HEAT STRESS AND THE CONTROL OF SKELETAL MUSCLE MASS

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ABSTRACT

PURPOSE: 1) Compare the effects of whole-body heat stress (HS) and resistance exercise (RE) on thermoregulatory responses and skeletal muscle heat shock and hypertrophy related signaling. 2) Examine the effects of acute heat stress (HEAT) on myotube growth and fusion compared to controls (CON), hypertrophy (HYPER), and atrophy (RAPA) treatments in-vitro. METHODS: 1) Eight healthy, physically active and resistance trained individuals (18-45 years) completed RE and HS. 2) 48 hours following treatments, C2C12 myotubes were assessed for myotube area and nuclear fusion index. RESULTS: 1) RE and HS similarly increased muscle but not core temperatures. HS but not RE increased HSPA1A/B protein expression, HSPC1-3 was unchanged. HS increased Akt-mTOR phosphorylation greater than RE. 2) HEAT increased myotube area greater than RAPA and CON, but to a lesser than
HYPER. HEAT increased fusion index greater than RAPA and CON but was not different than HYPER.
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CHAPTER I: INTRODUCTION

Introduction

Skeletal muscle is essential for vital functions including locomotion, breathing, and digestion, as well complex sport activities, and is a primary tissue controlling metabolic rate \(^1,2\). Therefore, the development and maintenance of muscle mass is an important factor modulating not only athletic performance but healthy aging and functional capabilities across the lifespan \(^1,3,4\). Under physiologic conditions skeletal muscle mass is balanced between anabolism and catabolism, with shifts towards muscle loss characterized by atrophy and growth by hypertrophy \(^3,5\). Many factors contribute to this balance including physical activity and resistance exercise, nutritional intake, health and disease status, as well as the progression of age \(^4–6\). Regardless of the stimulus, the mechanistic target of rapamycin (mTOR)–protein kinase B (Akt) pathway is widely considered an essential control point in maintaining and increasing skeletal muscle mass through its intracellular signaling effects \(^7\). Conversely, the inflammatory related nuclear factor kappa B (NF-κB) and forkhead box (FOXO) pathways mediate muscle degradation \(^3,8\). Importantly, while each of these mechanisms serve important independent roles in the physiologic control of skeletal muscle mass, they can additionally blunt the effects of one another \(^3,9\). Of considerable interest, systemic and cellular responses to heat stress may enhance hypertrophy related outcomes while additionally reducing atrophy related processes.

High temperatures, \(~40 \, ^{\circ}C\), have been extensively studied as a stressor in relation to recreational, occupational, and athletic events \(^10–12\). Though humans can experience temporary acclimation following repeated heat exposure \(^13\), the increased demands of high temperature reduces physiologic capacities \(^14,15\) and can be deadly in extreme or prolonged states \(^16–18\). In contrast to these risks, growing evidence demonstrates that heat stress alone or in combination with resistance exercise or exercise like stimuli enhances hypertrophy related signaling \(^19–22\) and reduces atrophy related signaling \(^19,23,24\). Furthermore, various forms of heat stress have been able to promote skeletal muscle growth \(^25–28\) and reduce atrophy \(^29–33\) or promote recovery following muscle loss \(^33–36\). Notably, the combined results from human
investigations, as well as animal and cellular models suggest a key role of the ubiquitously expressed, and stress inducible heat shock proteins (HSPs) \(^{37,38}\) in mediating these effects \(^{39}\).

Specific HSPs including HSP72 (HSPA1A), HSP70 (HSPA1B), HSP90-alpha (HSPC1), HSP90-alpha A2 (HSPC2), and HSP90-beta (HSPC3), HSPA1A/B and HSPC1-3, respectively \(^{40-42}\), are capable of pertinent interactions within the Akt-mTOR pathway \(^{43-45}\). Therefore, heat stress mediated responses may stimulate hypertrophic effects in part through activation of the Akt-mTOR cascade \(^{39}\). Nonetheless, evidence regarding the expression of HSPs in skeletal muscle \(^{46-48}\) and whether heat-based interventions can stimulate gross hypertrophy in humans are mixed \(^{49-51}\). Furthermore, comparisons across human, animal, and cellular investigations are severely limited by methodological constraints, including lack of control points such as contrast to known hypertrophic stimuli or heat stress alone. Therefore, this investigation aims to present comparative results regarding the acute thermoregulatory, heat shock, and hypertrophy related signaling responses in human skeletal muscle following a single session of whole-body heat stress and resistance exercise. Moreover, we will examine the effects of heat stress and growth stimulus in comparison to control and atrophy inducing conditions using an in-vitro model.

**Purpose**

The primary purpose of this investigation is to examine the effects of acute passive whole-body heat stress (60 minutes, 55-60 °C, 20-30% relative humidity) and resistance exercise (leg press & knee flexion 5x8-12 at 70% 1-RM, plus knee extension & calf raises 3x10 at 100% of 10-RM) on systemic temperature (core & muscle), hypertrophic (Akt, mTOR, S6K1, 4E-BP1) and heat shock (HSPA1A/B, HSPC1-3) related signaling factors in human skeletal muscle at pre-, 30 minutes, and 3 hours post-trial. This aim will allow a greater understanding of the acute effects of heat stress in comparison to a bout of resistance exercise on local signaling factors related to the control of skeletal muscle mass. Additionally, examination of systemic temperature and skeletal muscle heat shock responses will help elucidate the underlying mechanisms connecting heat stress and hypertrophy related responses. The secondary purpose of this investigation is to examine the cellular effects of
acute heat stress (60 minutes, 40 °C, 5% CO₂) in comparison to hypertrophy (grow media treatment) and atrophy (rapamycin treatment) inducing conditions on myotube area (MHC staining) and morphology (nuclear fusion index) in cultured C2C12 myotubes. This secondary in vitro model will build upon our human experiment, allowing for examination of myotube development as a proxy to functional hypertrophic outcomes in isolated skeletal muscle.

**Hypotheses**

Hypothesis 1: acute whole-body heat stress will increase systemic temperature and enhance heat shock and hypertrophy related signaling in human skeletal muscle.

- **1a:** Acute whole-body heat stress will increase core (T_c) and muscle (T_m) temperature compared to baseline and to a greater degree than resistance exercise.
- **1b:** Phosphorylation of the Akt-mTOR pathway will increase at either 30 min or 3 hours post heating compared to baseline but to a lesser degree than resistance exercise.
- **1c:** Protein expression of HSPA1A/B and HSPC1-3 will increase at either 30 min or 3 post heating compared to baseline and to a greater degree than resistance exercise.

**Rationale:**

- Ihasn et al. (2020) demonstrated that 60 min of whole-body heat stress (45-50°C, 50% relative humidity) acutely increased T_c and T_m to a similar degree.
- Evidence demonstrates protein expression of HSPA1A/B, HSPC1-3, and the Akt-mTOR cascade is enhanced in skeletal muscle following heat stress.
- There is variability in post-translational protein modifications of the Akt-mTOR pathway between 30 min to 3 hours post stimulus. Taking the highest point of expression between 30 min and 3 hours will account for individual responses to some extent.

Hypothesis 2: Acute resistance exercise will increase systemic temperature and enhance hypertrophic, but not heat shock signaling in human skeletal muscle.
- 2a: acute resistance training heat stress will increase $T_c$ and muscle $T_m$ temperature immediately post exercise but to a lesser degree than heat stress.

- 2b: post-translational phosphorylation of the Akt-mTOR pathway will increase at either 30 min or 3 hours post exercise compared to baseline and to a greater degree than heat stress.

- 2c: Protein expression of HSPA1A/B and HSPC1-3 will increase at either 30 min or 3 post heating compared to baseline but to a lesser degree than heat stress.

Rationale:

- High intensity and high-volume resistance exercise is well known to stimulate muscular hypertrophy, in part through acute Akt-mTOR mediated signaling and increased myofibrillar protein synthesis $^7,5^3$. Similar resistance training protocols including incline leg press (5x10), knee extension (4x8), and leg extensions (4x8) have been demonstrated to enhance muscle protein synthesis and total turnover at 3 hours post exercise $^5^3$.

- Limited evidence suggests prolonged resistance exercise can enhance skeletal muscle HSP expression $^5^4–5^6$, yet trained individuals may express higher basal levels and present impaired protein responses $^5^7$.

Hypothesis 3: Acute heat stress will enhance myotube development in cultured C2C12 cells.

- 3a: MHC expression area and fusion index will be greater at 48-hours post heat stress compared to control conditions.

- 3b: MHC expression area and fusion index will be greater following 48-hours of grow media enrichment compared to control conditions and to a greater degree than heat stress.

- 3c: MHC expression area and fusion index will be reduced following 48-hours of rapamycin treatment compared to control conditions, heat stress, and grow media enrichment conditions.

Rationale:
▪ 60 min of heat stress (40-42 °C) has been shown to enhance total cellular protein accumulation and fusion index in cultured myotubes compared to baseline\textsuperscript{25,58}.

▪ Previous findings have demonstrated that grow media treatment post-differentiation results in robust myotube hypertrophy\textsuperscript{59}.

▪ Rapamycin blunts protein synthesis and hypertrophic outcomes, although lower concentrations (164 nM) allow for maintenance of cellular viability\textsuperscript{6,60}.

**Scope:**

Eight healthy individuals aged 18-45 years, who regularly engage in exercise including ≥150 minutes of moderate to vigorous intensity aerobic activity per week and ≥2 days of resistance exercise per week while living in the Albuquerque area for a minimum of 6 months will be recruited for this investigation. Additionally, participants will not have a history of known heat related illness or regular exposure to other sources of heat stress (i.e., sauna usage or occupational demands) within 3 months of their experimental trials.

**Significance**

Skeletal muscle plays an essential role in maintaining functional capabilities across the lifespan of all individuals. Reductions in muscle mass increase health and mortality risks in a variety of healthy and diseased populations. Heat stress may enhance muscular growth or prevent its loss, yet further understanding is needed regarding the mechanisms and efficacy of heat-based interventions. Heat shock proteins, with an emphasis on HSPA1A/B and HSPC1-3, may play a key part in transmitting heat induced stimuli to hypertrophic pathways such as the Akt-mTOR cascade in skeletal muscle. By exploring the effects of heat stress compared to a resistance training bout in humans and growth conditions in *in-vitro* we may better present the magnitude of acute hypertrophic stimulus for such interventions. This will further practical understandings regarding the effects of whole-body and isolated cellular heat stress on stimulating skeletal muscle growth, especially in comparison to known hypertrophic stimuli.
Limitations

1. The use of healthy and active individuals as participants will limit the applicability of our findings to non-resistance trained, sedentary or clinical populations. Moreover, resistance trained individuals may present blunted hypertrophic responses to resistance exercise compared to untrained individuals per the law of diminishing returns.

2. Though relevant, Akt-mTOR signaling responses are not solely responsible for hypertrophic adaptations and we will be unable to directly comment on other mechanistic effects. Moreover, in humans we can only suggest acute responses but not chronic effects including gross muscular hypertrophy.

3. The C2C12 cells utilized for the in-vitro experiments are derived from mice, therefore these results must be interpreted with caution considering extrapolation to human investigations.
References


CHAPTER II: RESEARCH REVIEW

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The Heat Shock Connection: Skeletal Muscle Hypertrophy and Atrophy
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Abstract
Skeletal muscle is an integral tissue system that plays a crucial role in the physical function of all vertebrates and is a key target for maintaining or improving health and performance across the lifespan. Based largely on cellular and animal models, there is some evidence that various forms of heat stress with or without resistance exercise may enhance skeletal muscle growth or reduce its loss. It is not clear whether these stimuli are similarly effective in humans or meaningful in comparison to exercise alone across various heating methodologies. Furthermore, the magnitude by which heat stress may influence whole body thermoregulatory responses and the connection to skeletal muscle adaptation remains ambiguous. Finally, the underlying mechanisms, which may include interaction between relevant heat shock proteins and intracellular hypertrophy and atrophy related factors, remain unclear. In this narrative we examine the relevant literature regarding heat stress alone or in combination with resistance exercise emphasizing skeletal muscle hypertrophy and atrophy across cellular and animal models, as well as human investigations. Additionally, we present
working mechanistic theories for heat shock protein mediated signaling effects regarding hypertrophy and atrophy related signaling processes. Importantly, continued research is necessary to determine the practical effects and mechanisms of heat stress with and without resistance exercise on skeletal muscle function via growth and maintenance.

**Introduction**

Skeletal muscle is an integral component of all vertebrate organisms, serving as the force generator for locomotion, a major site of metabolism (1), and a tissue that coordinates across all physiologic systems to maintain homeostasis (2). Furthermore, as a remarkably plastic tissue which adapts to a plethora of internal and external stimuli (2), skeletal muscle is a key target for modulating basic functionality, health (3), and feats of human performance (4). At the basic level, skeletal muscle mass is regulated by an adaptive equilibrium of growth and breakdown, termed muscular hypertrophy and atrophy, respectively (2, 5). In a balanced state, skeletal muscle mass remains unchanged, yet various stimuli may shift this pendulum resulting in hypertrophy or atrophy. Importantly, alterations to the contractile elements of skeletal muscle not only affect gross size but its functional properties, affecting both health and performance (2–4). Accordingly, while physiologic stimuli including physical activity, exercise, and nutrient intake promote muscular growth, states of disuse and pathologic conditions can induce muscular degradation (6–8). Due to these well-known responses, skeletal muscle is an important target for not only improving physical health and performance but preventing or reducing the severity of many diseases (3, 9, 10).

Regular resistance training has been extensively researched for its ability to enhance post-natal skeletal muscle development, growth, and function (11). Unfortunately, many individuals do not meet the recommended levels of physical activity and resistance exercise whether by choice, lifestyle, or limited physical capabilities and health status (12–14). Of interest, results from cellular, animal, and human investigations suggest heat stress from various external sources (environmental, direct application, water immersion, microwave & diathermy) can beneficially influence outcomes supporting hypertrophy (15–22) and limiting atrophy (23–26), particularly when combined with resistance training. While promising, it is
unclear if heat-based stimuli alone or with resistance exercise can result in meaningful muscular adaptations related to mass and function in humans (27–29). Furthermore, the underlying mechanisms linking heat stress and these skeletal muscle adaptations, including the role of specific heat shock proteins (HSPs), remains ambiguous. Therefore, the purpose of this review is to examine the literature regarding the effects of heat stress alone or in combination with resistance exercise on the cellular and gross physiologic responses and adaptations underlying muscular hypertrophy and atrophy with an emphasis on the mechanistic role of HSPs. In this way, we will present working theories regarding the underlying mechanisms for heat induced signal transduction and adaptation in skeletal muscle. Additionally, we aim to provide researchers and practitioners with understandings of the applied effects for heat-based interventions with or out without resistance exercise on muscular hypertrophy and atrophy.

**Muscular Hypertrophy & Atrophy**

Though cooperatively balanced under normal physiologic conditions, hypertrophy and atrophy related processes are dysregulated by states of disuse or disease including but not limited to bed rest, sarcopenia, cancer, and muscular dystrophy (2, 6–8). Importantly, heat stress may influence pertinent underlying mechanisms of muscle hypertrophy and atrophy. The protein kinase B (Akt) - mechanistic target of rapamycin (mTOR) signaling cascade is well accepted as a central regulator of cellular growth and development with an emphasis on skeletal muscle mass (5, 30). Along with its primary components; mTOR complex 1 (mTORC1), mTOR complex 2 (mTORC2), P70-S6 kinase 1 (S6K1), ribosomal protein S6 (rps6), and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1); the Akt-mTOR cascade responds to physiologic stimuli including nutrient intake and exercise, resulting in greater myofibrillar protein synthesis and hypertrophy (5, 31). Furthermore, its effects are in opposition to catabolic signaling pathways operating primarily through the ubiquitin-proteasome and autophagy-lysosome systems which stimulate myofibrillar breakdown and atrophy (6, 8, 10, 32). This set of pathways is influenced by a host of upstream factors including circulating inflammatory factors like tumor necrosis factor alpha.
(TNF-α), reactive oxygen species (ROS), and cytosolic calcium accumulation under conditions of stress, disuse, or disease (8, 9). Downstream intracellular components include nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), forkhead box O (FOXO) transcription factors 1 and 3, as well as calpain and caspase activity (8, 9, 32). Together these components promote direct degradation (33) or indirect proteolysis and autophagy via enhanced expression of atrophy promoting factors including the muscle specific E3 ubiquitin ligases muscle RING-finger protein-1 (MuRF1) and muscle atrophy F-box gene (MAFbx/Atrogen-1), as well various autophagy related genes (Atg’s) (6, 8, 32).

Factors outside of the Akt-mTOR signaling cascade may also be relevant to the stimulation of muscular hypertrophy such as the mitogen-activated protein kinase (MAPK) family, extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal Kinase (34), as well as mitochondrial relevant signaling factors for their role in controlling atrophy (35, 36). Considering the scope of this review, we will focus primarily on the Akt-mTOR cascade and the closely associated atrophy signaling factors. Balance between the activity of these atrophic signals and that of the Akt-mTOR cascade are inherent to the control of skeletal muscle mass (5, 8). Key points of regulation include Akt mediated phosphorylation and inhibition of FOXO transcriptional activity (33, 37), as well as mTORC1 mediated phosphorylation and inhibition of autophagy inducing unc-51-like kinase 1 (ULK1) (8). Of interest, factors within each of these hypertrophy and atrophy related signaling cascades have been shown to be affected by heat stress in cellular (38), animal (19, 39–44), and human-based (15, 16, 24, 45, 46) investigations [Tables 2.1 & 2.2].

The Heat Shock Connection

The primary mammalian HSPs, ranging from approximately 15-110 kDa (47), are well known for their diverse molecular effects across multiple cellular domains, and have subsequently been the focus of extensive review (47–50). These ubiquitously expressed proteins are considered widely as stress responsive chaperones with key roles in maintaining cellular homeostasis (47–50). HSPs are regulated transcriptionally by heat shock
transcription factors (HSFs) (51) and are expressed in response to stimuli including heat stress (52) and exercise (53) across tissue domains, including skeletal muscle tissues (54). Of interest, various HSPs have been further suggested as protective against the progression of aging and related clinical disorders with an emphasis on skeletal muscle mass and function (55–57). Accordingly, in myopathy related conditions, HSP expression appears to coincide with immunologic responses to inflammatory states and may be inherent to processes including proteostasis (58) and muscle regeneration in humans (59). Furthermore, deleterious mutations in HSP coding genes are known to promote the development of various myopathies (60). Regardless, their exact biological roles in stress responses and disease states in skeletal muscle are still being unveiled (54). HSPs are grouped by molecular size and function such as the HSP70 (HSPA), HSP90 (HSPC), and small HSP (HSPB) superfamilies (47, 61, 62). There is some evidence that heat stress alone and in combination with resistance exercise or exercise-like stimuli can increase the translation and expression of specific members within these HSP families alongside mixed results regarding signaling factors related to gross muscular atrophy and hypertrophy (15, 21, 24, 26, 38–43, 45, 46, 63). Furthermore, research suggests there are interactions between factors within or parallel to the Akt-mTOR cascade, atrophy related signaling, and specific HSP regulatory factors and families; HSF1-2, HSP72 (HSPA1A), HSP70 (HSPA1B), HSP90-alpha (HSPC1) HSP90-alpha A2 (HSPC2), HSP90-beta (HSPC3), equivalent human and mouse HSP27/25 (HSPB1), respectively (44, 49, 61–68). As HSPA1A and HSPA1B, as well as HSPC1, HSPC2, and HSPC3 express minimal structural differences, present similar inducible actions, and localize within the cytoplasm they will be referred to collectively as HSPA1A/B and HSPC1-3 unless stated otherwise, respectively [Table 1] (61, 62, 69).

As upstream regulators of HSP expression, HSFs provide information regarding the necessity of heat shock responses for specific signaling effects (51). While various HSFs play a key role in survival and thermotolerance (70, 71), they have also been shown to relevant to normal myoblast development in-vitro (68), and their absence reduces hypertrophic responses to heat stress in mice (63). This suggests that the normal regulation and presence of mammalian HSFs and subsequently HSPs may be important not only for general cellular proliferation and survival but muscular hypertrophy and recovery from damage. Of note,
some evidence suggests HSF mediated hypertrophic responses to overload could include mechanisms other than HSP expression in mice such as cytokine and myokine responses (72). While HSFs and other potential mechanisms present an interesting field of inquiry, we will primarily focus on downstream HSP mediated responses in this review. Accordingly, findings from in-vitro models demonstrate that HSPA1A/B and HSPC1-3 interact with key targets of the Akt-mTOR signaling cascade including mTORC2, mTORC1 and Akt (66, 73, 74). As an example, Martin et al. (2008) demonstrated HSPA1A/B co-localization with mTORC2 following acute heat shock in human HeLa cells (73). Additionally, inhibition of HSPA1A/B in this model reduced mTORC2 formation and activity indicating a functional dependence following heat stress (73). While mTORC1 activity is the primary initiator of transient hypertrophic processes in this cascade, mTORC2 serves a key role by activating Akt via its serine 473 site, as shown in drosophila cells (75). In a similar fashion, HSPC1-3 appears to associate with and promote the downstream effects of mTORC1 in-vivo (74). Ohji et al. (2006) discovered that HSPC1-3 binds to mTORC1 via its raptor component and that the HSPC inhibitor geldanamycin impairs the hypertrophic effects of both S6K1 and 4E-BP1 in human embryonic kidney cells (74). A study by Sato et al. (2000) has additionally demonstrated that HSPC1/2 and HSPC3 can bind directly to, and form complexes with, Akt via its 229-309 and 327-340 sites, respectively, and protects against protein phosphatase 2 induced apoptosis in human embryonic kidney and mouse fibroblast cells (66). Furthermore, when HSPC3 binding was inhibited in human kidney cells, Akt activation was reduced compared to controls and cell death increased in apoptosis stimulated conditions (66). While not all these studies have utilized heat stress, there is additional evidence that heat induction plays a part in the discussed mechanisms. Accordingly, it has been shown that heat stress activates Akt in mouse fibroblasts and human kidney cells in a PI3K dependent manner, suggesting a heat inducible effect on HSPC1-3 related Akt-mTOR signaling in-vitro (76). Moreover, the work of Yoshihara et al. (2013) presents evidence for temperature dependent activation of Akt in response to heat stress in the skeletal muscle of rats (19). Together, these investigations illustrate potential mechanisms by which the heat inducible HSPA1A/B and HSPC1-3 may cooperatively stimulate the Akt-mTOR signaling cascade (77).
Beyond Akt-mTOR signaling, HSPs may play additional roles regarding the muscle remodeling process through calcium and calcineurin mediated responses (44, 67, 78). Following their earlier experiments (21, 41), Kobayashi et al. (2005) showed that heat stress upregulates calcineurin and HSPA1A/B in rat skeletal muscle (44). It has also been demonstrated that HSPC1-3 forms complexes with and activates calcineurin in epithelial cells through calmodulin dependent and independent means, respectively (67). Although the role of calcium mediated calcineurin signaling for hypertrophy in-vivo has been debated (79), it is a known regulator for muscle remodeling and fiber type transition in response to exercise stimulation (80, 81). The work of Lara-Pezzie et al. (2007) demonstrated that calcineurin inhibition impairs Akt function and increased FOXO3a activity along with MuRF1 and MAFBx expression in C2C12 mouse myotubes (82). Conversely, calcineurin overexpression inhibits FOXO3a and its downstream targets in cells, while promoting muscle regeneration from cardiotoxin induced damage in mouse skeletal muscle (82). Moreover, muscular contraction increases cytosolic calcium, resulting in calcium calmodulin and calcineurin activation which promote nuclear factor of activated T-cell (NFAT) nuclear translocation and increase expression of hypertrophic factors including myosin heavy chain, actin, myogenin, IGF-1, and various myokines (80, 82). In opposition, mechanical unloading can also increase chronic calcium concentration, which activates calpains, reducing the expression of HSPC3 along with Akt and mTOR activity, and enhances proteasome-mediated muscular protein degradation in rat muscle (78). Owing to this effect, others suggest that intracellular calcium accumulation and calpain protease activity play a role in disuse or unloading induced muscle atrophy processes (9, 82).

With regard to muscle loss, additional roles for HSPs as regulators of autophagy and atrophy related factors have been suggested (65, 78, 83). It has been demonstrated that HSPA1A/B expression is protective against TNFα-induced cell death in tumor cells (83) and that its inhibition promotes widespread cell death in breast cancer cells (84). Nylandsted et al. (2000) further provided evidence that HSPA1A/B localizes with the plasma membrane of lysosomes resulting in lower permeabilization and therefore impaired autophagic capabilities (65). Although these studies utilize cancer cells, they highlight the potential role of HSPAs to impair cellular autophagy, an atrophy related process, and this molecular mechanism may
exist in other contexts, such as in skeletal muscle. As example,Senf et al. (2008) showed that overexpression of HSPA1A/B reduces disuse induced muscle atrophy in rats through inhibition of FOXO3a, NF-κB, and subsequent reduction of MuRF1 and MAFbx E3 ligases (85). Paired with findings that hindlimb unloading stimulated atrophy in rats is accompanied by HSPA1A/B dissociation from newly forming ribosomal peptides alongside decreasing elongation rates, the role of HSPA1A/B as an atrophy reducing agent is promising (86). Similarly, HSPB1 transfection reduces muscle wasting during disuse atrophy in rats through inhibition of NF-κB, MuRF1, and MAFbx, but not FOXO transcriptional activity (87). Conversely, HSPA1A/B may enhance TNFα induced cellular apoptosis through inhibition of the NFκB deactivating region, kappa B alpha (IκBα), suggesting that increased HSP expression is not an inherently beneficial stimulus (88). Nonetheless, the combined findings presented here propose a set of interactions by which heat inducible families including HSPAs, HSPCs, and HSPBs may interact with hypertrophic and anti-atrophic signaling responses [Figure 1]. Yet, as the evidence for these proposed mechanisms is derived primarily from isolated cellular and animal models, including non-muscle models, they remain unproven, particularly in humans. Until appropriate muscle specific and human based investigations have been conducted, these theorized mechanisms should be viewed with caution and serve primarily to drive future research.

It is also necessary to recognize that exercise alone can initiate pertinent responses considering the discussed HSPAs, HSPCs, and HSPBs (53, 54), yet it is not entirely clear what type of stimulus or internal thermoregulatory responses are required (89) or how they are expressed in skeletal muscle (90–92). Though an important field of inquiry, we will primarily focus on heat induced HSP responses alone or in combination with exercise as opposed to exercise alone. Additionally, although HSPs appear to play a role in the development of and responses to muscular dystrophies (59, 60), the effects of heat stress and exercise in modulating these effects is not clear but presents an interesting field of inquiry for future researchers. Finally, while outside the scope of this review, we acknowledge that heat-based interventions and HSPs have been explored as effectors for other physiologic adaptations including changes to cardiovascular and mitochondrial properties (22, 93, 94). Regardless, with a focus on muscular atrophy and hypertrophy, continued mechanistic
investigation is necessary to parse out the specific roles of those discussed here and potentially other HSPs. Importantly we can gain further insights into the practical effects of heat stress and HSPs, on muscular hypertrophy and atrophy by exploring the basic and applied results of cellular, animal, and human studies.

**Cellular & Animal Models – Basic Signaling Outcomes**

Demonstrative to the importance of the heat shock response for muscular function, Ohno et al. (2015) showed that HSF1 null mice experienced reduced muscular hypertrophy and myogenesis following a 60 min bout of heat stress at 40 °C (63). Conversely, the heated controls from this experiment presented increased number of (paired box protein 7) Pax7 positive nuclei at 3 days, along with higher body weight and soleus muscle mass 7 days post-treatment. Furthermore, heat stress increased HSPA1A/B translation and expression, HSPB1 expression, and the phosphorylation ratio of Akt and S6K1 at days 3 and 7, and 7, respectively, despite higher baseline values observed in HSF1 null groups (63). In further murine models, heat stress has been shown to increase the phosphorylation ratio of Akt (17–19) and S6K1 (19, 40), along with muscular growth (40). Responses in Akt and S6K1 have in fact displayed temperature dependent phosphorylation immediately following 30 min of lower body water immersion from 39–41 °C in mouse muscle with Akt displaying greater temperature sensitivity than S6K1 (19). Curiously, the expression of HSPA1A/B and HSPC1-3 were not different between any condition despite the previously discussed mechanisms for HSP mediated Akt activation (19). Regardless, the acute sample collection timepoint for this investigation may have missed changes in intramuscular HSP expression, which are not well characterized, and corroborates an important *in-vitro* connection between heat stress and Akt-mTOR signaling. Wei and Heide (2008) similarly found that 20 min of conduction (42 °C) heating immediately stimulated Akt activity in rat myocardial cells and provided protection against ischemia induced cellular damage but they did not explore changes in HSPs (18). Conversely, others have shown 60 min of acute environmental (41 °C) (41) and 30 min of repeated (7-8 days) diathermy (41-41.5 °C) heating with (42) or without immobilization (43) increases cellular proliferation, muscular growth, and HSPA1A/B and
HSPB1 expression in rats. Similarly, Kobayshi et al. (2005) provide evidence that 60 min of environmental (41 °C) heat stress enhances rat muscle mass alongside HSPA1A/B and calcineurin expression within 7 days of recovery (44). An investigation by Goto et al. (2003) further found concomitant increase in HSPA1A/B and HSPC1-3 expression in rat myoblasts following heat stress, mechanical stretch, and the combination of both interventions (21). Interestingly, HSPA1A/B expression was more responsive to mechanical stretch, while HSPC1-3 to heat stress, indicating potentially divergent roles for these signaling proteins as molecular chaperones and heat-inducible factors (21).

When examining atrophy related signaling factors, Ohno et al. (2010) demonstrated that TNFα protein expression was decreased 3 days following 60 min of environmental heat stress (42 °C) in rat skeletal muscle, suggesting reductions in upstream inflammatory signals (39). This investigation also found increased protein expression of the NF-κB inhibitor IκBα along with decreased phosphorylation ratios of NF-κB in the same rats (39) and cultured mouse myotubes (38). Interestingly, an applied animal study by Ohno et al. (2012) displayed increased muscle mass and protein content following 60 min of environmental heat stress (41 °C) in old (106 weeks) and young (7 weeks) mice alongside similar increases to HSPB1 and HSPA1A/B (40). Specifically, irrespective of unchanged calpain 1 and 2 expression, aged mice had reduced expression of the lysosomal associated protease cathepsin L following heat stress, which was elevated at baseline in old but not young mice (40). A further finding from this investigation indicated that aged mice expressed greater HSPB1 and HSPA1A/B expression at baseline despite similar increases between groups following heat stress, suggesting the heat-induced stress response was an essential component to the observed hypertrophic effects (40). Although these investigations cannot provide direct evidence of HSP interactions with atrophy and hypertrophy related factors, they suggest heat stress in cellular and animal models is associated with the upregulation of HSPA1A/B, HSPC1-3, HSPB1, and Akt-mTOR related signaling. Additionally, there is applied evidence from these same and other similar investigations suggesting hypertrophic and atrophy reducing effects of heat stress.
Cellular & Animal Models – Applied Physiologic Outcomes

The early work of Naito et al. (2000) first presented evidence that environmental heat stress (60 min, 41 °C) attenuated muscular atrophy caused by hindlimb unloading in rats (95). This investigation set the stage for further experiments demonstrating that various forms of heat stress can not only reduce muscular atrophy but promote hypertrophy in cellular and animal models. For example, various heating methods; ambient air (21, 23, 38–41), hot water immersion (26), and direct (42); have been shown to affect atrophy and hypertrophy in mice and rat myotubes (21, 38), as well as in mice and rats (39–41). From these models it’s suggested that even a single dose of heat stress at 41-42 °C for 30-60 min can promote muscular growth (39–41). In fact, environmental heat has increased total body and relative muscle mass within 7 days following a single session of whole-body heating (60 min, 41-42 °C) in mice and rats (39–41). Heat stress has additionally demonstrated an ability to counter atrophy and promote muscular recovery in animal models (23, 26, 42, 43). Accordingly, muscle loss is reduced compared to non-heated controls following hindlimb suspension (41) and recovery enhanced following cardiotoxin induced degeneration injection (38) in rats. Further, repeated heat stress can enhance recovery from muscle atrophy when implemented concurrent to (26, 42, 43) and after (42, 43) atrophy inducing protocols.

Importantly, evidence from these experiments and others additionally suggests physical exercise in-vivo and exercise-like mechanical or electrical stimulation in-vitro can further enhance heat stimulated hypertrophic outcomes in muscle. For example, Goto et al. (2003) demonstrated that 96 hours of mechanical cyclic stretching paired with heat stress enhanced total protein expression compared to controls, heat stress, or mechanical stretch alone in cultured rat myotubes (21). Furthermore, the effects of heat and mechanical stress on total HSP expression in rat soleus muscles was greater than either intervention alone (21). Yoshida et al. (2013) similarly provided evidence that rats subjected to regular (3x weekly) hot water heating (20 min, 42 °C) prior to electrical stimulation mimicking low intensity exercise experienced greater protection from gastrocnemius muscle loss across two weeks of hindlimb suspension compared to heat and electrical stimulation alone (26). Although not an exercise stimulus per se, Selsby et al. (2007) also found greater protection from soleus muscle atrophy following 7 days of immobilization when every other day direct heating (30
min, 41-41.5 °C) was combined with 7 days of reloading compared to reloading alone (43). Evidence regarding the combined effects of heat stress and muscular stimulation is not unanimous, as despite improvements compared to non-heated controls, Frier and Locke (2007) found lower relative muscle mass following direct heat stress (15 min at target core temp of 42 °C) and plantaris overload compared to overload alone (27).

From the basic outcomes of these studies, it is apparent that heat stress applied through environmental, direct application, and water immersion may enhance hypertrophy in part through acute Akt-mTOR signaling to induce greater cellular growth. Additionally, reductions in atrophy related signaling factors following heat stress may further shift physiologic states towards anabolism and protein synthesis, ultimately promoting the accretion of skeletal muscle protein and hypertrophy. This is reflected in the applied investigations demonstrating that heat stress can protect from atrophy as well as promote skeletal muscle growth under normal or following atrophic conditions in animal and cell models. Additionally, heating may enhance myofibrillar protein synthesis and hypertrophy when combined with exercise or exercise-like stimuli, yet it may not be as beneficial as exercise-based stimuli alone. While evidence utilizing human participants is methodologically diverse in comparison and fails to include robust atrophy inducing models, there is similar promising evidence for heat stress as a stimulus for reducing muscle atrophy and promoting hypertrophy.

**Human Investigations – Basic Signaling Outcomes**

In humans heat stress alone also appears to directly enhance hypertrophy related and heat shock signaling (15) as well as in combination with resistance exercise (16, 46). Furthermore, it is possible that heat stress alone could protect against muscular loss in part through reduction of atrophy related signaling factors (15, 24, 45). The work of Ihsan et al. (2020) indicates that environmental heat stress (60 min, 45-50 °C, 50% Relative Humidity) alone increases phosphorylation of Akt, mTOR, p70S6K, ribosomal binding protein S6 (rpS6), and eukaryotic translation initiation factor 4E along with the gene expression of HSPA1A/B, HSPC1-3, and HSPB1 at either 30 min or 3 hours post-heating (15). Kakigi et
al. (2011) demonstrated increased signaling through the Akt-mTOR cascade comparing the combination of acute resistance exercise and direct muscle heating compared to resistance exercise alone (16). Specifically, 60 min following a bout of single leg resistance exercise (knee extension, isokinetic, 4 sets x 6 repetitions) plus direct microwave heating, 20-min pre and during exercise, the phosphorylation status of ERK 1/2, Akt, mTOR, S6K1, and 4E-BP1 were increased compared to non-heated exercise conditions. Unfortunately, this investigation did not include examination of HSPs (16). A final training study by Yoon et al. (2017) provides evidence that circulating anabolic hormones, GH and IGF-1, were similarly increased when comparing 12 weeks of high load without heating and low load resistance training plus prolonged (8 hours) direct sheet heating (46).

Although these investigations suggest acute heat stress alone or in combination with resistance exercise enhances hypertrophic signaling, particularly in relation to the Akt-mTOR pathway, there is some conflicting evidence. In an investigation of acute high load resistance exercise followed by single leg water immersion (20 min, 46 °C) there was no enhancement of myofibrillar protein synthesis rates between 2 and 5 hours post-exercise (28). Additionally, the phosphorylation status of mTOR, p70S6K, rpS6, and 4E-BP1 were not increased (28). Pertinent to this discrepancy, the work of Ihsan et al. directly compared whole-body to single leg water immersion heating (~49.5 °C) and found only whole-body heating was capable of increasing Akt-mTOR signaling factors in skeletal muscle (15). Additionally, atrophy related signaling factors are relatively unstudied in humans, and to our knowledge only one group has utilized disuse or atrophy simulating designs (24). Although these studies by Hafen et al. (2018, 2019) focused on mitochondrial signaling responses, which are beyond the focus of this review, they demonstrated that repeated diathermy treatments increase HSPA1A/B and HSPC1-3 expression, ERK 1/2 phosphorylation, but not HSPB1 expression while attenuating muscle atrophy during immobilization (24, 45). Moreover, in passively heated humans, the investigation by Ihsan et al. (2020) indicates that whole-body heat stress inactivates the atrophy related transcription factors FOXO3a and NF-κB in skeletal muscle (15). Though limited in absolute number, particularly considering the exploration of HSPs and atrophy related signaling these studies suggest that passive localized
and whole-body heating, as well as localized microwave plus resistance exercise can influence HSPs, atrophy, and hypertrophy related signaling in skeletal muscle.

**Human Investigations – Applied Physiologic Outcomes**

When exploring gross hypertrophic responses, especially in combination with resistance exercise, there is a larger body of evidence from human-based investigations. Goto et al. (2007) showed that 30 sessions of steam-heating sheets applied to the upper arm 30 min before, and throughout low load (<30% 1 RM) elbow extension exercise (3 sets x 30 reps) increased biceps brachii size and isometric torque (20). Compared to baseline, participants displayed an impressive 7.5 ± 5.5% increase in Cross Sectional Area (CSA) and 18.4 ± 11% increase in maximal isometric elbow flexion while non heated arms did not increase in either measure (20). Using a similar low load training design - 18 sessions, elbow extensions, 3 sets x 8 reps, <30% 1RM - Nakamura et al. (2019) demonstrated that heat retaining packs applied to the upper arm increased triceps CSA and 1RM compared to baseline and non-heated controls (96). Together these investigations suggest upper arm heating via direct methods promote muscular hypertrophy and strength development in combination with low load resistance exercise more than exercise alone. Another training study conducted by Yoon et al. (2017) compared the effect of 12 weeks (3 sessions per week) single leg resistance training at high loads (60% 1RM, knee extensions, 3 sets x 15-18 reps) or low loads (40% 1RM, knee extensions, 3 sets x 25) plus 8 hours of heat stress via heating sheets applied to the rectus femoris muscle (46). In this experiment, both high load and low load resistance groups with heat stress increased quadriceps CSA and 1RM knee extension to a similar degree while a heat stress only control group displayed no changes for either measure (46). Although low load resistance training plus heat stress enhanced muscular strength and size of the lower limbs, it appears no more beneficial than high load training alone. Additionally, the null effects of the heating only group indicates lower limb heating might not promote muscular adaptation on its own. In agreement with the previous findings, Labidi et al. (2020) found 8 hours of heating pad application to the gastrocnemius muscle 5 days per week for 6 weeks to have no effect on CSA (97). Similarly, Stadnyk et al. (2017) found direct heating
pads applied to the thigh for 20 min following resistance training did not improve quadriceps muscle mass or 3RM knee extension compared to non-heated contralateral controls (29). In fact, both control and post-training heat intervention groups demonstrated similar hypertrophic outcomes following 12 weeks of resistance training (29). Conversely, the results from Kim et al. (2020) suggest that single leg heat stress (90 min, 52 °C) via water perfused suit 5 days a week for 8 weeks improved peak torque of the knee extensors compared to control limbs (98). Despite these improvements to muscular function, no measures of hypertrophy were investigated (98).

In line with these results, others have also demonstrated heat-induced gains in muscular strength or improved force characteristics without changes to muscle cross sectional area (99, 100), which could reflect changes in neuromuscular capabilities. As example, an acute bout of environmental heat stress (73 °C) has been shown to increase resting motor evoked potentials in the hand musculature (99, 101), while 11 days of passive heat acclimation (60 min, 44-55 °C, 40% RH) can increase stimulated peak twitch and maximal voluntary torque of the soleus muscle (99). As exercise is known to enhance motor cortex excitability in healthy (102) and clinical populations (103), this may indicate that heat stress might be mimicking some of the neuromuscular effects of exercise. Since neural adaptations, not just changes in muscle fiber CSA, contribute strength gains (104), future researchers may consider including neuromuscular measures as a relevant outcome to muscle focused heat based interventions. With the only currently known atrophy model in humans, the work of Hafen et al. (2019) showed that daily diathermy heating (2 hours) reduced muscle atrophy across 10 days of single leg immobilization (24). While the immobilization alone decreased whole vastus lateralis and average myofibrillar CSA by 7.3% and 10.8%, losses were reduced to 4.5% and 5.8% following heat treatment, respectively (24).

Together these applied experiments provide evidence that site specific and whole-body heating can acutely enhance signaling cascades leading to, as well directly stimulate muscle hypertrophy either alone or in combination with resistance exercise. Yet, localized heating combined with low load resistance training, might not be preferential to high load training without heat. While this suggests traditional high load training as the greater
hypertrophic stimulus, certain populations unable to perform high load training could still find benefit utilizing low load plus heat training styles. Additionally, while limited in evidence, the simultaneous inhibition of atrophy related signaling factors may limit atrophy during disuse or further enhance hypertrophy. Importantly, as these outcomes are based on a relatively limited number of investigations, their universal occurrence remains unproven. Of concern, few studies discussed in this section included measures for relevant HSPs (15, 24, 45, 98) despite widespread acknowledgement of their potential roles in relation to heat induced muscular outcomes. Accordingly, it is currently difficult to extrapolate the role of specific HSPs for influencing gross skeletal muscle hypertrophy and atrophy in humans. It is also important to acknowledge that discrepancy between experimental results may in part be explained by factors including heating method effectiveness, targeted location, as well as intervention timing; pre-, post-, concurrent, acute, chronic. Reviewed elsewhere (105), the differential effects of heating methods; direct heating or steam pads (20, 29, 46, 96, 97), hot water immersion or circulation (15, 28, 98), environmental (15), diathermy (24, 45), microwave (16); on factors related to physiologic responses including muscular hypertrophy and atrophy have not been adequately evaluated or directly compared. Additionally, comparisons between upper (20, 96) and lower body (15, 16, 24, 28, 29, 45, 46, 97, 98) musculature or whole-body responses (15) are extremely limited. Furthermore, it is unclear how acute (15, 16, 28) or chronic (25, 29, 46, 96–98) heat-based interventions differ from passive (15, 97, 98) and exercise-combined (16, 25, 28, 29, 46, 96) protocols regarding the effects discussed in this review. This point is further confounded by differences in training studies which utilize pre- (16, 20, 96, 97), concurrent (16, 20, 29, 96), or post-exercise (16, 28, 29) heating interventions. Considering these points, it is important to briefly discuss thermoregulatory responses including skin, core, and muscular temperatures following heat stress, resistance exercise, and combined interventions.

Thermoregulatory Considerations

It has been well established that physical work and exercise increase metabolic heat production resulting in the elevation of skin, core, and skeletal muscle temperatures (106–
108). Though intrinsically linked with the ultimate goal of maintaining systemic temperature within physiologic ranges (109, 110), heat stress (52) and exercise (53) stimulated thermoregulatory deviations across these tissues are relevant stimuli for HSP induction and expression. Despite individual variability or differences in sampling techniques (111), moderate to high intensity resistance exercise appears to elevate skin, core, and muscular temperature modestly, with changes of <1°C (112), <0.5°C (113), and approximately 1°C (113, 114), respectively. This is notably less than increases observed during maximal aerobic efforts (115–117), but could in part be due to the small total volume of resistance exercise utilized in these experiments. Cumulatively, various forms of heat stress discussed have also been shown to enhance skin (15, 22), core (15), and muscular (15, 20, 28, 29, 46, 96, 97) temperatures. Yet, some methodologies differ in their ability to increase temperature systemically as well as stimulate hypertrophy and atrophy related processes. Accordingly, while passive whole-body heating can equivocally increase skin, core, and muscular temperatures, some localized methods do not appear to significantly alter core temperature (15, 118, 119). As an example, heating sheets and pads, water perfusion suits and immersion, as well as microwave and diathermy devices increase muscular but not core temperatures in the lower and upper limbs by approximately 0.5-4 °C (15, 16, 20, 24, 28, 29, 45, 98, 120), depending on application length (28, 46, 97). Conversely, whole-body heating interventions including environmental and water perfusion suits induce similar changes in muscle temperature while additionally elevating body temperature (15, 120). In agreement with this discrepancy, the results of Ihsan et al. (2020), showed that whole-body environmental but not single heating water perfusion influences HSP expression, atrophy, and hypertrophy related signaling in skeletal muscle (15). In fact, both methods induced similar muscular temperatures (~38 °C), while whole-body resulted in significantly greater skin temperature responses (33 °C vs 40 °C), and only whole-body elevated core temperature (~39 °C) (15). Therefore, in this instance systemic heating including changes in skin, core, and muscular temperatures were necessary to influence HSP, hypertrophy, and atrophy related signaling responses in humans. Likewise, Fuchs et al. (2020) demonstrated that lower body heating via water immersion following resistance exercise did not enhance HSP expression or myofibrillar protein synthesis while only elevating skin, core, and muscle temperatures by approximately 10 °C, 0.6 °C, and 2.3 °C, respectively (28). Ultimately, lower core and skin
temperatures represent greater ability to dissipate heat as a compensable stress (121), which might not be adequate to stimulate the hypertrophy and atrophy related responses discussed in this review. Since, even moderately hot environments (30 °C) can significantly increase skin and core temperatures passively (122) or during exercise (22), this possibility is worthy of further investigation. Of interest, certain localized heating techniques including microwave and diathermy have been shown to stimulate HSP and hypertrophy signaling responses (16, 45) or confer muscle sparing effects during disuse (24). Microwave and diathermy can increase skin and muscle temperatures between 5-9 °C within 30 min of application (24, 45, 119, 123). These are notably greater temperature increases for skin and muscle than some have reported following direct sheet (20, 96) and single leg or lower limb water heating (15, 97, 118) methods, and could account for some of the observed differences between investigations. Importantly, the thermoregulatory responses to heat stress, resistance exercise, or their combination are currently difficult to interpret. Pointedly, to our knowledge, no study to date has included a comparative measurement between heat stress, resistance exercise, or combined interventions. Furthermore only a handful have included or demonstrated increased translation (97) or expression (24, 45, 46) of HSPs in skeletal muscle. Finally, we note that various animal models have shown increases in core and muscular temperatures following whole-body, localized, and hindlimb heating techniques. Regardless, methodological incompatibilities and differences between murine and human thermoregulatory responses (124) make comparisons difficult, and deserve greater review elsewhere.

**Perspectives and Significance**

Here we have presented the relevant research regarding the effects of heat-based interventions alone or in combination with resistance style exercise on basic signaling and applied physiologic outcomes associated to muscle hypertrophy and atrophy with an emphasis on the role of HSPs across cellular and animal models, and humans. From a mechanistic perspective, we explored routes by which HSPA1A/B, HSPC1-3, and HSPB1 may participate with intracellular signaling pathways that protect from muscular atrophy or
confer hypertrophic benefits. Considering gross muscular outcomes, some evidence suggests that heat stress alone can reduce muscular atrophy and promote hypertrophy in animal and cell models as well as human-based investigations. If elicited, these effects might be enhanced when combined with resistance exercise or exercise-like stimuli, include low load training styles. Despite this, consensus has not been reached considering the ability of heat stress to stimulate muscle protein synthesis, promote hypertrophy, or reduce atrophy acutely or chronically. This is particularly true considering the effects of heat stress alone, and in experimental designs examining muscle loss in humans. Furthermore, while some evidence is present, the exact mechanisms by which HSPs might associate with or influence relevant signaling responses in skeletal muscle are not fully elucidated. As our proposed mechanisms of interaction between HSPs and muscular signaling responses derive from a mix of cellular, animal, and human models including non-muscle specific tissues, they should be viewed as part of a theoretical framework for future research inquiry. Future applied and mechanistic investigations following heat stress alone or in combination with resistance exercise, specifically utilizing skeletal muscle, and humans are required before the validity of HSP mediated responses, or the efficacy of heat-based interventions can be determined.

Table 1. Standard and Commonly Utilized HSP Nomenclature. Superfamily classifications are provided on the left while specific proteins are denoted on the right. Adapted with regards to the work of Kampinga et al. (2009) (61) and Hoter et al. (2018) (62).

<table>
<thead>
<tr>
<th>Superfamily Name (Common Analog)</th>
<th>Protein Name (Common Analog)</th>
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<tbody>
<tr>
<td>HSPA (HSP70)</td>
<td>HSPA1A (HSP72)</td>
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<tr>
<td></td>
<td>HSPA1B (HSP70)</td>
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<tr>
<td>HSPC (HSP90)</td>
<td>HSPC1 (HSP90-alpha)</td>
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<tr>
<td></td>
<td>HSPC2 (HSP90-alpha A2)</td>
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<td></td>
<td>HSPC3 (HSP90-beta)</td>
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<tr>
<td>HSPB (Small HSP)</td>
<td>HSPB1 (HSP27/HSP25)</td>
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</tbody>
</table>
### Table 2.1 Summary of Investigations Including Relevant Hypertrophy and Atrophy Related Responses in Humans.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Heat Intervention (Sample)</th>
<th>Protocols (Condition)</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goto et al. 2007</td>
<td>Direct heat pad &amp; steam sheet applied to the arm for 30 min pre- and during exercise for a total of 60 min (HS).</td>
<td>10 weeks/40 sessions single arm 3x30 elbow extensions at &lt;30% 1 RM (RT).</td>
<td>↔ Biceps brachii temperature.</td>
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<tr>
<td></td>
<td></td>
<td>RT + HS.</td>
<td>↔ Max isometric flexion and CSA.</td>
</tr>
<tr>
<td></td>
<td>(8 healthy males).</td>
<td></td>
<td>↑ Biceps brachii temperature.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑ Max isometric flexion and CSA.</td>
</tr>
<tr>
<td>Kakigi et al. 2011</td>
<td>Direct microwave (150W) applied to the VL for 20 min pre- and during resistance exercise (HS).</td>
<td>Single bout of 4x6 maximal isokinetic single-leg extensions (RE).</td>
<td>↑ S6K1 and ERK1/2 phosphorylation.</td>
</tr>
<tr>
<td>(Acute Study)</td>
<td></td>
<td>RE + HS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(8 Healthy males).</td>
<td></td>
<td>↑ Akt, mTOR, 4E-BP1, S6K1 and ERK1/2 phosphorylation.</td>
</tr>
<tr>
<td>Yoon et al. 2017</td>
<td>Direct heat sheet applied to the rectus femoris for 8 hours, 3 days per week for 12 weeks (HS).</td>
<td>12 weeks of 3x15-18 single leg knee extensions at 60% 1 RM, 3 times per week.</td>
<td>↑↑ CSA.</td>
</tr>
<tr>
<td>(Training Study)</td>
<td>(21 healthy, elderly females).</td>
<td></td>
<td>↑ 1 RM</td>
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<td></td>
<td></td>
<td></td>
<td>↑ GH circulatory concentration.</td>
</tr>
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<td></td>
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<td>↑ IGF-1 circulatory concentration.</td>
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<tr>
<td></td>
<td>12 weeks of 3x15-18 single leg knee extensions at 40% 1 RM, 3 times per week + HS.</td>
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<td></td>
<td>↑ Rectus femoris temperature.</td>
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<td>↑ CSA.</td>
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<td>↑GH circulatory concentration.</td>
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<td>↑ IGF-1 circulatory concentration.</td>
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<td>↑ Rectus femoris temperature.</td>
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<td>↑ IGF-1 circulatory concentration.</td>
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<tr>
<td>Stadnyk et al. 2017</td>
<td>Direct heat pad at 40 °C applied to the thigh during and for 20 min following resistance exercise (HS).</td>
<td>12 weeks/30 sessions of 4x8 single leg knee extensions at 70% 1 RM (RT).</td>
<td>↑ Quadriceps muscle mass, 3 RM, peak concentric torque, and rate of torque development.</td>
</tr>
<tr>
<td>(Training Study)</td>
<td>(10 healthy males).</td>
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<td></td>
<td></td>
<td></td>
<td>IM + HS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑ Quadriceps muscle mass, 3 RM, peak concentric torque, and rate of torque development.</td>
</tr>
<tr>
<td>Nakamura et al. 2019</td>
<td>Direct heat retaining pack (pre-heated at 75 °C) applied to the upper arm 20 min prior to resistance exercise (HS).</td>
<td>6 weeks/18 sessions of 3x8 supine elbow extensions at 30% 1 RM (RT).</td>
<td>↔ CSA and 1 RM.</td>
</tr>
<tr>
<td>(Training Study)</td>
<td>(30 healthy males).</td>
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<td>↑ CSA and 1 RM.</td>
</tr>
<tr>
<td>Hafen et al. 2019</td>
<td>2 hours diathermy heating of thigh for 10 days (HS).</td>
<td>10 days of single leg braced immobilization (IM)</td>
<td>↔ Vastus lateralis temperature (Day 1).</td>
</tr>
<tr>
<td>(Chronic)</td>
<td>(12 healthy males and 11 healthy females).</td>
<td></td>
<td>↓↓ Vastus lateralis and myofiber CSA (Day 10).</td>
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<td></td>
<td></td>
<td></td>
<td>↔ HSPA1A/B and HSPC1-3 protein expression (Day 10).</td>
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<td></td>
<td></td>
<td></td>
<td>IM + HS</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>↑ Vastus lateralis temperature (Day 1).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ Vastus lateralis and myofiber CSA (Day 10).</td>
</tr>
</tbody>
</table>
Labidi et al. 2020 (97). (Training Study)
8 hours of single leg direct heating pads applied to the gastrocnemius (8:00 am – 4:00 pm), 5 days per week for 6 weeks (HS).
(8 healthy males and 7 healthy females).

↑ HSPA1A/B and HSPC1-3 protein expression (Day 10).

Kim et al. 2020 (98) (Training Study)
90 min single leg water perfused suit at 52 °C, 5 days a week, for 8 weeks/40 sessions (HS).
(10 healthy males, and 2 healthy females).

↑ Peak torque.

Ihsan et al. 2020 (15) (Acute Study)
60 min passive whole body environmental at 44-50 °C, 50% RH (WB-HS).
60 min single leg water-perfused suit at 49.5 ± 1.5 °C (SL-HS)
(9 Healthy males).

↑ Vastus lateralis and core temperature.
↑ Akt, mTOR, S6K1, eIF4E, FOXO3a, and NF-κB phosphorylation.
↑ HSPA1A/B, HSPC1-3, HSPB1 mRNA expression.
↑ Vastus lateralis temperature. ↔ Core temperature.

Fuchs et al. 2020 (28) (Acute Study)
Sample: 12 healthy males (23 ± 1 years).
20 min SL water immersion at 46 °C (HS).
Resistance exercise: 4x10 leg press and knee extension at 80% 1RM (RE)

↑ Myofibrillar protein synthesis rate.
↔ mTOR phosphorylation.
↑ S6K1 phosphorylation.
↑ Vastus lateralis temperature.


Table 2.2 Summary of Investigations Including Relevant Hypertrophy and Atrophy Related Responses in cells or animals.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Heat Intervention (Sample)</th>
<th>Protocols (Condition)</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goto et al. 2003 (21). (Acute)</td>
<td>60 min environmental heating at 41 °C (HS).</td>
<td>96 hours cyclic stretching (Stretch).</td>
<td>↑ HSPA1A/B protein expression. ↑↑ HSPC1-3 protein expression. ↑ Total protein concentration.</td>
</tr>
<tr>
<td>Study and Conditions</td>
<td>Description</td>
<td>Contralateral Controls</td>
<td>Results</td>
</tr>
<tr>
<td>----------------------</td>
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<tr>
<td>Goto et al. 2004</td>
<td>60 min environmental heating at 41 °C. Hind limb suspension (HLS).</td>
<td>↑↑ Absolute body and relative soleus muscle weight (10% &gt; HLS).</td>
<td>↑↑ HSPA1A/B protein expression. ↑ HSPC1-3 protein expression. ↑ Total protein concentration.</td>
</tr>
<tr>
<td>Uehara et al. 2004</td>
<td>60 min environmental heating at 41 °C (HS).</td>
<td>↑ Soleus mass (Day 7).</td>
<td>↑ HSPA1A/B protein expression (1 hour). ↑ S6K1 phosphorylation (1 hour). ↑ 5-Bromo-2’-deoxyuridine and antigen-positive nuclei (≥1 day).</td>
</tr>
<tr>
<td>Selsby and Dodd, 2005</td>
<td>30 min thermal blanket heating under anesthesia 24 hours before and on alternating days. Tc maintained at 41-41.5 °C (HS).</td>
<td>↔ Absolute body and relative soleus mass. ↔ HSPA1A/B and HSPB1 protein expression. ↔ 4-hydroxyl-2-nonono protein expression.</td>
<td>↓↓ Absolute body and relative soleus mass. ↑ HSPA1A/B and HSPB1 protein expression. ↑ 4-hydroxyl-2-nonono protein expression.</td>
</tr>
</tbody>
</table>

**Note:** The table lists the conditions and outcomes of various studies involving heat stress (HS) and chronic or acute hind limb suspension (HLS), with comparisons to untreated controls and additional treatments such as cyclosporine A (CA) and vehicle injection (CON). The results include changes in protein expression and body mass metrics.
<table>
<thead>
<tr>
<th>Selsby et al. 2007 (43). (Chronic)</th>
<th>Untreated controls.</th>
<th>Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min thermal blanket heating under anesthesia 24 hours before and on alternating days. $T_c$ maintained at 41-41.5 °C (HS). (9-10 male Sprague-Dawley rats per group.) Soleus muscle collected at 9 days, (48 hours following treatments).</td>
<td>↔ Absolute body, relative soleus, and extensor digitorum longus weight (Days 1-14). ↑ HSPA1A/B protein expression (Days 1-14). ↑ Calcineurin protein expression (Days 1-7).</td>
<td>↔ Absolute body, relative soleus, and extensor digitorum longus weight (Days 1-14). ↑ HSPA1A/B protein expression (Days 1-14). ↑ Calcineurin protein expression (Days 1-7).</td>
</tr>
<tr>
<td>7 days of casted hindlimb immobilization (IM).</td>
<td>↔ Absolute body and relative soleus mass. ↔ HSPA1A/B and HSPB1 protein expression. ↔ 4-hydroxyl-2-noneno and Nitrotyrosine protein expression.</td>
<td>↓↓↓ Absolute body and relative soleus mass. ↓ HSPA1A/B and HSPB1 protein expression. ↑ 4-hydroxyl-2-noneno and Nitrotyrosine protein expression.</td>
</tr>
<tr>
<td>IM + weight reloading (RC).</td>
<td>↓↓ Absolute body and relative soleus mass. ↔ HSPC1 protein expression. ↑ HSPB1 protein expression. ↑↑ 4-hydroxyl-2-noneno and Nitrotyrosine protein expression.</td>
<td></td>
</tr>
<tr>
<td>IM + HS + RC.</td>
<td>↓ Absolute body and relative soleus mass. ↑↑↑ HSPA1A/B and HSPB1 protein expression. ↔ 4-hydroxyl-2-noneno and Nitrotyrosine protein expression.</td>
<td></td>
</tr>
<tr>
<td>Kojima et al. 2007 (25). (Chronic)</td>
<td>Physiologic saline injection (PS). *NC</td>
<td></td>
</tr>
<tr>
<td>60 min environmental heating at 41 °C 24 hours prior to or after conditions (HS). 25 male Wistar rats (7 weeks) per group. Venous blood and tibialis anterior muscles collected at 1, 3, 7, 14, and 28 days.</td>
<td>↑ Absolute body weight (Days 1-28). ↔ Relative tibialis weight (Days 1-28). ↔ HSPA1A/B protein expression (Days 1-28).</td>
<td>↑ Absolute body weight (Days 1-28). ↔ Relative tibialis weight (Days 1-28). ↔ HSPA1A/B protein expression (Days 1-28).</td>
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<tr>
<td><em>NX</em> HSPA1A/B protein expression (Days 3-7).</td>
<td>↑↑ Absolute body and relative tibialis weight (Days 1-3). ↓ Absolute body and relative tibialis weight (Days 7-28). ↔ HSPA1A/B protein expression (Days 1-28).</td>
<td>Cardiotoxin injection (CTX).</td>
</tr>
<tr>
<td><em>BX</em> HSPA1A/B protein expression (Days 1-28).</td>
<td>↑↑ Absolute body and relative tibialis weight (Days 1-3). ↔ Absolute body and relative tibialis weight (Days 7). ↓ Absolute body and relative tibialis weight (Days 14-28). ↔ HSPA1A/B protein expression (Days 1-28).</td>
<td>HS followed by CTX.</td>
</tr>
<tr>
<td><em>AX</em> HSPA1A/B protein expression (Days 1-28).</td>
<td>↑↑ Absolute body and relative tibialis weight (Days 1-3). ↓ Absolute body and relative tibialis weight (Days 7-28). ↔ HSPA1A/B protein expression (Days 1-3, 28). ↑ HSPA1A/B protein expression (Days 7-14).</td>
<td>CTX followed by HS.</td>
</tr>
</tbody>
</table>

Ohno et al. 2010 (39). (Acute) 60 min environmental heating at 42 °C (HS). (15 male Wistar rats).

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<tbody>
<tr>
<td>HS</td>
<td>↑ HSPA1A/B protein expression and IkBα phosphorylation (day 1). ↓ NF-κB protein phosphorylation (day 1). ↓ TNFα protein expression (day 3). ↑ Pax7-positive nuclei (day 3). ↑ Body weight, gastrocnemius mass, and protein content (day 7).</td>
<td>Ohno et al. 2010 (39). (Acute) 60 min environmental heating at 42 °C (HS). (15 male Wistar rats).</td>
</tr>
</tbody>
</table>

Ohno et al. 2011 (38). (Acute) 60 min environmental heating at 41 °C on day 7 of myotube differentiation (HS). (C2C12 mouse myotubes)

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<tr>
<td>BAY11-7082 (1.25µM) inhibition of NF-κB at day 7 of myotube differentiation (BAY).</td>
<td>↑ IkBα protein phosphorylation (12 hours). ↓ NF-κB protein phosphorylation (12 hours). ↔ HSPA1A/B protein expression (12 hours &amp; day 2). ↑ Relative protein content (2 days).</td>
<td>Ohno et al. 2011 (38). (Acute) 60 min environmental heating at 41 °C on day 7 of myotube differentiation (HS). (C2C12 mouse myotubes)</td>
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<tr>
<td>HS</td>
<td>↑ IkBα protein phosphorylation (12 hours). ↓ NF-κB protein phosphorylation (12 hours). ↑ HSPA1A/B protein expression (12 hours &amp; day 2). ↑ Relative protein content (day 2).</td>
<td>BAY + HS</td>
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<tr>
<td>BAY + HS</td>
<td>↑ IkBα protein phosphorylation (12 hours). ↓ NF-κB protein phosphorylation (12 hours). ↑ HSPA1A/B protein expression (12 hours &amp; day 2). ↑ Relative protein content (day 2).</td>
<td>Young-HS Day 7</td>
</tr>
<tr>
<td>Study</td>
<td>Treatment Details</td>
<td>Changes</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ohno et al. 2012 (40)</td>
<td>60 min environmental heating at 41 °C (HS). (6 young and 6 old male c57BL/6J mice.)</td>
<td>↑ HSPA1A/B and HSPB1 protein expression, and S6K1 phosphorylation. ↔ Cathepsin L protein expression. ↑ Absolute and relative soleus weight and protein content.</td>
</tr>
<tr>
<td>Frier and Locke, 2012 (27)</td>
<td>Direct covered heating pad at 55 °C. T_c maintained at 42 °C for 15 min (HS). (25 Sprague-Dawley rats per group).</td>
<td>↔ Absolute body and relative plantaris mass (Days 1-7). ↔ HSPA1A/B and HSPB1 protein expression (Days 1-7). ↑ HSPB1 protein expression (Days 2-7).</td>
</tr>
<tr>
<td>Yoshihara et al. 2013 (19)</td>
<td>30 min hindlimb water immersion at 37, 38, 39, 40, or 41 °C. (7 male Wistar rats per group).</td>
<td>↑ Akt and S6K1 protein phosphorylation (30 min). <em>Increases occurred in temperature-dependent manner</em></td>
</tr>
<tr>
<td>Yoshida et al. 2013 (26)</td>
<td>60 min hindlimb water immersion at 42 °C under anesthesia every 3 days (HS). (5 Male Wistar rats per group).</td>
<td>↓↓ Body and relative gastrocnemius weight. ↔ HSPA1A/B protein expression.</td>
</tr>
<tr>
<td></td>
<td>Untreated controls.</td>
<td>↔ Body and relative gastrocnemius weight. ↔ HSPA1A/B protein expression.</td>
</tr>
<tr>
<td></td>
<td>2 weeks of hind limb suspension (HLS).</td>
<td>↓↓ Body and relative gastrocnemius weight. ↔ HSPA1A/B protein expression.</td>
</tr>
<tr>
<td></td>
<td>HLS + HS.</td>
<td>↓↓ Body and relative gastrocnemius weight. ↑ HSPA1A/B protein expression.</td>
</tr>
<tr>
<td></td>
<td>HLS + 20 min low intensity neuromuscular electrical stimulation every 3 days (LEX).</td>
<td>↓↓ Body and relative gastrocnemius weight. ↔ HSPA1A/B protein expression.</td>
</tr>
<tr>
<td></td>
<td>HLS + 20 min high intensity neuromuscular electrical stimulation every 3 days (HEX).</td>
<td>↔ Body and relative gastrocnemius weight. ↔ HSPA1A/B protein expression.</td>
</tr>
<tr>
<td>Study</td>
<td>Treatment/Experimental Conditions</td>
<td>HLS + HS + LEX.</td>
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<tr>
<td>Ohno et al. 2015</td>
<td>60 min environmental heating at 41 °C (HS). (45 wild type, and 39 HSF1-null mice).</td>
<td>↓ Body and relative gastrocnemius weight.</td>
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<td></td>
<td></td>
<td>↑ Body and relative gastrocnemius weight.</td>
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<tr>
<td></td>
<td></td>
<td>↑ HSPA1A/B mRNA expression (Day 7).</td>
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<td></td>
<td></td>
<td>↑ HSPC1 mRNA expression (Days 3 &amp; 7).</td>
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<td></td>
<td></td>
<td>↑ HSPA1A/B and HSPB1 mRNA expression (Day 0).</td>
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<td></td>
<td>↓ Akt and S6K1 protein phosphorylation (Day 0).</td>
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<td></td>
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<td>↓ HSPA1A/B and HSPB1 mRNA expression (Day 0).</td>
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<tr>
<td></td>
<td></td>
<td>↓ Akt and S6K1 protein phosphorylation (Day 1 &amp; 7).</td>
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<tr>
<td></td>
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<td>↓ Pax7-positive nuclei (Days 1 &amp; 3).</td>
</tr>
<tr>
<td>Tsuzuki et al. 2018</td>
<td>30 min environmental heating at 40-41 °C (HS). (6-8 Long-Evans Tokushima Fatty, type 2 diabetic, rats per group).</td>
<td>Untreated controls.</td>
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<td>30 min treadmill running (20 meters/min) at 4 °C.</td>
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<td>↑ Tc and gastrocnemius temperature.</td>
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<td>HS.</td>
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</table>

Figure 1. Possible Mechanisms of Heat Shock Protein Interaction with Hypertrophy and Atrophy Related Responses in Skeletal Muscle. *1 HSPA1A/B co-localization and stabilization with mTORC2, *2 HSPC1-3 raptor-mTORC1 association, *3 HSPC1-3 Akt binding and activation, *4 HSPA1A/B and HSPC1-3 complex formation with calcineurin, *5 HSPA1A inhibition of lysosomal function via reduced permeabilization, *7 HSPB1 stabilization of IκB-α and MuRF1 & MAFbx expression, *8 HSPA1A inhibition of NF-κB and FOXO3a activity and MuRF1 & MAFbx expression. Arrows indicate activation while bars indicate inhibition. Green coloration confers hypertrophy related processes, purple atrophy related, while blue indicates a mixed role.
References


70. Zhang Y, Huang L, Zhang J, Moskophidis D, Mivechi NF. Targeted disruption of hsf1 leads to lack of thermotolerance and defines tissue-specific regulation for stress-


97. **Labidi M, Ihsan M, Behan FP, Alhammoud M, Smith T, Mohamed M, Tourny C, Racinais S.** Six weeks of localized heat therapy does not affect muscle mass, strength and contractile properties in healthy active humans.


CHAPTER III: RESEARCH ARTICLE

Novel Heat Stress Increases Heat Shock and Hypertrophy Related Signaling in Resistance Trained Individuals.

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2. Harvard University, Harvard Medical School, Joslin Diabetes Center.
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Introduction

Skeletal muscle is essential for basic motion, complex sport activities, and acts as a systemic regulator of homeostasis \textsuperscript{1-3}. With its adaptive capabilities, the development and maintenance of skeletal muscle mass is important not only for sports performance but healthy aging and the retention of functional capabilities across the lifespan \textsuperscript{1,4,5}. In basal states, skeletal muscle mass is balanced between hypertrophy and atrophy via underlying anabolic and catabolic processes \textsuperscript{2,6,7}. Many factors contribute to and can shift this equilibrium, including exercise, health and disease status, as well as the progression of age \textsuperscript{4,7}. The protein kinase B (Akt)-mechanistic target of rapamycin (mTOR) pathway including its downstream targets ribosomal S6 kinase beta 1 (S6K1), and eukaryotic elongation initiation factor 4E-binding protein 1 (4E-BP1) are important control points for increased protein synthesis and skeletal muscle mass in response to stimuli such as exercise \textsuperscript{2,8,9}. Furthermore, Akt-mTOR signaling inhibits relevant atrophy related pathways that stimulate myofibrillar protein degradation via ubiquitin proteasome and autophagy lysosome systems \textsuperscript{10,11}. Interestingly,
some evidence demonstrates that heat stress alone or in combination with resistance exercise can stimulate or enhance hypertrophic responses in skeletal muscle 12–19.

There is emerging evidence from cellular, animal, and human based investigations suggesting that heat stress of various forms can stimulate processes related to 12,13,15,16,19–23 as well as promote skeletal muscle hypertrophy 2,17,18,20,24, protect against atrophy 25–29, and enhance recovery following muscle loss or damage 30–32. Furthermore, limited evidence indicates that the combination of resistance exercise in humans 2,17,33 or exercise-like stimulation in cells and animals 18,25 could further promote the hypertrophic effects of heat stress. While some evidence regarding these effects in humans 33–36 and animals 37 are mixed, methodological differences including heating magnitude, type (environmental, direct, hot water immersion, microwave), duration (acute, chronic), the inclusion and type of resistance exercise (high load, low load), as well as timing of heating (pre, post, concurrent) may account for some of the observed differences 38. Regardless, heat induced responses in skeletal muscle including the expression, translation, and mediation via heat shock proteins (HSPs) may play a role in eliciting skeletal muscle adaptations 38. In fact, specific HSPs appear to interact with pertinent hypertrophy and atrophy related signaling factors 21,28,39–46 and may be implicit in the muscular responses discussed here 12,18,20–23,25–27,29,47–49.

HSPs are ubiquitously expressed across mammalian tissues, including skeletal muscle 50, in response to various stressors including heat stress 51 and exercise 52. Beyond their role in thermotolerance 51,53, these stress inducible chaperones are implicated for their effects on cellular immunity 54, protein homeostasis 55, and the regulation of skeletal muscle mass across the lifespan in healthy and clinical populations 56–58. Of interest, specific HSPs of the HSP70 (HSPA) and HSP90 (HSPC) families 40,41,59; HSP72 (HSPA1A), HSP70 (HSPA1B), HSP90-alpha (HSPC1), HSP90-alpha A2 (HSPC2), and HSP90-beta (HSPC3); can interact with key hypertrophy and atrophy related signaling factors with an emphasis on the Akt-mTOR and Nuclear Factor-κB and Forkhead BOX O pathways 21,43–45,58. Reviewed elsewhere 38, these mechanisms and others may help explain evidence that heat stress alone or combined with resistance exercise can promote factors related to as well as enhance muscular hypertrophy, or reduce atrophy 12,18,20–23,25–27,29,47–49. Nonetheless, these mechanisms have yet to be robustly proven in skeletal muscle and in human investigations.
Moreover, there is no consensus regarding the ability of heat stress alone or combined with resistance exercise to meaningfully promote muscular hypertrophy or reduce atrophy in comparison to resistance exercise alone.

The primary purpose of this investigation was to compare the individual effects of acute whole-body heat stress and resistance exercise on thermoregulatory responses, as well as skeletal muscle heat shock and hypertrophy related signaling factors in humans. We hypothesized that resistance exercise would increase hypertrophic related signaling to a greater degree than heat stress. Conversely, we proposed that heat stress would induce greater thermoregulatory and heat shock responses than resistance exercise. Our secondary purpose was to examine the effects of acute heat stress on the growth and development of cultured mouse myotubes in comparison to hypertrophy and atrophy inducing conditions. Our hypotheses included that heat stress would induce myotube growth and development compared to controls but to a lesser degree than hypertrophy conditions. Additionally, we proposed that atrophy conditions would reduce growth and development compared to controls, heat stress, and hypertrophy conditions.

Methods

Participants

All study protocols were approved by the University of New Mexico (UNM) Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Participants were recruited by word of mouth from the University of New Mexico and surrounding Albuquerque area. All study activities were completed in the UNM Exercise Physiology Laboratories including the dedicated gym space, environmental heat chamber, and private rooms. Participation in this study was voluntary with all participants providing verbal and written informed consent. Following consent, volunteers completed health history and physical activity questionnaires to determine health and fitness status. All participants were deemed healthy, did not participate in regular heat training, and reported participation in at least 150 min of moderate intensity physical activity plus two or more days of structured resistance exercise per week and lived in the Albuquerque area for a minimum of 6 months.
Following consent and health screening, participants visited the laboratory to complete baseline testing followed by two experimental trials including resistance exercise (RE) and whole-body passive heat exposure (HS). Experimental trials were completed in a randomized and counterbalanced fashion with at least 14, but not more than 21 days of washout between conditions.

**RE & HS Protocols**

During baseline testing, participant demographics (age, sex, height, weight, body fat %), estimated 1 repetition maximum (RM) for seated leg press and knee flexion, as well as 10 RM for seated knee extension and standing calf raise [Table 1] were determined. Sex-specific three-site skinfolds and density equations for males (chest, abdomen, thigh) and females (triceps, iliac crest, thigh) were used to estimate body fat percentage via the Siri equation. RMs were determined following a 5-min warm-up on a cycle ergometer at a self-selected pace by progressively increasing weight lifted until no more than 5 or 10 repetitions could be completed for leg press and knee flexion, and knee extension and calf raise, respectively, with 3 min of rest between sets. Participants arrived for all experimental trials following an overnight fast having refrained from vigorous exercise and alcohol consumption for at least 24 hours, and caffeine for 4 hours. Prior to the heat conditions, participants were determined to be hydrated via urine specific gravity <1.020. RE was preceded by a cycling warm up, then a set of 10-15 repetitions for leg press and knee flexion at 50% of 1 RM. In total, resistance exercise included five sets of 8-12 repetitions of leg press and knee flexion at 75% of 1 RM followed by three sets of 10 repetitions of knee extension and calf raises as at 100% of 10 RM. Each paired exercises (leg press & knee flexion, knee extension & calf raise) were alternated with 3 min of rest between sets. The number of repetitions, rating of perceived exertion (RPE), and heart rate (HR) were measured at the end of each set, while core temperature (Tc) was measured pre- and post-trial. HS included 60 total min of passive, seated heat exposure in an isolated environmental heat chamber maintained at 55-60 °C and 20-30% relative humidity. To assist in participant comfort, participants were given 5-minute breaks every 20 minutes. During these breaks, participants were allowed 1 min in a thermoneutral environment (~20-22 °C) and the remaining 4 min in the environmental antechamber (~10% cooler than the isolated chamber). Every 10 minutes, RPE, HR, thermal
sensation, $T_c$, as well as Dry (DB) and wet bulb (WB) temperatures were recorded. Participants were allowed water *ad-libitum* throughout all trials. Following each experimental condition, participants rested in a thermoneutral environment between the 30 min and 3-hour post-trial biopsies.

*Muscle Biopsy & Temperature Protocols*

For each experimental trial, muscle tissue samples were collected from the *m. vastus lateralis* muscle pre-, 30 minutes post-, and 3 hours post-trial. The limb was sanitized with sterile alcohol swabs, antiseptic (iodine), followed by superficial and deep injection of approximately 3-5 cc of local anesthesia (2% lidocaine). Following verification of superficial numbness, the superficial fascia of the muscle was pierced with a 14-gauge hollow needle. A 14-gauge biopsy needle (Argon Medical Devices, Frisco, TX) was inserted via the pilot incision twice to obtain approximately 10-20 mg of total tissue. Incision sites were cleaned and wrapped in sterile bandaging. Tissue samples were cleaned of debris, washed in cold phosphate buffered saline (PBS), flash frozen in liquid nitrogen, and stored at -80°C for Western blot analysis. Muscle temperature ($T_m$) was measured pre- and immediately post trial for each experimental condition. $T_m$ was performed in the same site as the baseline biopsy for all trials. During the pre-trial muscle biopsy, a sterile implantable temperature probe (IT-18, Physitemp Instruments, Clifton, NJ) was inserted past the superficial fascia (~2-3 cm) through the hollow pilot needle prior to collecting tissues. $T_m$ was recorded from a calibrated device (BAT-12, Physitemp Instruments) with the pilot needle removed. Post-trial $T_m$ was measured within 5 min of trial cessation using the same methods excluding tissue collection.

*Western Blot Protocols*

Muscle tissue was lysed with approximately 300 ul of ice-cold lysis buffer (General Cell Lysis Buffer, Millipore Sigma, St. Louis, MO) containing protease and phosphatase inhibitors plus EDTA (Halt, Thermo Fisher Scientific, Waltham, MA) for 60-120 sec in beadbug tubes and a microtube homogenizer (Millipore Sigma). Protein content was quantified using a protein assay kit (Pierce BCA, Thermo Fisher Scientific). Following the addition of 4X Laemmli buffer (Bio-Rad Laboratories, Hercules, CA) with 5% β-
mercaptoethanol, samples were incubated at 95°C for 10 minutes. Proteins were separated by electrophoresis on 4-20% polyacrylamide gels and 1x tris/glycine/SDS running buffer (Bio-Rad Laboratories), then transferred using 1x tris/glycine transfer buffer with 10% methanol (Bio-Rad Laboratories) to 0.45 µm PVDF membranes (Thermo Fisher Scientific) via Transblot Turbo Transfer using the SD protocol (Bio-Rad Laboratories). Membranes were blocked for 90 min in 5% bovine serum albumin (BSA) or dry milk in Tris buffered saline plus 0.05% Tween 20 buffer solution (TBST), cut according to molecular weight, washed with TBST, and incubated overnight at 4°C with primary antibodies in BSA-TBST; Phospho-Akt (Ser473), Akt, Phospho-mTOR (Ser2448), mTOR, Phospho-S6 Kinase 1 (Thr389), S6 Kinase 1 (949D7), Phospho-4E-BP1 (Thr37/46), 4E-BP1 (53H11) (all antibodies purchased from Cell Signaling Technology, Danvers, MA); diluted 1:1000 per manufacturer recommendations. Membranes were washed with TBST and incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology) diluted 1:1000 or 1:2000 for 1 hour at room temperature. Membranes were incubated in luminol reagent (100 mM Tris-HCL pH 8.8, 1.25 mM Luminol-DMSO, 2 mM 4IBPA-DMSO, 5.3 mM H₂O₂) for 3 minutes and imaged with a ChemiDoc Touch Imaging System (Bio-Rad Laboratories). Image Lab software was used to quantify protein expression (Bio-Rad Laboratories). Following initial phospho-protein probing, membranes were stripped in mild stripping buffer (200 mM glycine, 3.5 mM SDS, 1% tween-20, pH 2.2) twice for 5 min on a rocker followed by washing in PBS, TBST, then 90 min bocking in milk-TBST. Membranes were then incubated for corresponding total Akt-mTOR proteins as described above. All proteins were standardized to total protein staining (Ponceau S Stain, Cell Signaling Technology) and expressed as ratio of phospho to total protein where applicable, as densiometric units with the pre-trial timepoint for each condition serving as baseline.

**in-vitro Experiments**

C2C12 myoblasts (American Type Culture Collection, passages 3-7) were used for all in-vitro protocols, initially seeded in 150 mm culture plates (~5x10⁶) until approximately 50-70% confluency then passed 1:6 into 35mm, 6-well culture plates (Corning) in Dulbecco’s
Modified Eagle’s Medium (DMEM) media (Sigma Aldrich) with 4.5 g·mL\(^{-1}\) glucose, L-glutamine, sodium pyruvate, 1% penicillin-streptomycin, and 10% fetal bovine serum (Sigma Aldrich). Myoblasts were cultured to approximately 90% confluence under standard conditions (37 °C and 5% CO\(_2\)) with media changes occurring every 24-36 hours (5-7 days). Myotubes were differentiated in DMEM containing 2% horse serum (Sigma Aldrich) in place of FBS and changed every 24 hours (4-5 days) until myotubes were fully developed.

**Cell Treatment Protocols**

Experimental conditions included untreated controls (CON), heat stress (HEAT), hypertrophy (HYPER), and atrophy (RAPA) conditions. CON replicates (n=9) were maintained in standard conditions throughout. HEAT (n=8) included 60 min at 40 °C and 5% CO\(_2\) followed by return to standard conditions in differentiation medium. HYPER (n=9) included exposure to grow medium under standard conditions for 48 hours. RAPA (n=9) included exposure to rapamycin (Thermo Fisher Scientific) treated differentiation medium (164 nM, 0.25% DMSO) for 48 hours. A single media change occurred for all conditions at 24 hours.

**MHC Staining & Analysis Protocols**

48 hours following experimental treatments, myotubes were aspirated of media, rinsed in PBS, and fixed in 4% paraformaldehyde (PFA) on a plate rocker for 5 min. Myotubes were then permeabilized with 0.3% Triton X-100 in PBS for 5 min, followed by another 5 min of fixation in PFA. Myotubes were rinsed twice in PBS with 0.01% tween-20 then blocked in 3% BSA in PBS on a plate rocker for 60 min. Myotubes were incubated in 3% BSA containing primary MHC antibodies (MF20, DSHB) at 1.5 ug/ml on a plate rocker overnight. Following three rinses in PBS, myotubes were incubated in PBS with secondary antibody diluted 1:500 (Alexaflour 488, Thermo Fisher Scientific) on a plate rocker for 60 min. Myotubes were washed three times with PBS, incubated for 5 min in 5 µM DAPI, then rinsed three times. Three images were taken per well for each replicate with randomly selected locality using an inverted microscope in a 20x20 field (Olympus CKX53, Life Science Solutions, San Diego, CA). Images were analyzed with using MyoCount software.
via MATLAB Runtime (v9.4, R2018a, MathWorks, Natick, MA) for myotube area (% of area occupied by functional myotubes), total number of nuclei, and nuclear fusion index (FI, number of nuclei within functional myotube/total nuclei). Functional myotubes were identified as those containing at least 3 nuclei. Images were averaged as a single measure for each condition replicate.

Statistical Analyses

Participant recruitment numbers were based on a priori effect size (0.8) power analysis in G*Power from an investigation of whole-body heat stress on $T_m$, $T_c$, and protein responses. As 6 participants were required to reach estimated power, we set a recruitment goal of 8. The distribution of all data was examined visually, with a Q-Q plots to assess statistical assumptions and normality. Perceptual data including thermal sensation and RPE, as well as environmental data including DB and WB were analyzed with repeated measures T-tests. Due to technical consideration or participant discomfort one post-trial $T_m$ measure as well as several individual HR data points were not recorded. Data were examined for normality, skewness, kurtosis, and analyzed using repeated measures ANOVAs with Geisser-Greenhouse epsilon corrections, as well as Tukey’s, Bonferroni, or Holm-Sidak multiple comparisons where relevant. If data points were missing, linear mixed effects models were utilized as they are robust to missing data. Our western blot data did not conform to tests of normality, and therefore were log transformed (Base 10), checked for approximate normality, and analyzed using repeated measures ANOVAs. For all conditions, protein expression data were analyzed using the individual peak value determined at either the 30 min or 3 hour time point to account for individual variability in signaling responses. Magnitude of change for main effects were examined with using Eta squared ($\eta^2$) for ANOVAs where relevant. Repeated measures correlations were additionally performed to determine statistically significant relationships between peak and delta $T_c$, $T_m$, and peak fold changes in the expression of all measured proteins. Statistical significance for all analyses was set at $p \leq 0.05$ and were performed in R (v 4.2.0, R Foundation for Statistical Computing, Vienna, Austria) via RStudio (v 2022.02.), GraphPad Prism, (v 9.3.1, GraphPad Software, La Jolla, California), or Excel (v16.0, Microsoft Corp., Redmond, WA).
Results

Physiological & Thermoregulatory Responses

$T_c$ increased from trial baseline for both conditions (F(1,28)=50.48, p<0.01, $\eta^2=0.60$) but $\Delta T_c$ was greater following HS compared to RE (F(1,28)=9.78, p<0.01, $\eta^2=0.25$) with average values of $\Delta 1.30 \pm 0.63$ and $0.53 \pm 0.35$, respectively (p<0.01). $T_m$ was also increased from baseline (F(1,34.91)=32.68, p<0.01, $\eta^2=0.58$) but was not different between conditions with average values of $\Delta 1.79 \pm 1.40$ and $2.49 \pm 1.59 \, ^\circC$ for RE and HS, respectively (p=0.56). HR was increased from baseline across all averaged exercise sets during RE (F(1.75,12.07)=68.36, p<0.01) and at all timepoints during HS (F(2.40,16.06)=21.87, p<0.01). Additionally, average HR was greater during RE compared to HS with values of $135.4 \pm 19.9$ and $99.7 \pm 12.80 \, \text{bpm}$, respectively (p<0.01) (Figure 1). Additionally, average RPE was additionally greater during RE ($14.8 \pm 1.0$) than HS ($10.4 \pm 2.5$) (p<0.01)

Protein Expression

HSPA1A/B protein expression was increased with time (F(1,28)=9.75, p<0.01, $\eta^2=0.32$) and was significantly different from baseline following HS (p=0.04) but not RE (p=0.35) with average peak change from fold values of $\Delta 0.27 \pm 0.11$ and $0.16 \pm 0.08$, respectively. HSPC1-3 was not changed with time (F(1,28)=0.51, p=0.48, $\eta^2=0.01$) with average change from fold values of $\Delta 0.03 \pm 0.14$ and $-0.17 \pm 0.11$ for RE and HS, respectively. Akt protein expression (n=7) increased with time (F(1,24)=7.14, p=0.01, $\eta^2=0.22$) but neither RE (p=0.15) or HS (p=0.39) were different from baseline with average peak change from fold values of $\Delta 0.31 \pm 0.21$ and $0.42 \pm 0.17$, respectively. mTOR protein expression increased with time (F(1,28)=20.20, p<0.01, $\eta^2=0.41$) and was significantly different from baseline following RE (p=0.01) and HS (p=0.03) with average peak change from fold values of $\Delta 0.28 \pm 0.17$ and $0.49 \pm 0.16$, respectively. S6K1 protein expression increased with time (F(1,28)=9.22, p<0.01, $\eta^2=0.24$) and was significantly different from baseline following HS (p=0.05) but not RE (p=0.40) with average change from fold values of $\Delta 0.61 \pm 0.21$ and $0.50 \pm 0.13$, respectively. 4E-BP1 protein expression (n=5) did not change with time (F(1,16)=4.02, p=0.34, $\eta^2=0.20$) with average change from fold values of $\Delta -0.15$
± 0.20 and -0.45 ± 0.22 for RE and HS, respectively (Figure 2). A representative image including phosphorylated and total protein expression, at the 30 min and 3-hour time points, as well as total protein staining for one subject is presented (Figure 3).

*in-vitro Results*

Myotube area was influenced by condition (F(3,31)=26.36, p<0.01, R²=0.71) and all treatments were different than controls. RAPA was lower than CON (p=0.02) with average area of 35.3 ± 3.7 and 39.6 ± 3.8 %, respectively. HS was greater than CON (p=0.03) and RAPA (p<0.01) with average area of 43.0 ± 2.43 %. HYPER was greater than CON, RAPA, and HS (p<0.01) with average area of 48.7 ± 2.8%. Fusion index was also influenced by condition (F(3,31)=11.57, p<0.01, R²=0.52). RAPA was not different than CON (p=0.44) with average values of 0.37 ± 0.04 and 0.39 ± 0.03, respectively. HS was greater than RAPA (p<0.01) and CON (p=0.02) and with average values of 0.45 ± 0.02. HYPER was greater than RAPA (p<0.01) and CON (p<0.01) with an average value of 0.46 ± 0.03. Fusion index was not different between HS and HYPER (p=0.65). Total number of nuclei was not different between any condition (F(3,31)=1.90, p=0.14, R²=15) (Figure 4).

*Correlation Analysis*

Repeated measures correlations demonstrated statistically significant interactions for thermoregulatory and protein expression responses. Both Δ $T_c$ and $T_m$ displayed strong positive associations (r=0.98 and r=0.88) with their respective peak values (p<0.01). Peak $T_m$ additionally demonstrated strong positive associations with peak (r=0.90, p<0.01) and Δ $T_c$ (r=0.88, p<0.01). Δ $T_m$ further demonstrated non-significant moderate positive associations with peak (r=0.62, p=0.09) and Δ $T_c$ (r=0.63, p=0.09). Akt protein expression demonstrated strong positive associations with S6K1 (r=0.80, p<0.01) and 4E-BP1 (r=0.91, p=0.01) expression. Finally, S6K1 protein expression demonstrated a strong positive association to 4E-BP1 (r=0.99, p<0.01). HSPA1A/B protein expression also demonstrated moderate positive association to Δ $T_c$ (r=0.59) though it was not statistically significant (p=0.09). Finally, Akt protein expression displayed non-significant moderate association to Δ $T_m$ (r=-0.66, p=0.07) (Figure 5).
Discussion

The primary purpose of this investigation was to compare the individual effects of acute whole-body heat stress and resistance exercise on thermoregulatory responses, as well as skeletal muscle signaling factors related to heat shock and muscular hypertrophy in humans. Contrary to our hypothesis, our results show that a single session of resistance exercise (RE) and passive whole-body heat stress (HS) similarly increased muscle, but not core temperature. In line with our hypothesis, HS but not RE increased heat shock protein expression in skeletal muscle. Moreover, and contrary to our hypothesis, HS resulted in greater activation of the Akt-mTOR cascade in comparison to RE. Finally, in partial agreement with our hypotheses, results from our in-vitro experiment demonstrate that heat stress (HEAT) enhanced myotube development to a lesser degree than hypertrophy (HYPER) but greater degree than control (CON) and atrophy (RAPA) conditions. These findings provide novel comparative evidence regarding the acute thermoregulatory, as well as the skeletal muscle heat shock and hypertrophy related signaling responses to whole-body heat stress alone in comparison to resistance exercise in humans. Additionally, they provide supplemental evidence regarding the functional effects of heat stress in isolated mouse skeletal muscle cells.

Thermoregulatory Responses

Body temperature responses to heat stress and aerobic exercise have been relatively well studied in comparison to resistance exercise. Both passive whole-body heat exposure and prolonged endurance exercise can raise core temperatures (T_c) above 39 °C, depending on the magnitude of thermal stress and exercise environment, respectively. Conversely, previous evidence suggests resistance exercise has minor effects on core temperature, stimulating increases of less than a half a degree. Our findings agree, showing that RE increased T_c by approximately 0.5 °C, which was significantly lower than the responses to HS (~1.3 °C). Albeit understudied in humans, previous findings have demonstrated that acute resistance exercise results in minor (~1 °C) changes to muscle temperature. Contrary to this evidence, our data demonstrate increases of approximately 2.4 °C after RE, which was not different from the effects of HS. This may in part be due to
differences in exercise protocols considering our use of higher volume and intensity whole-body exercise compared to low volume or isolated exercise sessions \textsuperscript{72,73}. Regardless, the fact that RE increased muscle temperature similarly to HS is surprising and raises questions regarding the potential role of muscular temperature for hypertrophic adaptations to exercise. The necessity of increases in muscle temperature for growth has not been explicitly demonstrated, however, cold stress has been shown to impair hypertrophic adaptations in skeletal muscle \textsuperscript{74–76}. In fact, cultured human myotubes exposed to 48 hours of cold stress (32 °C) demonstrate impaired morphologies, as well as nutrient mediated mTOR pathway signaling, and protein synthesis \textsuperscript{74}. Moreover, repeated post-resistance exercise cooling reduces anabolic signaling, strength gains, hypertrophy, and heat shock responses in humans \textsuperscript{75,76}. Even though greater evidence is necessary to determine the role increases in muscle temperature could play in hypertrophic signaling and adaptations, this could represent a connection between heat stress, HSP expression, and hypertrophy related signaling in skeletal muscle \textsuperscript{38}.

\textit{Heat Shock Responses}

Our results have shown that the skeletal muscle expression of HSPA1A/B is increased following HS but not RE in humans. Additionally, HSPC1-3 expression was unchanged by either RE or HS. It would stand to reason that whole body and tissue specific temperature responses are important for the local induction and expression of various HSPs \textsuperscript{51}, particularly in skeletal muscle \textsuperscript{52}, yet evidence in this regard is not unanimous \textsuperscript{77,78}. Previous data has shown that whole-body heating but not single-leg hot water immersion increases the skeletal muscle gene expression of HSPA1A/B and HSPC1-3 \textsuperscript{12}. Further, whole-body heating increased both core and muscular temperatures while single limb heating only increased muscle temperature. As HS in our experiment similarly resulted in greater $T_c$ responses than RE despite nondifferent increases in $T_m$, it’s possible that whole-body thermoregulatory strain is necessary stimulus for the local induction of HSPA1A/B. Conversely, others have shown increasing core (~1.5 °C) and muscle (~3.6 °C) temperatures does not influence the expression of HSPA1A/B in human skeletal muscle 48 hours post-heating \textsuperscript{77}. Yet as we demonstrate peak increases for HSPA1A/B expression within 3 hours of heat stress, acute signaling may be of greater relevance for this HSP. Conversely, others
have demonstrated that 30 min of hot water immersion ranging from 37 to 41 °C does not immediately increase the skeletal muscle expression of HSPA1A/B or HSPC1-3 in mice. Irrespective of these differences, the effects of HS alone observed here agree with evidence suggesting that heat can increase HSPA1A/B gene and protein expression in human skeletal muscle. Of note, others have shown acute whole-body heat stress can comparably increase the gene expression of HSPC1-3. Nonetheless, HSP gene responses are not absolutely congruent to post-transcriptional responses and in our sampling timeframe, HSPC1-3 protein expression is not responsive to our RE or HS protocols.

In contrast to our findings, previous research has demonstrated resistance exercise alone can increase HSPA1A/B protein expression in humans. Important to consider, these limited previous studies have utilized damage inducing training plus downhill running or chronic resistance training protocols in humans, making direct comparisons to our study complicated. Regardless, our design allows for direct comparison between interventions and presents novel findings indicating acute resistance exercise does not acutely stimulate HSPA1A/B protein expression to the same degree as whole-body heat stress. To our knowledge, no study to date has examined the effects of resistance exercise compared to heat stress on muscular heat shock expression in humans. Conversely, cell and animal models have demonstrated similar responses when comparing exercise or exercise like stimuli and heat stress. In fact, in cultured rat myotubes undergoing cyclic stretching (96 hours) or heat stress (60 min, 41 °C) HSPA1A/B and HSPC1-3 expression was increased to a similar degree. Another investigation induced soleus overload in rats and showed overload alone increased HSPA1A/B expression in skeletal muscle within three days, while acute heat stress (15 min, Tc maintained at 42°C) resulted in a comparatively greater HSPA1A/B protein expression within one day. Although comparison between cell, animal, and human models are quantitatively imperfect, these experiments provide indications that are contrary to ours. While transcriptional and translational HSP responses may be discordant, more research is required to determine if resistance exercise increases heat shock activity in human skeletal muscle and under what exercise intensity or volumes, and time frames it might occur.

*Hypertrophy-Related Signaling*
Our protein expression results show that both acute RE and HS increased the phosphorylation status of mTOR, while only HS increased S6K1 phosphorylation in skeletal muscle. Through activation of growth pathways including the Akt-mTOR cascade, resistance exercise and growth stimulation such as nutritional intake increase myofibrillar protein synthesis ultimately resulting in greater muscle cross sectional area \(^2,9\). The necessity of this cascade has been questioned \(^84\), yet multiple investigations indicate its importance for maintaining muscular function and stimulating hypertrophic outcomes \(^85–88\). Therefore, despite lacking the demonstration of muscular growth, our results suggests that pro-hypertrophic signaling responses were greater following HS compared to RE as indicated by increased phosphorylation status of the Akt-mTOR cascade.

Resistance exercise has been well established as a hypertrophy inducing stimulus \(^89\), and exercise protocols similar to ours acutely increase protein synthesis \(^90\) and skeletal muscle mass over time \(^91\). In this regard, our RE protocol served as a comparative control which is known to induce muscle hypertrophy with progressive application. Curiously, we hypothesized a greater effect of RE alone on Akt-mTOR mediated signaling but only observed significant activation of mTOR. While unexpected, individuals with prior strength training history, like those in our study, can demonstrate blunted hypertrophic responses to resistance exercise. As example, the acute phosphorylation status of Akt, mTOR inhibiting tuberous sclerosis complex 2 (TSC2), and S6K1 are unchanged in trained individuals contrary to their activation in untrained but physically active individuals \(^92\). Importantly, we also acknowledge that muscle hypertrophy is not solely dependent on Akt-mTOR associated signaling. Growing perspectives propose divergent hypertrophic pathways including myostatin and transforming growth factor (TGF) - activin receptor related pathways \(^93–95\). Furthermore, 4E-BP1 has been demonstrated unessential for inducing skeletal muscle hypertrophy in response to mechanical overload in mice \(^96\). Nevertheless, inhibition or knockout of the primary factors within the Akt-mTOR cascade are detrimental to muscular development and growth \(^85,94,97\). Moreover, mTOR explicitly, appears to be a key mediator of muscular growth which can be activated through Akt independent mechanisms \(^98\) resulting in the gene expression of eukaryotic initiation factor 2B (eIF2B) \(^99\). This is particularly notable as muscle protein synthesis rates following resistance exercise in rats display greater
dependance on eIF2B activity than 4E-BP1\(^{99,100}\). Thus, lacking increased activation of Akt or 4E-BP1 following RE or HS, we show that both protocols stimulated phosphorylation of the upstream control point, mTOR, to a similar degree. Further, HS resulted in downstream phosphorylation of S6K1 where RE did not, cautiously suggesting an acutely enhanced hypertrophic stimulus in this group of humans.

Others have previously presented hypertrophic\(^{2,17,18,20,24}\) and related signaling\(^{12,13,15,16,19–23}\) effects following various forms of heat stress, with some mixed results\(^{33–37}\). Our findings correspondingly agree with those suggesting a hypertrophic signaling effect following heat stress. As example, a comparable heat stress model utilizing 60 continuous min of whole-body heat stress (45-50 °C, 50% relative humidity) found increased phosphorylation status compared to baseline for similar acute markers including Akt, mTOR, S6K1, and eukaryotic initiation factor 4E (eIF4E)\(^{12}\). This is additionally matched by various cell and animal investigations which cumulatively indicate heat stress increases the activity the Akt-mTOR cascade\(^{14,16,22,47}\). Despite the increased signaling response of HS compared to RE, we don’t know the chronic effects of heating, and to date no study has demonstrated a gross hypertrophic effect of heat stress alone in humans\(^{36,49}\). In fact, two investigations including the repeated (over 6-12 weeks) use of direct heating pads (8 hours, 3-5 times per week) found no significant changes in muscle cross sectional area\(^{36,49}\). Importantly, differences in experimental design including heating methodology and timing, explored elsewhere\(^{38}\), should be noted. As the only known study contrasting resistance exercise and whole-body heat stress alone across the same participants, this adds novel information suggesting an acute hypertrophic signaling effects of heat stress which was greater than a bout of resistance exercise. Notwithstanding, more research is necessary to determine if these signaling effects result in meaningful outcomes and if heat stress alone can progressively elicit gross muscle hypertrophy in humans. Conversely, evidence from cell and animal models indicates that heat stress alone is capable of promoting muscular growth\(^{18,20,22,23,28,32,47}\).

**in-vitro Experiments**
Like previous reports showing myotube treatment with grow medium (10% FBS) enhances MHC protein expression and myotube area \textsuperscript{65}, our in-vitro results demonstrate that HYPER enhances average myotube area as well as the number of nuclei contained within differentiated myotubes. Furthermore, we show that a single bout of HEAT increases myotube growth and fusion compared to controls, albeit with comparably lower myotube area but similar FI increases as HYPER. Moreover, as the number of total nuclei did not change across any condition, it suggests increased an myonuclear domain for HYPER and HEAT, while RAPA present a reduction.

Multiple animal and cell models have demonstrated hypertrophic effects of heat stress under various conditions \textsuperscript{18,20,22,23,28,32,47}. Yet, few have included isolated hypertrophy or growth control conditions \textsuperscript{18,37}. From these limited experiments, it appears that mechanical stimulation in cells enhances protein accumulation to a similar degree as heat stress \textsuperscript{18}, but does not increase rat muscle mass without limb overload \textsuperscript{37}. Our results partially agree with both findings, suggesting acute heat stress can enhance cellular hypertrophy within 48 hours, albeit to a lesser degree than grow factor mediated growth stimuli. Similarly, others have shown that heat stress increases myonuclear qualities in cells and animals \textsuperscript{20,47,101}. In fact, 60 min of heat (42 °C) increases the percentage of formed multinuclear myotubes by 7 days compared to controls \textsuperscript{101}. Interestingly, HSPA1A/B overexpression has been indicated as a promotor of C2C12 fusion and myotube diameter \textsuperscript{102}. Notably, HSPA1A/B treatments did not alter myoblast proliferation, instead resulting in increased myonuclear fusion and diameter for a similar amount of cells compared to controls \textsuperscript{102}.

Similar to our rapamycin treated cells, cellular atrophy models have similarly demonstrated loss in myotube area without changes in total nuclei, indicating reduction in myonuclear domain \textsuperscript{103}. While classically a focus for muscle development, atrophy, or regrowth models, evidence indicates a role of myonuclear development in post-natal muscle hypertrophy \textsuperscript{104–107}. The exact nature of myonuclear domain expansion to hypertrophy is still a topic of debate \textsuperscript{108}, yet growing evidence suggests nuclear development occurs alongside \textsuperscript{104–107} and is possibly necessary for muscular growth \textsuperscript{109}. Research has even demonstrated that individuals with greater hypertrophic responses to prolonged training presented profound satellite cell and myonuclear responses compared to lower responders \textsuperscript{104}. In line with
potential myonuclear responses, a single 60 min heat session (42 °C) is capable of increasing satellite cells alongside skeletal muscle mass in rats. Importantly, we have not measured satellite cell responses, yet our results do demonstrate that HEAT stimulates a robust change in myotube fusion index comparable to HYPER and in contrast to the reductions induced by RAPA. While speculative, these findings indicate the potential for both myotube growth and myonuclear expansion, which may be relevant for elucidating the hypertrophic effects of heat stress in-vitro.

Heat Shock Interactions

Previous research has indicated potential points of interaction between various HSPs, including HSPA1A/B and HSPC1-3, and Akt-mTOR-mediated hypertrophic signaling. HSPA1A/B appears to interact with mTOR complex 2 and is mechanistically relevant for subsequent Akt activation upon heat stimulation. Additionally, HSPC1-3 can interact with mTOR complex 1 preventing phosphatase mediated apoptosis, while HSP inhibition impairs the phosphorylation of S6K1 and 4E-BP1. Moreover, evidence demonstrates that HSP reduction in mice, via upstream heat shock transcription factor 1 knockout, impairs the hypertrophic effects of heat stress. Though not measured here, other intracellular mechanisms including atrophy-related signaling should be considered with regards to resistance exercise and heat stress. Despite these possible but relatively unstudied mechanisms, our results show that changes in the skeletal muscle expression of HSPC1-3 were not necessary for the induction of hypertrophy related signaling following RE and HS. Conversely, HS increased the expression of HSPA1A/B and promoted greater hypertrophic signaling than RE. These findings are undoubtedly interesting, yet we have not measured direct interactions between HSPs and Akt-mTOR proteins. Subsequently, our data only suggest that increased HSPA1A/B expression as well as activation of the Akt-mTOR cascade occurred with HS compared to RE. Greater evidence, including interaction-based analyses are needed to directly examine these effects in humans.

Regression Analysis Outcomes

Regression analysis in this experiment indicated significant relationships between core and muscle temperature responses, as well as between hypertrophy related but not HSP
protein expression. Accordingly, Δ and peak Tc and Tm demonstrated strong positive associations for their respective values. Strong positive associations were similarly found for Δ and peak Tc to peak Tm while Δ Tm was moderately but non-significantly associated to Δ and peak Tc. In total, these results simply suggest that greater changes in core or muscle temperature are associated with higher peak temperatures for the same measure, and vice versa. Furthermore, greater Δ and peak Tc responses are associated with greater peak Tm responses. Admittedly not surprising, these findings help characterize whole-body thermoregulatory responses, and particularly the connection between Tc and Tm during resistance exercise and heat stress. Interestingly, while we found no significant associations between thermoregulatory responses and HSPs, HSPA1A/B was moderately but non-significantly associated to Δ Tc. In line with our previous discussion, local HSPA1A/B responses in skeletal muscle may not be entirely temperature dependent. However, as heat stress in other experiments including humans, cells, and animals increases HSP gene and protein expression12,18,20,22,23,28,32,37,47,48, it is unlikely that core and muscular temperature responses are entirely irrelevant. Nevertheless, the regulatory role of local and systemic temperatures for the expression of HSPs, particularly in skeletal muscle, remains unclear. We further found no associations between thermoregulatory responses and hypertrophy related factors, though note a non-significant moderately negative association between Δ Tm and Akt protein expression. Cautiously, it’s possible that excessive local heat accumulation results in down-regulation of hypertrophy related responses. Yet, we find this speculation unlikely as models comparing heating methodologies have demonstrated greater hypertrophy related effects when local and system temperature responses are greater12.

Limitations

A primary limitation of this study includes the use of a physically active and resistance trained population who performed acute RE and HS in the fasted state. This sample may present higher basal HSP content as well as impaired stress responses to exercise compared to untrained individuals112. Yet, as we demonstrate increases in HSPA1A/B expression following HS, it is expected these responses would be further pronounced in untrained individuals. Resistance trained individuals also express different magnitudes of hypertrophic responses than untrained persons91. Even so, trained individuals present
increased myofibrillar protein synthesis rates in response to fasted resistance exercise\textsuperscript{113}. Despite impaired net protein balance in a post-absorptive state\textsuperscript{114}, the underlying mTOR related signaling responses are activated\textsuperscript{115}. A primary limitation to our \textit{in-vitro} designs includes the lack of investigation for heat shock and hypertrophy related protein expression. Nevertheless, others have shown similar acute heat stress increases the expression of HSPA1A/B alongside changes in protein content or myotube development \textit{in-vitro}\textsuperscript{18,101}. Finally, the results from our cellular investigation cannot be directly applied to our findings in humans. Though related in design and tissue domain, their independent results should be viewed with caution until empirically demonstrated across models.

**Conclusions**

The results from this experiment demonstrate that the acute whole-body heat stress increases core but not skeletal muscle temperature to a greater degree than resistance exercise as well as the skeletal muscle expression of HSPA1A/B. Furthermore, heat stress resulted in greater local hypertrophy related protein expression than resistance exercise in humans. Although the mechanistic role of HSPs remains ambiguous, heat stress appears to present a novel stimulus which increases hypertrophy related signaling in humans. Our cellular model additionally demonstrates that acute heat stress increases myotube development but to a lesser degree than growth factor mediated conditions. Interestingly, both heat stress and growth conditions similarly increased myonuclear fusion. While direct comparison between our human and cellular experiments is complicated, together these findings further propose hypertrophic signaling responses following acute heat stress have the potential to stimulate gross hypertrophic outcomes. Nonetheless, greater investigation is needed to determine if these acute effects of heat stress can similarly induce chronic muscular hypertrophy in humans.
Table 1. Participant Descriptive Data

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Body Fat (%)</th>
<th>1 RM Leg Press (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>27.0 ± 4.6</td>
<td>170.7 ± 10.2</td>
<td>68.0 ± 8.9</td>
<td>9.76 ± 5.0</td>
<td>257.6 ± 45.8</td>
</tr>
<tr>
<td>Females</td>
<td>29.0 ± 7.0</td>
<td>169.4 ± 13.5</td>
<td>66.0 ± 12.0</td>
<td>16.28 ± 4.2</td>
<td>196.0 ± 35.9</td>
</tr>
</tbody>
</table>

Participant descriptive data for physically active (≥ 150 min moderate activity per week), resistance trained (≥ 2 days per week ≥ 6 months) males (n=5) and females (n=3). All data presented as group mean ± standard deviation.

Figure 1. Thermoregulatory Responses to Resistance Exercise (RE) and Heat Stress (HS). A = pre and post trial Tc values for RE and HS. B = pre and post trial Tm values for RE and HS. A & B = each plotted shape represents the same participant throughout conditions. Group average pre values represented by white bars and group average post values gray bars with standard deviation error bars. C = RE, pre and post for RE and HS. Each plotted point represents the group average for that temperature with standard deviation error bars. HS includes averaged data at 0.5 min intervals, RE includes averaged data across repeated exercise sets for leg press & knee extension and knee extension & calf raise. RE represented by white data and HS by black. Annotate: * over fall bar indicate significant effect of time and difference from baseline, asterisk over brackets indicate significant difference between average values for indicated condition. Tc = rectal core temperature, Tm = lateral muscle temperature, HP = heart rate, bpm = beats per minute.
Figure 2. Protein Expression Following Resistance Exercise (RE) and Heat Stress (HS). Each plotted shape represents the same participant throughout conditions. Bars represent average fold change for respective conditions with standard error of the mean error bars. All proteins presented as phosphorylated to total, taken from peak value occurring at either 30 min and 3 hours post-trial compared to baseline (pre), corrected to total protein (ponceau staining), and log transformed (base 10). Asterisk (*) over full bar indicates significant effect of time and change from baseline for both conditions. Asterisk over half bar indicates significant effect of time and change from baseline only for indicated condition. HSP = heat shock protein, Akt = protein kinase B, mTOR = mechanistic target of rapamycin, S6K1 = ribosomal S6 kinase beta 1, 4E-BP1 = eukaryotic elongation initiation factor 4E-binding protein 1.

Figure 3. Representative Protein Expression Image. Phosphorylated and total protein expression for all protein targets at baseline (pre), 30 min post, and 3 hours post-trial for RE and HS for one subject. Two Iblots were required to obtain all targets, ponceau total protein stain for each presented on right of image. P- indicated phosphorylated protein form. HSP = heat shock protein, Akt = protein kinase B, mTOR = mechanistic target of rapamycin, S6K1 = ribosomal S6 kinase beta 1, 4E-BP1 = eukaryotic elongation initiation factor 4E-binding protein 1, RE = resistance exercise, HS = whole-body heat stress.
Figure 4. *in vitro* Experimental Outcomes. A = myocyte area following control (CON), rapamycin (RAPA), heat stress (HEAT), and hypertrophy (HYPER) conditions. B = fusion index (FI) following CON, RAPA, HEAT, and HYPER conditions. Individual points represent averaged values for individual replicates and white bars group averages with error bars as standard deviation. C = representative images for CON, RAPA, HEAT, and HYPER conditions. DAPI = nuclear staining in blue, MHC = myosin heavy chain staining in green, Merge = merged DAPI and MHC staining. Asterisk (*) over half bar indicates significant difference compared to CON for indicated conditions, asterisk significant difference from RAPA, point (•) significant difference from HEAT, and obelisk (†) significant difference from HYPER.

Figure 5. Thermoregulatory, Heat Shock, and Hypertrophy Related Protein Correlations. Correlation (r) and significance (p) values for repeated measures correlations considering thermoregulatory responses, HSP protein, and Akt-mTOR protein expression. Positive r-values and blue coloration indicate positive relationships while negative r-values and red coloration indicate negative relationships. * indicates a significant relationship. Δ. Delta = change from baseline, Peak = max value recorded, Tc = rectal core temperature, Tm = recorded muscle temperature, HSP = heat shock protein, Akt = protein kinase B, mTOR = mechanistic target of rapamycin, S6K1 = ribosomal S6 kinase beta 1, 4E-BP1 = eukaryotic elongation initiation factor 4E-binding protein 1.
References


84. Ham, A. S. *et al.* mTORC1 signalling is not essential for the maintenance of muscle mass and function in adult sedentary mice. *Journal of Cachexia, Sarcopenia and Muscle* **11**, 259–273 (2020).


CHAPTER IV: SUMMARY & PERSPECTIVES

Across this dissertation, we have collectively examined the role of heat stress in the control of skeletal muscle mass. In our research review, we first presented evidence suggesting that various forms of heat stress can promote muscular hypertrophy, prevent atrophy, or the recovery from disuse and damage. Furthermore, we provided a series of mechanistic perspectives by which the stress and heat inducible HSPs could potentially confer these muscular effects. With consideration to local and systemic thermoregulatory responses, we suggested that certain forms of heat stress including whole-body methodologies show promise for promoting hypertrophic responses, with an emphasis on Akt-mTOR related signaling in human skeletal muscle. Importantly, we acknowledged major methodological limitations to current understandings, chiefly lack of contrast between heat stress alone and known hypertrophic stimuli such as resistance exercise.

In our research manuscript, we therefore set out to examine the acute effects of whole-body heat stress on thermoregulatory, heat shock, and hypertrophy related responses in comparison to resistance exercise in a trained population. With this design, we aimed to provide evidence regarding the magnitude of hypertrophic effects and their relationship to thermoregulatory and heat shock responses following acute whole-body heat stress. Moreover, using an in-vitro model we additionally examined the effects of acute heat stress on developed myotube development compared to hypertrophic conditions. In this way, we sought to provide further indication regarding the functional effects of heat stress at the cellular level in comparison to known hypertrophic conditions. The results of our human investigation indicated that acute whole-body heat stress activated greater hypertrophy related Akt-mTOR signaling responses in skeletal muscle compared to resistance exercise in a trained population. Heat stress additionally stimulated greater systemic but not thermoregulatory responses alongside increased HSP expression in skeletal muscle compared to resistance exercise. Despite this, no significant associations were found regarding thermoregulatory, HSP, and Akt-mTOR responses for either condition. Our in-vitro experiments further demonstrated that heat stress increased the growth of myotubes, albeit to
a lesser extent than grow mediated hypertrophy conditions. Conversely, heat stress increased myotube nuclear fusion to a similar degree as growth conditions. These changes further contrast to rapamycin mediated atrophy conditions which reduced myotube area but not myonuclear fusion, suggesting differing mechanisms of myotube development comparing atrophy to hypertrophy and heat conditions.

Cumulatively, our findings indicate that whole-body heat stress can acutely increase systemic thermoregulatory responses as well as heat shock and hypertrophy related signaling in skeletal muscle to a greater extent than resistance exercise in trained individuals. While acute signaling responses are necessarily indicative of gross hypertrophic outcomes, we suggest that novel heat stress can stimulate Akt-mTOR signaling in trained individuals which may experience blunted activation in responses to resistance exercise. Additionally, HSP expression increased only following heat stress, indicating a potential role of heat mediated factors in enhancing these hypertrophy related responses. Yet, evidence of this in humans remains equivocal, and our lack of significant association between variables indicates continued mechanistic research is necessary. We have also shown that a single session of heat stress can enhance the development of myotubes *in-vitro*, but to a lesser degree than grow mediated hypertrophy. Despite the imperfect comparisons, this provides indication that the acute heat induced effects of our human experiments have the potential to induce gross hypertrophic outcomes like our *in-vitro* experiments. Importantly, we make this extrapolation cautiously, but suggest it justifies continued investigation. Accordingly, greater investigation of whole-body heat stress including extended examination of hypertrophic outcomes or chronic applications are warranted.
APPENDIX

A. Screening & Consent Forms.

   1. Health and Physical Activity Questionnaire.
   2. Quiz to Assess Comprehension.
   3. Informed Consent.

B. Data Collection Forms.

   1. Data collection packet.
   2. 24-Hour Food Log.

C. Miscellaneous Forms.

   1. Biopsy Care Sheet.
A. Screening & Consent Forms.

1. Health and Physical Activity Questionnaire.

HEALTH & PHYSICAL ACTIVITY QUESTIONNAIRE

Family history questions are included because certain conditions of your first degree relatives can incur risk to you during maximal exercise.

Participant # (researcher will fill in) ___________ Date _____/_____/_____

Age ____ yrs Sex _____

Emergency contact (first name, phone #)
____________________________________________________

INITIAL SCREENING

Do you engage in at least 150 minutes of exercise per week? ___Yes ___No
Do you engage in at least 2 session of resistance training per week? ___Yes ___No
Do you have a history of heat stroke or heat-related illness? ___Yes ___No
Are you pregnant or planning on getting pregnant? ___Yes ___No ___N/A

HEALTH HISTORY

Physical injuries:
____________________________________________________

Current Limitations
____________________________________________________
Have you ever had any of the following? Please check all that apply.

- High blood pressure
- High triglycerides
- Asthma
- High cholesterol
- Diabetes (specify type)
- Liver disease
- Emphysema
- Kidney disease
- Stroke
- Heat illness

Describe any checked conditions:

Have you ever had any of the following cardiovascular problems? Please check all that apply.

- Heart attack
- Heart surgery
- Valve problems
- Chest pain or pressure
- Swollen ankles
- Dizziness
- Arrhythmias/Palpitations
- Heart murmur
- Shortness of breath
- Congestive heart failure
- Pacemaker

Do immediate blood relatives have any of the conditions listed above? Yes No
If yes, list the problem, and family member age at diagnosis
___________________________________________________________________________
___________________________________________________________________________

Do you currently have any other medical condition not listed? Yes No
If yes please explain
___________________________________________________________________________
___________________________________________________________________________

Are you taking any medications, vitamins or dietary supplements now? Yes No
If yes, list the medication or supplement
___________________________________________________________________________
___________________________________________________________________________
Are you allergic to latex?  ____Yes  ____No

Have you ever experienced any adverse effects during or after exercise (fainting, vomiting, shock, palpitations, hyperventilation)?  ____Yes  ____No

If yes, please elaborate

LIFESTYLE FACTORS

Do you now or have you ever used tobacco including e-cigs, vape?  ____Yes  ____No

If yes:  type ____________  How long did you use these products? _______ years

Quantity ____/day  Months/Years (circle one) since quitting________

EXERCISE HISTORY

Endurance exercise

Days per week (circle one):  0  1-2  3-5  6-7

Minutes per exercise session (circle one):  <30  30-60  60-240  240-360  >360

Describe your usual exercise modalities:

Resistance exercise

Times per week (circle one):  0  1-2  3-5  6-7

Minutes per day (circle one):  30-60  60-240  240-360  >360

Describe your usual exercise modality:
2. Quiz to Assess Comprehension.

The effects of whole-body heat stress on skeletal muscle responses
Assessment of understanding
Circle True or False for each question

1) This research requires me to do two bouts of resistance exercise
   T   F

2) Participation in the study will take a total of 2 hours
   T   F

3) My blood will be drawn multiple times
   T   F

4) I will be exposed to high altitude twice during the study
   T   F

5) Muscle biopsies will be done on my upper arm
   T   F

6) Muscle biopsies will be done 9 times over the study
   T   F

7) I can withdraw from the study anytime without consequences
   T   F

8) I will sit in a very hot room and use a rectal thermometer
   T   F

9) I can participate even if I don’t lift weights regularly
   T   F

10) I am required to wear a face mask during the exercise
    T   F
3. Informed Consent.

The effects of whole-body heat stress on skeletal muscle responses

Consent to Participate in Research
Version date: 11/30/21

Purpose of the study: You are being asked to voluntarily participate in a research study that is being done by Christine Mermier, Ph.D., who is the Principal Investigator, and Zac Fennel from the Department of Health, Exercise & Sport Sciences and their associates. The purpose of this study is to investigate the effect of passive whole body heat stress 131-140°F (55-60°C) alone or following a resistance exercise bout.

You are being asked to participate in this study because you are a healthy individual (no heart or lung disease or disorders such as diabetes, kidney disease and other diagnosed medical conditions, and no current bone or joint injury) between the ages of 18 – 45 years old. We are seeking participants who are engaged in regular exercise (>150 minutes of moderate to vigorous intensity aerobic activity and at least 2 resistance training sessions per week for a minimum of 1 year). You must also not have a history of heat stroke or heat related illness. Lastly, if you know or think you may be pregnant, you will not be able to participate.

Key information for you to consider:

- Participation in the project will take a total of 15 hours over 4 visits across 5-6 weeks.
  - Diagram provided below.
- The study requires a total of 2 bouts of exercise, 2 passive heat exposures, 6 muscle temperature readings, 9 skeletal muscle micro-biopsies, 1 visits of 1 hour, and 3 visits of 5 hours each.
  - Muscle biopsies may cause muscle discomfort and soreness.
- The exercise includes 60 minutes of moderate to vigorous intensity resistance training on two separate occasions.
- The passive heat exposure includes 75 minutes of resting in a room at 131-140°F (55-60°C) on two separate occasions.
- Exercise may cause muscle soreness, fatigue, nausea, or dizziness.
- Heat exposure may cause fatigue, discomfort, and/or dizziness.
Up to 12 people will be recruited for this study at the University of New Mexico. This form will explain what to expect when joining the research, as well as the possible risks and benefits of participation. If you have any questions at any time during or after reviewing this form, please ask one of the study researchers.

What you will do in the study:

- After reading the consent form and discussing the details with the research team, you will be sent the informed consent to review, a health history and physical activity questionnaire as well as a COVID-19 symptoms screening checklist via email. If after you read the consent and discuss the requirements and risks with your friends and family you want to participate, you will send back the completed health history and physical activity questionnaire. In addition, if you qualify based on the questionnaire you will be sent a COVID-19 symptom checklist via email prior to your visit. During your first visit you will sign the consent form if you decide to participate in the study and before any study activities take place.
- Before every visit, a research team member will call you and inquire whether you have any COVID symptoms according to the COVID-19 symptoms checklist. You will also be asked if you have been exposed to anyone with suspected or known COVID-19. You will be approved to come to the laboratory if you have no signs and symptoms of COVID-19 and if you have not been exposed to anyone who has COVID-19 symptoms or has tested positive for the virus. You must wear a mask every time you visit our facilities for the study. Prior to entering the lab, your body temperature will be measured by a no-touch forehead thermometer. If your temperature is over 37.5 °C (99.5 °F) you will not be allowed into the lab and the visit will be rescheduled.
- You will be required to visit the Exercise Physiology Laboratory for an initial visit, then a total of three trials. Each trial will include 5 hours of your time.
- We will request that you wear exercise attire during each visit.
- Across each trial and follow up visit this study requires you to allow us to collect 6 muscle temperature measurements, and 9 skeletal muscle micro-biopsies.
  - Muscle temperature and biopsy procedures are described in detail below.
- Upon arrival for the first visit, we will measure your resting blood pressure after 5 min of sitting quietly. This will involve you sitting upright while a blood pressure cuff is wrapped around your upper arm. A stethoscope will be placed against your arm and the cuff which will be inflated and then deflated while systolic (upper number) and diastolic (lower number) blood pressure is measured. If your resting blood pressure is over 140 (systolic) or 90 (diastolic), you will not be able to participate in the study. Women will be asked to take a urine pregnancy test. If the test shows that you could be pregnant, you will not be able to participate in the study and you will be referred to your health care provider for follow-up.
- You will also have your body composition (body fat) estimated, using the skinfold caliper technique. This will require a slight pinching of the skin at three sites of the body (chest, stomach, and thigh for men and upper arm, hip and thigh for women), done two-three times at each site.

COVID-safe practices:

- As long as the New Mexico State of Public Health Emergency declared in Executive Order 2020-004 remains in effect, this study will use procedures intended to reduce the risk of transmitting COVID-19. When we schedule your visit and again when you arrive at Johnson Center, we will ask you questions about COVID-19 symptoms. We will also check your temperature using a touchless thermometer. If you have a temperature above 99.5°F or have any COVID-19 symptoms we will reschedule your visit for another day.
By participating in this study, you are saying that as far as you know that you do not have any current symptoms of COVID-19, including: fever or chills; mild or moderate difficulty breathing; new or worsening cough; sustained loss of smell, taste, or appetite; sore throat; vomiting or diarrhea; aching throughout your body. If you have any of these, you must let us know, and we will delay this study until your symptoms are gone and you feel better. We will test your temperature using a remote thermometer. In addition, if you develop any of these symptoms within two weeks of participating in this study, you should contact us to let us know. You will be contacted if a research team member you interacted with develops COVID-19.

Any friends or family members who come with you to a visit must wait outside of the building. Use of masks is required; if you do not have a mask or if your mask is not sufficient, one will be provided for you. A social distancing limit of 6’ will be followed whenever possible except when necessary for data collection, for example when performing the skinfold measurement, blood draws, muscle temperature readings, and muscle biopsies. We will use additional personal protective equipment like face shields or Plexiglas barriers when we need to remain within 6’ of you. Please contact Dr. Mermier immediately (cmermier@unm.edu/ (505) 715-0455) if you test positive for COVID-19 or develop symptoms of COVID-19 within 14 days of your visit to Johnson Center.

Strength Testing
You will have refrained from alcohol for 24 hours, all exercise for 24 hours, and caffeine for 4 hours.

During the first visit, you will perform a set of resistance training exercises to determine your 3-5 repetition maximums (RM) for two exercises, seated leg press and knee flexion and your 10 RM for two exercises, seated knee extension and calf raise. Prior to RM testing you will perform a 5-min warm-up on a cycle ergometer at a self-selected pace. Afterward you will perform increasingly heavier repetitions until you can complete no more than 3-5 or 10 repetitions for each exercise. Between sets you will be given adequate time to recover (1.5-3 min). Your 3-5 RM will be used to calculate your 1 RM for use in subsequent resistance training sessions.

At the end of visit 1 you will be given a food log and asked to track your diet in the 24 hours leading up to visit 2. You will be asked to mimic the recorded diet for all following visits (3-4).

Trials: Visits 2-4

You will be assigned to one of the following conditions first:
  - Resistance exercise.
  - Passive heat exposure.
  - Resistance exercise + passive heat exposure.

You will complete all conditions in a randomized order, meaning the order will be determined by picking a number from a hat. These trials will take 5 hours each. All three conditions will be separated by at least 7 days. You will be asked to arrive to the laboratory fasted overnight, having refraining from exercising for 24 hours, drinking alcohol for 24 hours and caffeine for 4 hours. Also, we ask that you mimic your diet recorded 24 hours prior to the first visit.

The exercise bout will consist of moderate to high intensity resistance exercise for approximately 60 minutes. You will complete alternating sets of seated leg press and knee flexion at 70% of determined 1 RM for five sets of 8-12 repetitions followed by alternating sets of seated knee extension and calf raises at 100% of determined 10RM each. All bouts of exercise will be preceded with a warm-up.

The heat exposure bout will consist of passive, seated resting in UNM’s environmental heat chamber for 75 minutes. The heat chamber will be maintained at 131-140 F° (55-60 C°).

You will wear a heart rate monitor around your chest during all exercise and heat exposure bouts.
• To continuously monitor your core body temperature to ensure you do not overheat, you will insert a rectal thermometer during all heat exposure bouts.
• Skeletal muscle tissue samples will be collected before, 30 minutes, and 3 hours after each experimental condition.
• Muscle temperature readings will be collected before, and immediately following resistance exercise (resistance exercise trial) and passive heat exposure (passive heat exposure and resistance exercise plus passive heat trials).

Resistance Training Protocol:

• You will complete two separate resistance training sessions within the UNM Exercise Physiology Laboratory.
  o One for the resistance exercise + heat and one for the resistance exercise only trials.
• The exercises will include seated leg press and seated leg flexion.
  o You will perform a warm up of increasing intensity at ~30-65% of your previously estimated RM for all exercises.
  o Leg press and leg extension will be completed in an alternating fashion beginning with leg press.
    ▪ You will be performing ~70% of your previously obtained estimated 1 RM for each exercise.
    ▪ The number of sets for each exercise will be five and the number of repetitions will fall between 8 and 15. You will attempt to complete all 15 repetitions for each set. If you cannot complete at least 8 repetitions for two consecutive sets the weight will be reduced by 10%.
  o Knee extension and calf raise will be completed in an alternating fashion beginning with knee extension.
    ▪ You will be performing ~100% of your previously estimated 10RM for each exercise.
    ▪ The number of sets for each exercise will be 10. You will attempt to complete 10 repetitions for each set. If you cannot complete at least 8 repetitions for a single set the weight will be reduced by 10%.
  o Between each alternating exercise you will be given 3 min to recover.
• Your perception of difficulty (RPE: rating of perceived exertion) will be obtained at the end of each exercise set for both exercises.
• You will be allowed to consume water ad-libitum (as needed) during the resistance training.

Passive Heat Exposure:

• You will complete two separate 60 min passive heat exposures in an environmental chamber within the UNM Exercise Physiology Laboratory.
  o One for the resistance exercise + heat and one for the passive heat only exposure trials.
• To accumulate a total of 60 min of heating, you will complete three 20 min bouts in a chamber held at 131-140°F (55-60°C) interspaced with 5 min of resting in an ante-chamber that is approximately 20% cooler 104-113°F (40-45 °C).
• You will be monitored by a technician throughout the heat exposure and you will be required to have a rectal thermometer inserted to monitor your core temperature.
  o You will be instructed by a technician how to insert a rectal thermometer ~4 inches (10 cm) past the anal sphincter to obtain an accurate reading. You will be given gloves and lubricant.
If your core temperature reaches 103.1°F (39.5°C) you will be moved from the heat chamber to an external anti-chamber until core temperature begins to drop. If core temperature does not drop within 5 min you will be removed to a temperate room 71°F (22°C), given cool water and wet towels around your neck and head and you will sit in front of a fan. You will be continuously monitored to ensure a return to a normal temperature.

If you are feeling dizzy or nauseous at any time, you will be removed from the heat chamber and core temperature will be monitored until you reach normal body temperature.

- Your perception of heat (thermal sensation) will be obtained at the end of every 10 min of heat exposure.
- You will be allowed to consume water *ad-libitum* (as needed) during the heat exposure.
- During the heat exposure you will be asked to rest passively in a seated position.
  - You may complete tasks on a computer or phone during this time.

**Skeletal Muscle Micro-Biopsies & Muscle Temperature Readings:**

- Skeletal muscle tissue samples will be collected before, 30 minutes, and 3 hours after each experimental condition for a total of 9 biopsies for each participant across this study.
  - Each of the biopsies for a given trial (pre, 30 minutes after, 3 hours after) will be performed at slightly different location than the previous but in the same general area on the same leg, just above or below the previous measure (within a few millimeters).
- Muscle temperature readings will be collected before, and immediately following resistance exercise (resistance exercise trial) and passive heat exposure (passive heat exposure and resistance exercise plus passive heat trials).
  - All muscle temperature readings for a given trial will be measured from the pre-trial biopsy incision site.
- **Key Points & Overview:**
  - Biopsy samples will be taken from the *vastus lateralis* (side of your upper leg) of your dominant leg by a trained laboratory technician using sterile processes.
  - During the entire biopsy process you will lie relaxed on an examination table.
  - The skin site will be cleaned, and prepared in a sterile manner.
  - The site (both shallow and deep) will be numbed with a numbing agent (lidocaine).
    - You may experience slight pinching and tingling sensation as the lidocaine is applied.
  - Once we have confirmed the target site is completely numb a small pilot hole will be made with a sterile 16-gauge needle.
    - You should not be able to feel any anything except some pressure from this point onward. If you inform your technician of sensation or pain we will re-apply additional lidocaine to ensure your comfort.
  - The pre-test muscle temperature readings will be completed during the pre- biopsy procedure just after the 16-gauge pilot needle insertion.
    - A sterile flexible 23-gauge (about ½ mm) temperature probe will be inserted along the pilot path to measure muscle temperature. You will not feel this procedure.
    - The reading will take a few seconds and afterwards the biopsy will continue as described.
  - A sterile biopsy needle (14-gauge) will be inserted into the site to obtain samples.
• We require two insertions of the biopsy needle into the same location, one right after the other, to collect adequate muscle tissue.
• In total, 25-50 mg (size of several sesame seeds) of muscle tissue will be collected during each biopsy timepoint.
  o The biopsy site will then be cleaned with sterile materials and securely covered with bandaging that provides pressure.
  o The post resistance exercise and passive heating muscle temperature measurements will be performed at the same site as the first reading.
    ▪ Following the same sterile preparation procedures as described for the muscle biopsy, your leg will be cleaned and numbed before acquiring a measurement.
    ▪ Following numbing, a sterile 24-gauge pilot needle/catheter (smaller than the pilot) will be inserted into the pre-biopsy pilot hole path and a sterile temperature probe will be inserted along the pilot needle/catheter to measure muscle temperature.
  o The measurement site will be cleaned and secured as described above.
  o You will be given written instructions for the proper care of the biopsy and muscle temperature site.

*You may feel mild to moderate pain when the lidocaine wears off. This pain will quickly go away and you will likely be able to perform normal daily activities or exercise without problem. However, it is common for participants to feel a sense of tightness and potentially may feel a sensation of a deep bruise or “Charlie Horse” at the biopsy site. This sensation should get better within 2 days.

*There may be a possibility of a small scar forming at the biopsy site.

*There have been no major complications reported as a result of taking small tissue samples from skeletal muscle.

*Muscle temperature measurement is less invasive than muscle biopsy.

Your consent is voluntary and may be withdrawn at any time. At the time of withdrawal, your data will be deleted. Your personal data collected for this research will be deleted or anonymized by the end of your participation in the study. Projected future use of your blood and muscle samples includes measuring proteins related to exercise.

**Risks:**

There is risk of COVID-19 exposure due to your participation in this study as the visits involve face-to-face interaction with research personnel. In order to minimize the risk, several strategies will be implemented. You and research personnel must follow social distancing requirements (>6 ft.) except for when it is necessary to collect data (e.g. to draw blood and to place equipment on you). Research personnel will be screened for symptoms or exposure to COVID-19 positive individuals before they will be allowed to work with you. The lab and environmental chamber areas will be cleaned and disinfected regularly and between participants. Hand sanitizer will be available in every room. All research personnel have been trained on any new procedures adopted to prevent exposure to COVID-19. There will be no more than 2 research team members working with you at a time. They will wear face masks at all time when working with you. You are also required to wear a mask at all times. When it is necessary for a research team member to touch your skin, such as during skinfolds measurements, research personnel will wear disposable gloves. For the muscle biopsy, research
personnel will wear disposable gloves (sterile gloves for biopsy), a mask, eye protection and lab coat. If you or a research team member reports exposure to, develops symptoms possibly associated with, or tests positive for COVID-19 within 14 days of a visit, the study will be paused and you will start a self-quarantine for at least 14 days. You will not be allowed to continue participating in this study unless you show no symptoms and test negative after quarantine.

There are risks associated with maximal exercise testing including the following: muscle soreness, fatigue, nausea, or dizziness during or after completion of exercise. The incidence of risk of fatal and nonfatal events during maximal exercise testing are very low, approximately <0.8 per 10,000 tests or 1 per 10,000 hours of testing. We will minimize these risks by checking your medical history questionnaire for any medical conditions or history that could increase your risk, and by using trained personnel to conduct your testing.

The risks associated with a muscle biopsy include momentary discomfort or moderate pain during the time the needle is inserted. To minimize the occurrence of discomfort and pain, an effective numbing agent (Lidocaine) will be used to numb the area to sampled. You will likely experience a brief and small pinching sensation while the numbing agent (Lidocaine) is injected. A minimal amount of muscle tissue (25-50 mg) will be extracted from your leg for each biopsy. You may feel a brief sensation of pressure in the leg and potentially some moderate pain during the tissue sampling. This pain will quickly get better, and you will likely be able to perform exercise and normal daily activities without problem. There is a risk that you may feel a sense of dizziness or feeling faint. Your leg may feel tight and you may feel a sensation of a deep bruise or “Charlie Horse” afterwards; however, this tightness in the muscle typically gets better within 2 days and you may begin exercising immediately, and begin exercising at normal capacity within 2 days. There is also a risk of the possible appearance of a scar, bruising or soreness, and infection. To limit the potential risks, only trained technicians using sterilized instruments and supplies will perform the biopsy procedure. Additionally, the sampling site will be sterilized prior to and after the procedure.

The risks associated with a muscle temperature measurement include momentary discomfort or slight pain during local anesthetic application which will be done for both the muscle biopsies and temperature measurement at the same time. To minimize the occurrence of discomfort and pain, the numbing agent (Lidocaine) will be applied superficially before being used to numb deeper tissues. You will likely experience a brief and small pinching sensation while the numbing agent (Lidocaine) is injected superficially. There is a chance you may feel light pressure as the temperature probe touches the muscle but this is unlikely and the risk for sensation is far less than that of tissue extraction.

Exposure to high temperature increases physiologic strain and can present the risk for heat related illnesses. Prolonged heat stress can reduce the ability of the body to cool and control body temperature resulting in heat exhaustion or heat stroke, especially during physical activity. Symptoms of heat exhaustion can include headache, dizziness, and nausea, as well as body weakness and elevated blood pressure. If body temperature increases to temperatures near 104°F (40 °C) and remains elevated, there is a chance for heat stroke. Symptoms of heat stroke can include hot, dry skin, excessive sweating or the cessation of sweating, mental confusion, slurred speech, loss of consciousness, seizures, and potentially death. To limit these potential risks your core temperature, perceived thermal strain, and responsiveness will be closely monitored by trained physiologists with experience conducting research in the heat. You will be sitting quietly during the heat exposure. You will be rapidly removed from the heat and cooled down if your body temperature reaches 103°F (39.5°C) or if you request to exit. You will furthermore have access to water at all points during heat exposure.

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There are risks of stress, emotional distress, inconvenience and possible loss of privacy and confidentiality associated with participating in a research study.

While we have protocols in place to minimize your exposure as much as possible, participation in this study may increase your risk of contracting COVID-19 or some other illness. Due to the COVID-19 pandemic, we are required by UNM to keep a contact log that includes all individuals involved in all interactions that have occurred in our lab. This log will use your subject ID, and will be destroyed within one month after the end of this study, or longer if required.

Benefits: You will receive no direct benefit from your participation in the study. We will provide you with the results of your body composition assessment and strength tests. Knowledge of body composition is of benefit in that it is indicative of health status and risk which can be helpful in directing an individual exercise program. Additionally, it is hoped that the information gained from this study will help us understand the effects of heat stress combined with resistance exercise on skeletal muscle responses.

Confidentiality of your information: Privacy will be maintained by conducting screening and testing in private rooms in the Johnson Center Exercise Physiology lab with no access to anyone but the study team. You will be given a random number to identify the samples and data collected for confidentiality There will be no link to personal identifiers (name, date of birth, etc.) on any study material, including the COVID-19 screening sheets. Only approved research team members will have access to your information through a password protected computer, with hard copies stored in a locked file cabinet. We will take measures to protect the security of all your personal information, but we cannot guarantee confidentiality of your data. The University of New Mexico Institutional Review Board (IRB) that oversees human subject research may be permitted to access your records. Your name will not be used in any published reports about this study.

What will happen if I am injured or become sick because I took part in this study?

If you are injured or become sick as a result of this study, UNM Health Science Center will provide you with emergency treatment, at your cost. No commitment is made by the University of New Mexico Health Sciences Center (UNMHSC) to provide free medical care or money for injuries to participants in this study. In the event that you have an injury or illness that is caused by your participation in this study, reimbursement for all related costs of care will be sought from your insurer, managed care plan, or other benefits program. If you do not have insurance, you may be responsible for these costs. You will also be responsible for any associated co-payments or deductibles required by your insurance. It is important for you to tell the investigator immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues or believe that you have been treated carelessly in the study, please contact the Office of the Institutional Review Board (OIRB) at the (505) 277-2644 for more information.

What will happen if the study has to stop unexpectedly?

If the study has to stop on short notice in the event that the university stops all on-campus operations and research again due to public health concerns related to COVID-19, research personnel and participants who have not yet started or have not completed all the 4 visits will be immediately notified to stop coming to the lab until further notice.
Payment: You will be paid up to $100 for your participation in this study. ($25 for visit one and $25 for each subsequent trial visit: visits 2, 3 & 4). You will be reimbursed with cash.

Right to withdraw from the study: Your participation in this study is completely voluntary. You have the right to choose not to participate or to withdraw your participation at any point in this study without penalty. In addition, the research team will stop your participation in the study if you are not willing to wear a mask when required or do not follow other COVID-safe practices.

If you have any questions, concerns, or complaints about the research study, or contract COVID-19 (including showing symptoms or testing positive) within 14 days of a visit to the lab please contact the principal investigator: Christine Mermier, Ph.D., Department of Health, Exercise & Sport Sciences, 1 University of New Mexico, Albuquerque, NM, 87131. She may be reached Monday-Friday 8:00 a.m. – 5:00 p.m. at (505) 277-2658, or anytime via email at cmermier@unm.edu.

If you would like to speak with someone other than the research team or have questions regarding your rights as a research participant, please contact the IRB. The IRB is a group of people from UNM and the community who provide independent oversight of safety and ethical issues related to research involving people:

UNM Office of the IRB, (505) 277-2644, irbmaincampus@unm.edu. Website: http://irb.unm.edu/

OPTIONAL: The UNM Exercise Physiology Laboratory personnel conduct studies that may include tests and procedures that are involved in this study. Data from this study may be useable in other studies. Your data including all measured variables (BF%, 1RM, HR, RPE, thermal sensation, core temperature, muscle temperature, muscle samples, etc.) will be de-identified (your name, date of birth or any other identifiable information will not be associated with any data). Muscle samples may be kept for up to 5 years and all other physiologic data will be kept indefinitely. If you are willing to allow the results from this study to be used in other future studies, please initial below. By initialing, you are not required to perform any additional tasks, tests, or paperwork. The researchers will only use your previously collected data.

I agree to allow my data to be used in other studies

__________________________
Initials
3. CONSENT

You are making a decision whether to participate in this study. Your signature below indicates that you have read this form (or the form was read to you) and that all questions have been answered to your satisfaction. By signing this consent form, you are not waiving any of your legal rights as a research participant. A copy of this consent form will be provided to you.

I agree to participate in this study.

______________________  __________________________  Date______
Name of Adult Participant   Signature of Adult Participant

Researcher Signature (to be completed at time of informed consent)

I have explained the research to the participant and answered all of his/her questions. I believe that he/she understands the information described in this consent form and freely consents to participate.

______________________  __________________________  Date______
Name of Adult Participant   Signature of Adult Participant
B. Data Collection Forms.

1. Data Collection Packet.

Data Collection Packet

**RM Pre-trial Checklist:**

Date: _____
Technician(s): _____
Consent/HH/PARQ Forms Printed/Signed ____ (check)
COVID-19 participant form ____ (check)
Technician COVID-19 forms ____ (check)
No exercise for 24 hrs. Y N
Resting BP (systolic/diastolic) /
No caffeine for 4 hrs.? Y N
No alcohol for 24 hrs.? Y N
Pregnancy test negative? Y N N/A

**RM Trial**

Height: ____ (check)
Weight: ____ (check)
Skinfold (M/W):

- Chest/Triceps: ____  ____  ____  Avg: ____
- Abdomen/Hip: ____  ____  ____  Avg: ____
- Thigh: ____  ____  ____  Avg: ____

General 5-min Warm Up ____ (check)
Leg Press 3-5 RM: ____  Estimated 1-RM: ____  85% 1-RM: ____
Knee Flexion 3-5 RM: ____  Estimated 1-RM: ____  85% 1-RM: ____
Knee Extension 10 RM: ____
Calf Raise 10 RM: ____
Participant Given Food Log ____ (check)

**Passive-HS Pre-Trial Checklist:**
Date: ____
Technician(s): ____
COVID-19 participant form ____ (check)
Technician COVID-19 forms ____ (check)
No exercise for 24 hrs. Y N
Resting BP (systolic/diastolic) /
No caffeine for 4 hrs.? Y N
No alcohol for 24 hrs.? Y N
Pregnancy test negative? Y N N/A

**Post-Trial Checklist**
Post-HS Muscle Temp ____ (check)
Participant Rest 30 (min) ____ (check)
30 (min) Post-Biopsy ____ (check)
Participant Rest 150 (min) ____ (check)
3 (hour) Post-Biopsy ____ (check)
Participant Given Biopsy Care Sheet ____ (check)

**Passive-HS Trial**
Pre-Trial Biopsy & Muscle Temp ____ (check)
Thermistor ____ (check)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>WB (°C)</th>
<th>DB (°C)</th>
<th>Core Temp (°C)</th>
<th>Thermal (0-8)</th>
<th>RPE (6-20)</th>
</tr>
</thead>
</table>
### RE Pre-Trial Checklist:

Date: ____

Technician(s): ____

COVID-19 participant form ____ (check)

Technician COVID-19 forms ____ (check)

No exercise for 24 hrs.  Y  N

Resting BP (systolic/diastolic)  /

No caffeine for 4 hrs.?  Y  N

No alcohol for 24 hrs.?  Y  N

Pregnancy test negative?  Y  N  N/A

### Post-Trial Checklist

Post-HS Muscle Temp ____ (check)

Participant Rest 30 (min) ____ (check)
30 (min) Post-Biopsy ____ (check)
Participant Rest 150 (min) ____ (check)
3 (hour) Post-Biopsy ____ (check)
Participant Given Biopsy Care Sheet ____ (check)

**RE Trial**

Pre-Trial Biopsy & Muscle Temp ____ (check)
General 5-min Warm Up ____ (check)
RE Warm Up ____ (check)

Leg Press 85% 1-RM: __ Knee Flexion 85% 1-RM: __ Knee Flexion 10 RM: __ Calf Raise 10RM: __

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Set</th>
<th>Repetitions</th>
<th>RPE (6-20)</th>
<th>*Alterations</th>
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<tbody>
<tr>
<td>Leg Press</td>
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<tr>
<td>Calf Raise</td>
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RE + HS Pre-Trial Checklist:

Date: ____
Technician(s): ____
COVID-19 participant form ____ (check)
Technician COVID-19 forms ____ (check)
No exercise for 24 hrs. Y N
Resting BP (systolic/diastolic) /
No caffeine for 4 hrs.? Y N
No alcohol for 24 hrs.? Y N
Pregnancy test negative? Y N N/A

Post-Trial Checklist

Post-HS Muscle Temp ____ (check)
Participant Rest 30 (min) ____ (check)
30 (min) Post-Biopsy ____ (check)
Participant Rest 150 (min) ____ (check)
3 (hour) Post-Biopsy ____ (check)
Participant Given Biopsy Care Sheet ____ (check)

RE Trial

Pre-Trial Biopsy & Muscle Temp ____ (check)
General 5-min Warm Up ____ (check)
RE Warm Up ____ (check)
Leg Press 85% 1-RM: ___ Knee Flexion 85% 1-RM: ___ Knee Flexion 10 RM: ___ Calf Raise 10 RM: ___

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Notes:

**Passive-HS**

Thermistor ____ (check)

<table>
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<tr>
<th>Time (min)</th>
<th>WB (°C)</th>
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<th>Core Temp (°C)</th>
<th>Thermal (0-8)</th>
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</tr>
</thead>
</table>
2. **24-Hour Food Log.**

**24-Hour Food Record**

**Participant ID #: _____________________________________**

**Date: ______________________________**

Please complete the following food record. The more accurate you are in recording, the more accurate your nutritional analysis will be.

**Instructions:**

1. Please write down everything you eat and drink in the 24 hours prior to:
   __________________________________________

2. Try not to change your diet in any way. This day should represent your normal eating pattern.

3. Be as accurate as possible when recording amounts. Try to record your food intake as soon as you eat, otherwise it can be hard to remember at the end of the day. If you have mixed foods or casseroles, write down all the ingredients and amounts (salad: type of lettuce, croutons, vegetables, cheese, meat, dressing). Write down if foods are fresh, frozen, or canned and the brand name. For example, include specifics such as ½ cup 1% milk, 8 oz. fat free Dannon yogurt, 12 oz. calcium fortified orange juice.
   a) Enter only one food item per line
   b) Use measuring spoons for items such as jelly and condiments.
   c) Use measuring cups for items such as vegetables, pasta, rice and cereals.
   d) Use ounces or dimensions for meat, cheese, pizza and desserts.
   e) Use number and size (small, medium and large) for bread rolls, raw fruits, etc.
   f) Use ounces or cups for beverages.

4. Record the date, time and place (home or restaurant) of every meal and snack, as well as the method of preparation (fried, baked, barbecued, grilled, etc.).

5. Feel free to bring in recipes or labels of unusual foods.

6. Please list dietary supplements (vitamins, minerals, etc.).
C. Miscellaneous Forms.
1. Biopsy Care Sheet.

Patient Instructions for Biopsy Site Care

1. Leave your wound dressings in place for the rest of the day of the biopsy and keep them dry.

2. Change band-aids daily starting the day after the biopsy.

3. Showers are fine after the biopsy. Leave the band-aids in place while you shower and change them after you dry off.

4. During the time period of daily band-aid changes, do not soak in a bath or swim.

5. The average time for daily band-aid changes is 5 to 6 days.

6. If you need to use anything to clean the wounds, hydrogen peroxide is recommended. If the wounds are fine (i.e., no signs of infection), all that is required is a daily band-aid change.

7. The wounds may or may not form a scab as they heal; either way is fine.

8. Continue to change the band-aids daily until there are no open wounds.

9. The local anesthetic used for the biopsy will usually last for 1 to 2 hours after the procedure. After it wears off, you may have some mild, localized soreness and tenderness at the biopsy sites over the next day or two.

10. Once you are without the band-aid, the biopsy sites may look slightly red or darker than the rest of your skin. This discoloration will gradually fade and blend back with your normal skin color. This fading process usually takes several months.

11. It is very rare for people to have any problems during the healing period. It is normal for the biopsy sites to bleed a little bit or drain pink fluid for a day or two after the biopsies. They should not bleed excessively (i.e., through the band-aid) after that time. They should never drain pus. If you do experience problems with significant bleeding, redness, infection, or other problems, contact one of the primary investigators, Dr. Christine Mermier, 505-277-2658/ cmermier@unm.edu or Zac Fennel at 360-490-8750/ zfennel@unm.edu