set enrichment analysis on WebGestalt.

*Volcano plot*
A volcano plot was created to illustrate the ES_{IR} (log ES_{IR}) relative to the log fold change in expression of transcripts that had a calculable ES_{IR} [i.e. SD(OR_{RE}) > SD(OR_{CON}); see equation (3)].

3.3 Results

Samples from one participant in RE and two participants in CON were not analyzed due to inadequate size of muscle sample/amount of RNA extracted (1), leaving 13 participants included in the present analysis (RE: n = 7; CON: n = 6).

Variability in mRNA expression responses to RE

Figure 4 presents a volcano plot that illustrates the fold change and ES_{IR} for each transcript with a calculable ES_{IR}. The number of transcripts with an ES_{IR} that was incalculable, less than 0.1, and within each effect size category is presented in Figure 5. 15849 transcripts were incalculable because the variability in observed responses to CON exceeded the variability in observed responses to RE (Fig. 5). Conversely, 1721 transcripts with 1486 unique mRNA (i.e. 207 transcripts targeted the same mRNA; see Figure 2 for illustration) had large variability (i.e. ES_{IR} > 0.6) in the true responses to RE (Fig. 5). 16 signaling/functional pathways had mRNA with large variability in the true responses to RE. Table 1 presents the mRNA with large variability in the true responses to RE that constitute these pathways. Interestingly, some of these pathways have been previously identified to play key roles in regulating the hypertrophic response to RT; see discussion for details.
Figure 4. Volcano plot of log fold change vs. effect size of individual responses (ES$_{IR}$) for transcript responses to resistance exercise. Dotted lines represent log values for each effect size.
Figure 5. Number of transcripts with no calculable effect size of individual responses (ES\textsubscript{IR}), an ES\textsubscript{IR} < 0.1, and within each effect size category.
Table 1. Signaling/functional pathways of the 1486 mRNA with large variability in the mRNA expression responses to resistance exercise.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Signaling Pathway</td>
<td>ADCY7, ADRB1, AGTR1, ATP2B1, ATP2B2, CYSLTR1, EDNRA, GNAS, HRH1, HTR2A, HTR7, ITPR2, P2RX5, P2RX7, PDE1C, PRKCA, PRKCB, PTGER3</td>
</tr>
<tr>
<td>TNF Signaling Pathway</td>
<td>BIRC3, CASP3, CASP8, CCL2, CCL20, CREB3L2, CREB3L3, CXCL1, CXCL3, CXCL5, LIF, MAPK1, MAPK13, MMP3, PIK3CD, PIK3CG, PTGS2, SELE, SOCS3, TNGA1P3</td>
</tr>
<tr>
<td>Jak-STAT Signaling Pathway</td>
<td>CDKN1A, CSF2RB, CSF3R, CSH1, IL11RA, IL12RB2, IL13RA2, IL23A, IL24, IL6R, IL7, JAK3, LIF, MYC, PIK3CD, PIK3CG, STAM2</td>
</tr>
<tr>
<td>Rap1 Signaling Pathway</td>
<td>ADCY7, ANGPT2, CTNNB1, FGF14, FGFRI, FPR1, FYB, GNAS, IGF1R, ITGB2, ITGB3, KRAS, LCP2, MAPK1, MAPK13, PIK3CD, PIK3CG, PRKCA, PRKCB, RAC1, RAC2, RGS14</td>
</tr>
<tr>
<td>Chemokine Signaling Pathway</td>
<td>ARRB2, CCL2, CCL20, CCL7, CXCL1, CXCL3, CXCL5, CXCL6, CXCL8, CXCR2, CXCR4, GNB4, GNG4, GNGT1, HCK, JAK3, KRAS, LYN, MAPK1, NCF1, PIK3CD, PIK3CG, PPBP, PRKCB, PTK2, RAC1, RAC2, SOS1, VAV1</td>
</tr>
<tr>
<td>Sphingolipid Signaling Pathway</td>
<td>BID, MAPK1, MAPK13, PIK3CD, PIK3CG, PDL1, PPPP2RC2, PRKCA, PRKCB, PTEN, RAC1, RAC2, SPHK1, SPTLC2, TP53</td>
</tr>
<tr>
<td>Neuroactive Ligand-Receptor</td>
<td>ADRB1, AGTR1, CSH1, CYSLTR1, EDNRA, F2RL1, FPR1, FPR2, FPR3, GABRA5, GNRHR, HRH1, HRH4, HTR2A, HTR7, MC2R, NPY1R, OPR1, OPRM1, P2RX1, P2RY13, PRLR, PTGER2, PTGER3</td>
</tr>
<tr>
<td>Interaction</td>
<td>ABLIM2, CXCR4, ENAH, MAPK1, PIK3CD, PIK3CG, PLXNA2, PLXNA4, PRKCA, PTK2, RAC1, RAC2, ROBO2, SRGAP1, SSH2, TRPC4, TRPC5, TRPC6</td>
</tr>
<tr>
<td>Axon Guidance</td>
<td>BIRC3, CCND1, IGF1R, ITGA2, ITGA4, ITGB3, ITGB8, MAPK1, PARVG, PIK3CD, PIK3CG, PRKCA, PRKCB, PTEN, PTK2, RAC1, RAC2, SOS1, SPP1, THB1, TNC, VAV1, ZYX</td>
</tr>
<tr>
<td>Focal Adhesion</td>
<td>CLDN6, CXCR4, EZR, ITGA4, ITGB2, MAPK13, NCF1, NCF2, NCF4, PIK3CD, PIK3CG, PRKCB, PTK2, RAC1, RAC2, RASSF5, VAV1</td>
</tr>
<tr>
<td>Leukocyte Transendothelial</td>
<td>ATP6V0A2, ATP6V1C2, ATP6V1H, CLEC7A, CORO1A, CTS, CTS, CYBB, FCAR, FCGR2A, FCGR3A, FCGR3B, ITGA2, ITGB2, ITGB3, NCF1, NCF2, NCF4, OLR1, RAC1, TFC, THBS1, TLR4, TUBB2A</td>
</tr>
<tr>
<td>Migration</td>
<td>ADRB1, ARAP2, ARFAGP3, ARRB2, CXCR2, CYTH4, DAB2, EHD1, EHD4, HSPA6, IGF1R, IQSEC3, PIPLKL1, RAB35, SNX5, STAM2, WIPF2</td>
</tr>
<tr>
<td><strong>Natural Killer Cell-Mediated Cytotoxicity</strong></td>
<td>BID, CASP3, ITGB2, KRAS, LCP2, MAPK1, PIK3CD, PIK3CG, RAC1, RAC2, SOS1, SYK, TNFRSF10C, TYROBP, VAV1</td>
</tr>
<tr>
<td><strong>Viral Carcinogenesis</strong></td>
<td>CASP3, CASP8, CCNA1, CCND1, CREB3L2, CREB3L3, HIST1H2BC, HIST1H2BG, HIST1H2BK, HIST1H4A, HIST1H4H, MAPK1, PIK3CD, PIK3CG, PMAIP1, RAC1, SYK, TP53, YWHAH, YWHAZ</td>
</tr>
<tr>
<td><strong>Transcription Misregulation in Cancer</strong></td>
<td>ATM, BCL2A1, BIRC3, BMP2K, CCNA1, CXCL8, DOT1L, IGF1R, ILIR2, MMP3, MYC, PLAY, PTK2, RUNX1, RUNX2, SUPT3H, TCF3, TP53, WHSC1</td>
</tr>
<tr>
<td><strong>MicroRNAs in Cancer</strong></td>
<td>ABCC1, ATM, CASP3, CCND1, CD44, EZR, ITGB3, KRAS, MAPK1, MIR155, MIR17, MYC, PLAU, PRKCA, PRKCB, PTEN, PTGS2, SLC7A1, SOCS1, SOS1, TGFB2, THBS1, TNC, TP53, TP63</td>
</tr>
</tbody>
</table>
The 1486 mRNA with large variability in the mRNA expression responses to RE were compared to the 2756 mRNA with acute responses that were previously found (2) to correlate with skeletal muscle 1-RM and CSA adaptations to RT. 109 of the 1486 mRNA with large variability in the true mRNA expression responses to RE were also among the group of mRNA previously found to have acute responses that correlate with both chronic 1-RM and CSA adaptations (Figure 6). Not surprisingly, several of these 109 mRNA were involved in signaling/functional pathways known to regulate the hypertrophic response to RT (Table 2).
Figure 6. Number of mRNA with large variability in the responses to RE (effect size of individual responses $[ES_{IR}] > 0.6$; orange circle) and, as previously demonstrated by Raue et al. (2) number of mRNA that predict chronic changes in quadriceps cross sectional area (CSA; yellow circle) and one-repetition maximum (1-RM; blue circle).
Table 2. Signaling-functional pathways of the 109 mRNA with large variability in the mRNA expression responses to resistance exercise and with acute responses that correlate with chronic skeletal muscle strength and size adaptations.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K/Akt Signaling Pathway</td>
<td>ANGPT2, CDKN1A, EIF4E, IL6R, MYC, SOS1</td>
</tr>
<tr>
<td>MAPK Signaling Pathway</td>
<td>IL1R1, MYC, SOS1, TGF-β2</td>
</tr>
<tr>
<td>TGF-β2 Signaling Pathway</td>
<td>BMP2, MYC, NOG, TGF-β2</td>
</tr>
<tr>
<td>FoxO Signaling Pathway</td>
<td>ATM, CDKN1A, SOS1, TGF-β2</td>
</tr>
<tr>
<td>Jak-STAT Signaling Pathway</td>
<td>CDKN1A, IL6R, MYC, SOS1</td>
</tr>
<tr>
<td>HIF-1 Signaling Pathway</td>
<td>ANGPT2, CDKN1A, EIF4E, MYC</td>
</tr>
<tr>
<td>ErbB Signaling Pathway</td>
<td>CDKN1A, MYC, SOS1</td>
</tr>
<tr>
<td>Ras Signaling Pathway</td>
<td>ANGPT2, RASAL2, SOS1</td>
</tr>
<tr>
<td>mTOR Signaling Pathway</td>
<td>EIF4E, SOS1</td>
</tr>
<tr>
<td>Insulin Signaling Pathway</td>
<td>EIF4E, SOS1</td>
</tr>
<tr>
<td>NF-κB Signaling Pathway</td>
<td>ATM, IL1R1</td>
</tr>
<tr>
<td>IL-17 Signaling Pathway</td>
<td>CCL2, IL17RB</td>
</tr>
<tr>
<td>Chemokine Signaling Pathway</td>
<td>CCL2, SOS1</td>
</tr>
<tr>
<td>NLR Signaling Pathway</td>
<td>CCL2, PANX1</td>
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<tr>
<td>Calcium Signaling Pathway</td>
<td>P2RX5</td>
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<tr>
<td>TNF Signaling Pathway</td>
<td>CCL2</td>
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<td>PPARδ Signaling Pathway</td>
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<td>Apelin Signaling Pathway</td>
<td>EGR1</td>
</tr>
<tr>
<td>Hedgehog Signaling Pathway</td>
<td>CSNK1G1</td>
</tr>
<tr>
<td>Acting Binding/Cytoskeleton</td>
<td>ABLIM2, ANKRD1, CAMSAP1, ENAH, MAEA, SOS1</td>
</tr>
<tr>
<td>Cell Cycle Regulation</td>
<td>CDKN1A, MYC, TGFB2</td>
</tr>
<tr>
<td>Functional Category</td>
<td>Relevant Genes</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Centrosome Formation</td>
<td>CKAP2, CLASP2, LTZFL1</td>
</tr>
<tr>
<td>mRNA Transport/Surveillance</td>
<td>BICD1, NXT1, THOC6</td>
</tr>
<tr>
<td>Lysine Degradation</td>
<td>DOT1L, NSD1, SUV420H1</td>
</tr>
<tr>
<td>Lipid Metabolism</td>
<td>DBP, DGKD, SGSM2</td>
</tr>
<tr>
<td>Cell/Focal Adhesion</td>
<td>PVR, SOS1</td>
</tr>
<tr>
<td>Autophagy</td>
<td>KIAA0226</td>
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<tr>
<td>Carbohydrate Metabolism</td>
<td>PGM3</td>
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<tr>
<td>Protein Processing</td>
<td>ERO1L</td>
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<tr>
<td>Purine Metabolism</td>
<td>PDE7A</td>
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<tr>
<td>Ribosome Biogenesis</td>
<td>NXT1</td>
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<tr>
<td>Ubiquitin Mediated Proteolysis</td>
<td>UBE2Z</td>
</tr>
<tr>
<td>Unknown</td>
<td>ABHD2, ADAMTS17, AQP3, ARID5B, BZW1, CLCN5, CRLF3, DST, ENAM, FAM102A, FAM134B, FJX1, FNDC3B, IFRD1, ITGF1, ITIH4, LIMS3, METTL7B, NT5DC3, PDS5B, PHLD8, PTPRD, RBM33, RTFN2, S100A2, SERTAD1, SLC36A1, TBL2, THAP2, THBD, TMEM43, TP63, TTC17, WDR1, ZNF292</td>
</tr>
<tr>
<td>Signaling/Functional Pathway</td>
<td>ANKRD13C, ARHGAP28, ASB5, BCAR3, DOCK5, DOCK7, FNBP1, GEM, GAPATCH2, HECTD2, HIVEP3, HS2ST1, KBTBD8, KLF12, KLF5, NFIA,</td>
</tr>
<tr>
<td>Unclear</td>
<td>NPAS2, NPC1, OSBP5, RAB3IP, RFX3, RUNX1, SLC35F5, SPRK2, SOX6, SOX9, STRN, TBX3, TRIO, UCK2, ZIC1</td>
</tr>
<tr>
<td>Analysis conducted</td>
<td>Number of transcripts/mRNA</td>
</tr>
<tr>
<td>-----------------------------------------------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Total number of transcripts in Murton et al.’s (1) freely available dataset</td>
<td>54,675</td>
</tr>
<tr>
<td>Transcripts with a calculable $\text{SD}<em>{\text{IR/ES}</em>{\text{IR}}}$</td>
<td>38,826</td>
</tr>
<tr>
<td>[SD($\text{OR}<em>{\text{EX}}$) &gt; SD($\text{OR}</em>{\text{CON}}$)]</td>
<td></td>
</tr>
<tr>
<td>Transcripts with a large $\text{ES}_{\text{IR}}$</td>
<td>1,721</td>
</tr>
<tr>
<td>($\text{ES}_{\text{IR}} &gt; 0.6$)</td>
<td></td>
</tr>
<tr>
<td>Unique mRNA with a large $\text{ES}_{\text{IR}}$</td>
<td>1,486</td>
</tr>
<tr>
<td>($\text{ES}_{\text{IR}} &gt; 0.6$)</td>
<td></td>
</tr>
<tr>
<td>mRNA with a large $\text{ES}_{\text{IR}}$ and identified by Raue et al. (2)</td>
<td>228</td>
</tr>
<tr>
<td>to correlate with chronic adaptations in skeletal muscle strength and/or size</td>
<td></td>
</tr>
<tr>
<td>mRNA with a large $\text{ES}_{\text{IR}}$ and identified by Raue et al. (2)</td>
<td>109</td>
</tr>
<tr>
<td>to correlate with chronic adaptations in both skeletal muscle strength and size</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 7.** Schematic of number of transcripts/mRNA at each stage of analysis.
3.4 Discussion

The current study utilized freely available microarray data (1) to examine individual variability in the mRNA expression responses to RE. Additionally, the current study sought to determine whether the mRNA with large variability in the true responses to RE were also a part of the subset of mRNA with acute responses that correlate with chronic adaptations to RT, as demonstrated by Raue et al. (2). The major novel findings of the present study are: 1) individual variability in the mRNA expression responses to RE existed for many, but not all, transcripts, 2) several of the pathways with mRNA that had large variability in the responses to RE have been previously identified to regulate the hypertrophic response to RT, and 3) 109 mRNA with large variability in the responses to RE were also among a group of mRNA that (2) Raue et al. found have acute responses that correlate with chronic skeletal muscle adaptations to RT.

**Individual variability in the mRNA expression responses to RE**

Although few studies have reported individual mRNA expression responses to RE (25–27), there appears to be variability in the observed responses with participants demonstrating increases, decreases, or no changes in mRNA expression. However, because these studies did not include a CON group, it is unknown whether the variability in the observed mRNA expression responses reflects a large degree of measurement error/within-subject variability rather than inter-individual differences in the responses to RE (35,36). Utilizing freely available microarray data (1), we observed that although the variability in the observed responses to CON exceeded RE for ~35% of the measured transcripts, individual variability in the responses existed (i.e. variability in observed
responses to RE exceeded CON) for the majority of transcripts (~65%) with ~4% of transcripts having a large effect size of individual variability in the responses to RE (Fig. 4). Interestingly, despite previous presentations of inter-individual heterogeneity in IGF-1 mRNA expression responses to RE (26,27), we found greater variability in the observed IGF-1 mRNA expression responses to CON than to RE (data not shown), which suggests that the variability previously presented reflects a large degree of measurement error/within-subject variability instead of variability in the responses to RE. Thus these results demonstrate the necessity of including a CON group to determine whether variability in observed responses reflects variability, independent of measurement error/within-subject variability, in the responses to exercise.

The 1486 mRNA with large variability in the mRNA expression responses are involved in several signaling/functional pathways proposed to regulate the hypertrophic response to RT; a myriad of cellular events that result in increased MPS (3,20,38,39). For example, the calcium and Jak-STAT signaling pathways activate several factors that regulate satellite cell-mediated myogenesis (55,132), which promotes MPS by increasing skeletal muscle transcriptional capacity (3,20,38,133,134). Conversely, through regulating a number of factors that increase skeletal muscle translational machinery (e.g. ribosomal translation-initiation complexes), the TNF signaling pathway promotes MPS by increasing skeletal muscle translational capacity (20). Collectively, our results highlight several signaling/functional pathways with mRNA that are largely variable in the responses to RE. Although some of these pathways do not have clear roles in regulating adaptations to exercise (e.g. transcription misregulation in cancer, microRNAs
in cancer, etc.), future research should investigate whether variability in acute activation of these pathways predicts the variance in hypertrophic responses to RT.

*109 mRNA with large variability in the responses to RE are also among the group of mRNA previously found (2) to have acute responses that correlate with skeletal muscle adaptations to RT*

Raue et al. (2) recently found that expression responses in 2756 mRNA to RE correlate with skeletal muscle strength (i.e. 1-RM) and/or size (i.e. CSA) adaptations following RT. Although the variability in the observed mRNA expression responses to RE explained, at least in part, the variance in chronic adaptations, Raue et al. (2) did not include a CON group to determine whether individual variability in the mRNA expression responses exists. Utilizing the freely available data from Raue et al. (2), we determined that 109 of the mRNA found to have large variability in the responses to RE in the present study were also among a group of mRNA that Raue et al. (2) found have acute responses that correlate with both skeletal muscle strength and size adaptations to RT (Fig. 6). Because 109 mRNA with large variability in the responses to RE were among the group of mRNA previously found to correlate with chronic adaptations, these 109 mRNA (listed in table 2) may play key roles in regulating the hypertrophic response to RT. Indeed, several of these 109 mRNA are involved in pathways known to regulate the hypertrophic response to RT including the PI3K/Akt, MAPK, and mTOR signaling pathways (3,20,21). Future work is needed to validate whether acute changes in expression of these 109 mRNA can predict the magnitude of an individual’s chronic adaptations to RT.
Limitations

Although calculating SD_{IR}, and the corresponding ES_{IR}, purportedly quantifies the variability in the responses to exercise (35–37), this statistical approach assumes that measurement error and within-subject variability identically contribute to variability in the observed responses between exercise and CON groups. Murton et al. (1) followed Affymetrix’s protocol (72) in an attempt to reduce measurement error; however, the many biological factors (e.g. diet, habitual exercise, sleep, etc.) that may influence mRNA expression poses a challenge in controlling for within-subject variability. Murton et al.’s (1) experimental protocol provided standardized dinners/post-exercise meals and instructed participants to refrain from exercise 24 hours before each biopsy (1). Therefore, within-subject variability between mRNA expression measurements may have resulted from participants following their own dietary/exercise habits. For example, because mRNA expression can remain altered 48-120 hours following RE (25,26,57–59,62,65,69,70,135,136), exercise completed up to 120 hours before the collection of the PRE biopsy may have altered baseline mRNA expression and thus contributed to the variability in observed responses to RE/CON. Due to the difficulties in controlling within-subject variability, it is unknown whether the differences in variability in the observed responses between RE and CON were a result of differences in within-subject variability between groups. The potential difference in within-subject variability between groups impairs the ability to discern whether a given SD_{IR}, and corresponding ES_{IR}, reflects variability in the responses to exercise. Therefore, it is unclear whether our results reflect individual variability in the mRNA expression responses to RE or
differences in within-subject variability between RE and CON. In an attempt to reduce potential differences in within-subject variability between participants, future work should determine the variability in mRNA expression responses to RE using study designs that standardize diet/exercise habits ~120 hours prior to collecting muscle biopsies.

In addition to differences in within-subject variability between groups, the small sample size in RE (n = 7) and CON (n = 6) also limits the findings of our study. It is possible that increasing the sample size, and thus more adequately representing the recruited population (i.e. young [age: 22 ± 2 years], non-smoking, recreationally active, and healthy males), alters the calculated SD_{IR}/ES_{IR} for each transcript. For instance, although our results demonstrate that SD_{IR}/ES_{IR} could not be calculated for ~35% of transcripts (i.e. the variability in observed responses to CON exceeded RE), our small sample size limits the confidence to conclude that these transcripts are more variable in response to CON than RE. Therefore, future research should use a larger sample size to determine whether variability, independent of measurement error and/or within-subject variability, in mRNA expression responses to RE exists.

Murton et al. (1) reduced the potential incidence of false negatives in statistical analysis of group responses by excluding probe sets that did not meet their filtering criteria: probe sets had to be classified as “present” on > 50% of arrays according to microarray analysis software and thought to be important in muscle processes according to pathway analysis software. Although false positives/negatives are not necessarily an
issue for analysis of individual variability, restricting analysis to probe sets that pass a given filtering criteria is thought to increase the reliability of microarray data analysis (137). However, because information regarding which probe sets passed Murton et al.’s (1) filtering criteria is not made available in the original paper or on GEO, we calculated a \( \text{SD}_{IR}/\text{ES}_{IR} \) for each probe set in the dataset. To reduce the number of transcripts analyzed, future studies can opt to only conduct individual variability analysis on probe sets that pass a given filtering criteria.

**Conclusions**

The results from the present study suggest that individual variability in the mRNA expression responses to RE exists for many mRNA that are involved in pathways that regulate the hypertrophic response to RT. Our results also highlight 109 mRNA that have large variability in the responses to RE are among a group of mRNA previously found (2) to have acute responses that correlate with RT-induced skeletal muscle adaptations. While responses in these 109 mRNA may represent acute mechanisms that can predict variability in chronic adaptations, the mechanisms that underlie variability in mRNA expression responses to RE are unclear. Murton et al. (1) show that the number of altered transcripts is related to the magnitude of muscle damage incurred during RE. However, future studies that control for participants’ exercise/dietary habits, and other biological factors that can contribute to within-subject variability, are needed to confirm whether variability in the mRNA expression responses exists and whether muscle damage incurred during RE explains this variability.
4.1 Summary of results

The purpose of this thesis was to examine the individual variability in the mRNA expression responses to acute exercise. This was accomplished by utilizing freely available microarray data (1) to compare the variability in observed mRNA expression responses to RE and CON. The key findings of this thesis are as follows:

1) Individual variability in the mRNA expression responses to RE existed (i.e. variability in observed responses to RE exceeded variability in observed responses to CON) for many, but not all, transcripts.

2) 1486 mRNA had large variability in the responses to RE and were involved in several pathways known to regulate the hypertrophic response to RT.

3) 109 mRNA with large variability in the true responses to RE also were among a group of mRNA previously found to have acute responses that correlate with skeletal muscle strength and size adaptations to RT.

Collectively these results suggest that individual variability in the mRNA expression responses to RE exists and highlight 109 mRNA that may play key roles in predicting individual skeletal muscle adaptations to RT.
4.2 Implications of key findings

Elucidating the mechanisms that contribute to individual variability in exercise training responses represents an important step toward prescribing exercise as an individualized medicine (19). Because acute mRNA expression responses represent the induction of chronic adaptations (3, 21, 22), variability in mRNA expression responses to acute exercise may represent a mechanism that contributes to individual variability in exercise training responses; however, no study had yet to convincingly demonstrate individual variability in mRNA expression responses to acute exercise. Therefore, the findings from this thesis contribute to the growing appreciation of inter-individual differences in responsiveness to exercise by demonstrating variability in the mRNA expression responses to RE.

We also found that 109 mRNA with large variability in the responses to RE were also among a group of mRNA that Raue et al. (2) found have acute responses that correlate with skeletal muscle strength and size adaptations to RT. Indeed, many of these 109 mRNA are involved in several pathways known to regulate the hypertrophic response to RT. Whether the magnitude of mRNA expression responses of these 109 mRNA to RE can predict individual responsiveness to RT remains unknown. These findings suggest that individuals who demonstrate large mRNA expression responses of these 109 mRNA to a given prescription of RE will likely subsequently demonstrate large chronic adaptations to RT (i.e. repeated bouts of that prescription of RE). Conversely, if minimal mRNA expression responses to RE of these 109 mRNA implicates minimal chronic adaptation, then perhaps altering the parameters of the RE prescription (i.e.
number of repetitions/sets, intensity, modality, etc) is needed to induce large mRNA expression responses and thus large skeletal muscle adaptations to RT. Although altering exercise training prescription appears to induce different chronic adaptations (10,43,138), it remains unknown whether modifying acute exercise prescription results in different mRNA expression responses within the same individuals.

4.3 Future directions

Although several studies have reported individual mRNA expression responses to acute exercise (25–29), the study presented in this thesis was the first to look at variability in the mRNA expression responses. Thus, while we present individual variability in the mRNA expression responses for many, but not all, mRNA at 24 hours post-RE, there remain many important questions to be answered by future research:

1) Because several time-course studies (i.e. studies that collect multiple post-exercise muscle samples) indicate that mRNA expression responses transiently peak 1-120 hours post-RE in a mRNA-specific manner (63,65,68,136,139), does examining mRNA expression at different post-RE time points impact individual variability in the true responses?

2) Given the many studies (30,31,140–143) demonstrating alterations in human skeletal muscle mRNA expression in response to varying aerobic exercise protocols (i.e. endurance exercise, high-intensity/sprint interval training), is there individual variability in the mRNA expression responses to aerobic exercise?
3) Does using RT-qPCR to measure mRNA expression also reveal individual variability in the true responses to RE? Although the degree of measurement error in RT-qPCR and microarrays is not clearly understood, it is highly unlikely that these two laboratory techniques have identical measurement errors. Because measurement error contributes to variability in the observed responses, and thus the calculation for variability in the exercise responses, it is unknown whether the presumable difference in measurement error between RT-qPCR and microarrays results in a different ES<sub>IR</sub> calculated for each mRNA. Thus the findings of the current study are not generalizable to future work aiming to measure mRNA expression with RT-qPCR; an important consideration given that the majority of studies utilize RT-qPCR due to the greater cost, tissue required, and potential technical complications associated with microarrays.

4) Would using a within-participants design, where the same participants complete both RE and CON, reduce the magnitude of individual variability in the mRNA expression responses to RE (i.e. reduce the number of transcripts with an ES<sub>IR</sub> > 0 and/or decrease the ES<sub>IR</sub> value for each transcript)? Some biostatisticians have recently argued that without ascertaining an individual’s response to both exercise and CON, it is unclear whether responses to exercise are truly exercise responses per se (34,144). Calculating SD<sub>IR</sub>/ES<sub>IR</sub> with a between-participants design, as done in the present study, assumes that within-subject variability, and thus its contribution to the variability in observed
responses, is equal between exercise and CON groups. Because a within-participants design decreases the risk of differences in within-subject variability between groups of participants (consisting of different individuals), it should increase the confidence in identifying the variability in the responses to exercise (34,144).

5) What are the mechanisms that contribute to variability in the mRNA expression responses to RE? Although Murton et al. (1) suggest that muscle damage incurred during RE influences the number of differentially altered transcripts, this finding is limited by their very small sample size (n = 7 participants). Thus much work is needed to elucidate the mechanisms that underlie individual variability in the mRNA expression responses to RE.

Collectively, answering these questions will improve our understanding of individual variability in mRNA expression responses to acute exercise and contribute to the growing the body of literature supporting the movement toward considering exercise as an individualized medicine.
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