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## The Effect of Hyperthermic Whole Body Heat Stimulus (Sauna) on Heat Shock Protein 70 and Skeletal Muscle Hypertrophy in Young Males during Weight Training

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**The Effect of Hyperthermic Whole Body Heat Stimulus (Sauna) on  
Heat Shock Protein 70 and Skeletal Muscle Hypertrophy in Young  
Males during Weight Training**

By:

**Brandon Creed Jones**

THESIS

Submitted to

Northern Michigan University

In partial fulfillment of the requirements for the

For the degree

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School of Health and Human Performance

May 2017

## SIGNITURE APPROVAL FORM

The Effect of Hyperthermic Whole Body Heat Stimulus (Sauna) on Heat Shock Protein 70 and Skeletal Muscle Hypertrophy in Young Males during Weight Training

The thesis by Brandon Jones is recommended for approval by the student's Thesis Committee and Department Head in the Department of Health and Human Performance and by the Assistant Provost of Graduate Education and Research.

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## ABSTRACT

The Effect of Hyperthermic Whole Body Heat Stimulus (Sauna) on Heat Shock Protein 70 and Skeletal Muscle Hypertrophy in Young Males during Weight Training

By:

Brandon C. Jones

The traditional Finnish steam sauna is theorized to aid skeletal muscle hypertrophy by stimulating Heat Shock Protein 70 (HSP70), which acts as a molecular chaperone to the folding of functional skeletal muscle. The aim of this study was to investigate if stimulating HSP70 by using a sauna (45 – 50°C, 80% Humidity) three times per week, for 15 minutes, could aid skeletal muscle hypertrophy during six weeks of resistance training in a young ( $21.38 \pm 1.9$  yrs.), recreationally trained male population. Thirteen subjects were randomly distributed into 3 groups [resistance Training + sauna (RT+S, n=5), RT + Relaxation (RT+R, n=5), and complete control (CON, n=3) or no training]. Primary dependent variables, observed in a pre- and post-test format, included: lean body mass (LBM), HSP70 concentration, and a 5 repetition maximum (5RM) back squat.

When comparing groups (i.e., RT+S, RT+R, and CON), no significant main effects or interactions were observed ( $p > 0.05$ ) over the 6-week intervention period for LBM, HSP70, and 5RM. The hypothesis that HSP70 would be upregulated to a greater extent with concurrently larger LBM and 5RM improvements in RT+S vs. the other groups was not supported. Interestingly, although HSP70 and LBM were highest in RT+S after 6-weeks of heavy resistance training, RT+R improved the most on 5RM. Sauna use in combination with resistance training does not appear to augment muscle hypertrophy or strength.

Key Words (Sauna, Heat Shock Protein, Skeletal Muscle Hypertrophy, Hyperthermia)

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The thesis follows the Journal of Strength and Conditioning Research submission format along with the School of Health and Human Performance at Northern Michigan University

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## Symbols and Abbreviations

5RM.....	5 Repetition Maximal Weight Back Squat
CON.....	Complete Control Group
DEXA.....	Dual energy x-ray absorptiometry
DOMS.....	Delayed Onset Muscle Soreness
HSPs.....	Heat Shock Proteins
HSP70.....	Heat Shock Protein 70
LBM.....	Lean Body mass
NSCA.....	National Strength and Conditioning Association
PEIF.....	Physical Education Instruction Facility
RT+S Group.....	Resistance Training + Sauna (Experimental Group)
RT+R Group.....	Resistance Training + Relaxation
VAS.....	Visual Analog Scale
Volume Load.....	Weight x sets x reps

## Chapter I

### **Introduction:**

Increasing skeletal muscle mass through muscle hypertrophy is a common goal for untrained athletes who hope to increase strength and improve aesthetic appeal. It is well documented that resistance training (RT) increases muscle hypertrophy by causing small tears in the myofibril near the z-line, followed by the addition of muscle mass during the recovery period (44). While numerous physiological responses have been postulated to aid in this process, a relatively new hypothesis involves the interaction of heat shock proteins (HSPs) and the structural development of skeletal muscle (24). The exact physiological mechanism of this process is unknown but researchers have suggested that HSPs may act as molecular chaperones to aid in the removal of denatured proteins and refold proteins into functional muscle mass (4,10). If HSPs are augmented by sauna use it could hypothetically lead to more protein being chaperoned into protein synthesis and enhanced skeletal muscle hypertrophy.

In rats, the concentration of HSPs has been shown to increase in response to physical activity (5,7,14,15). While few studies have examined the HSP response in humans, all studies point to a similar increase in HSP concentration following aerobic exercise and resistance training (25,26,37). It is unknown how exercise leads to an increase in HSPs but a viable hypothesis is that the observed increase in HSPs is a response to excessive heat produced by skeletal muscle during intense exercise.

In rats and other animal models, heat alone has been demonstrated to increase HSPs along with reducing atrophy of immobilized muscles (36,42,45). This increase in HSPs in response to heat is part of a generalized response to stress and serves as a protective mechanism against cellular damage. Another group of researchers utilizing a rat model demonstrated an

increase in muscle mass from heat stress alone (46). It therefore seems reasonable to postulate that if heat can aid in the recovery process of skeletal muscle through increased concentrations of HSPs, then the application of heat after RT induced damage may also augment muscle hypertrophy.

As a case in point, using an in-vitro model of rat muscle cells, researchers observed that a combination of applying both heat stress and mechanical stress resulted in a greater increase in the concentration of cellular muscle protein than either heat or mechanical stress alone (9). It therefore stands to reason that if the combination of heat stress and mechanical stress has such an effect on muscle cells in-vivo, the increased concentration of HSPs by heating the muscle following resistance training may additionally increase muscle hypertrophy. This suggests the possibility that heat may be used to promote cell proliferation and increase muscle hypertrophy. Strength enhancement related to the enhanced skeletal muscle hypertrophy still needs to be investigated.

As suggested from these animal studies, it is possible that the increased concentration of HSPs through full body hyperthermia may be beneficial to protein concentrations and muscle hypertrophy. However, to the best of the current authors' knowledge, there have been no studies which directly examined the use of full body heat application (sauna) and resistance training (RT) on muscle hypertrophy in humans. Therefore, the primary goal of this study was to examine the effects of hyperthermic whole body heat stimulus (sauna) on HSPs and skeletal muscle hypertrophy following RT in humans. If the findings of this study are similar to those observed in animal models, it could significantly change the methods used to increase muscle mass during resistance training.

## **Methodology:**

### *Experimental Approach to the problem*

The current study was designed as a within group mixed repeated measure analysis examining the relationship between use of a traditional Finnish sauna and changes in lean body mass (LBM) during six-weeks of resistance training in a recreationally trained population. The convenience sample was randomly assigned to three intervention groups, indicated below in the subject section. The independent variable was a 15 min sauna session (45-50° Celsius, 75-80% Humidity) completed three times per week. The dependent variables, related to skeletal muscle hypertrophy, were: a) LBM, b) HSP70 concentrations, and c) 5 Repetition Maximum Back Squat (5RM). Each group was tested on dependent variables before and after a six-week resistance training protocol.

### *Subjects*

This study was approved by an Institutional Review Board (IRB) and all subjects were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed consent document to participate (Appendix I). Thirteen untrained male students from a local university were enrolled (See Table 1 for group assignments). Participant's (n = 13) mean age, height, weight, BMI, and LBM were:  $21.38 \pm 1.9$  ( $\pm$ SD) years,  $182.3 \pm 5.9$  cm, weight:  $82.1 \text{ kg} \pm 11.36$ , BMI:  $24.7 \pm 3.4$ , and LBM:  $62.8 \text{ kg} \pm 5.5$ , respectively. In order to ensure health and safety of participants, all subjects completed a PAR-Q prior to the initiation of the study (Appendix II). All enrolled participants were untrained in resistance training but recreationally active, classified by less than one year of consistent resistance training three times per week for the past year (40). To document the subject's training history, each subject completed a recall survey which included the approximate frequency and load per week of resistance training the subjects had completed three (3) months prior to the start of the study (Appendix III). The

subjects were recruited from health classes, and most were Reserve Officers' Training Corps (ROTC) cadets at the local university. Randomly assigned research groups included: (1) resistance training + sauna (RT+S), (2) resistance training + relaxation (RT+R), and (3) complete control (CON). Notably, thirty subjects were initially recruited and anticipated; however, despite this high initial group number, only a total of thirteen subjects completed the six week intervention, primarily due to a 40% drop out rate. In future studies it may be necessary to provide a stronger incentive to participate, such as monetary reward and greater buy-in from ROTC instructors or other supervisors.

Table 1: Overall study design format with definition of groups and protocols.

Group	Number of Subjects (N)	Protocol
<b>Experimental (RT+S)</b>	5	Sauna + Resistance Exercise
<b>Resistance Training Control (RT+R)</b>	5	Resistance Exercise + *Relaxation
<b>Complete Control (CON)</b>	3	No Intervention

\*Relaxation = Placebo Sauna for 15 min.

### *Procedures*

During the initial meeting all subjects signed an informed consent approved by Northern Michigan University's IRB board that ensured they understood the risks and benefits as well as the purpose and procedures of the study. The initial meeting also ensured the participants met all inclusion criteria, including: age 18-39 yrs, male, and recreationally trained with  $\leq 1$  year of previous RT experience three times per week or more. A health questionnaire (Appendix III) was given to the participants in order to collect basic health information such as exercise experience and supplement usage. Participants in the RT+S and RT+R groups completed a weekly

validation log of their resistance training program (Appendix IV) to determine volume load [volume load = weight (lbs.) x reps x sets]. Nutritional intake was recorded week 1 and week 6 by participants and the United States Department of Agriculture (USDA) nutrient database was used to analyze protein and caloric intake (Appendix V). The final form completed was a Visual Analog Scale (VAS) that assessed baseline fatigue and muscular pain. The VAS was given to the participants at the end of every week, along with a checklist of study protocol adherence to analyze muscle soreness and protocol compliance, respectively (Appendix VI).

RT+S (n = 5) completed the resistance training protocol (Appendix VII) three times per week followed by a fifteen minute sauna session at 45-50° Celsius and 75-80% humidity the same day. RT+R (n = 5) completed the same resistance training protocol as RT+S followed by a 15 minute relaxation protocol at room temperature (21° Celsius, 25% Humidity). Relaxation served as a placebo sauna session (Appendix VIII). The control group (CON), recruited two weeks after the start of the study due to high dropout rates in the intervention groups, did not participate in the resistance training or sauna/relaxation protocols and were instructed not to alter their daily exercise/activity routines throughout the duration of the study.

During the first week of the study, 3 ml of blood was drawn from the antecubital vein to analyze HSP70 concentrations. The second week began by taking a baseline strength measurement of a 5RM Back Squat. This strength assessment was performed during the second week to allow for proper instruction time and safety precaution. Once the baseline measurements were taken, the participants started their assigned group sessions three times per week for a total of six weeks. After the six-week intervention all subjects had two days of complete rest to reduce training fatigue, followed by a post-intervention Dual-energy X-ray absorptiometry (DEXA) scan for LBM, HSP70 blood draw, and 5RM back squat test.

### *Sauna Protocol*

The RT+S group completed a 15 minute sauna session three times per week (45-50° Celsius, 75-80% Humidity). The sauna was located in the Physical Education Instruction Facility (PEIF) and participants used the sauna between 8:00am-3:00pm after completing a 6:30 am resistance training protocol. The traditional sauna created a whole body hyperthermic stimulus whereby participants poured water on electrically heated rocks. The sauna was insulated with wood paneling and temperature was recorded at participant hip height. Participants in the RT+R group completed a relaxation protocol between 8:00am-3:00pm at room temperature (21° Celsius, 25% Humidity) for 15 minutes, three times per week. The relaxation protocol was designed to address psychological relaxation associated with sauna usage, see appendix VIII.

### *Resistance Training Protocol*

RT+S and RT+R participants completed the supervised resistance training protocol Monday, Wednesday, and Friday mornings from 6:30-7:45 am for six weeks. Training took place at the PEIF and primarily used barbells and dumbbells. The protocol consists of multi-joint compound exercises for 3-4 sets of 8-10 repetitions with minimal rest (see Appendix VII for list of exercises and sets/reps/rest). The resistance training protocol was based on the National Strength and Conditioning Association's (NSCA) recommendations for skeletal muscle hypertrophy (10).

### *DEXA scan for LBM*

A full body DEXA scan using a 3 compartment model was completed during week 1 and week 7 to analyze body composition. The DEXA was located in the offices of Advanced Orthopedics for Sports Medicine as part of the Upper Peninsula Medical Center under the

supervision of Dr. Bryan Dixon. LBM was the primary dependent variable examined pre and post-intervention. LBM has shown a strong relationship with cross sectional area and strength enhancements (33,39). Body fat percentages and changes in total bodyweight were also measured to further analyze body composition changes during the 6-week intervention.

### *5RM Back Squat Testing*

After 1 week of familiarization with the barbell back squat, a 5RM was assessed for baseline strength measurements and supervised by a certified strength and conditioning specialist. The familiarization period was used due to an untrained/recreational population, which required an adjustment period related to utilizing a loaded squat in order to accurately test maximal strength (10). Participants were given three attempts to find the maximal weight where they could still complete five repetitions to exhaustion with the barbell placed on the upper trapezius muscle. A complete repetition included: participant reached a squat depth where the femur was parallel with the ground in the bottom position or as close as possible due to flexibility limitations of an untrained population (10). The same 5RM protocol was used after completion of the 6 week resistance training protocol.

### *HSP70 Concentration Analysis*

During the first week of the study, 3 ml of blood was drawn from the antecubital vein in the Clinical Lab Science Department at NMU and a 3 ml EDTA test tube was used as temporary storage. The serum was then centrifuged at 1000 x g for 15 minutes. The samples were extracted and pipetted into a clean cryogenic tube and stored at 4° C. HSP70 was evaluated from the serum by a HSP70 ELISA kit (ADI-EKS-77B, Enzo Life Sciences, Farmingdale, NY) with a sensitivity of 200 pg/mL. Each sample was tested in triplicate along with seven (7) standard HSP-70



dilutions. Optical density of the ELISA was read using a spectrophotometer in the Chemistry Department on campus. HSP-70 concentrations were calculated from the optical density using the trend line of the known standard curve, see Figure 4.

*Statistical Analysis*

IBM SPSS Statistics, version 24 (Armonk, NY: IBM Corp.) software was used for analysis. A mixed study group design with repeated measures analysis of variance (rANOVA) was utilized to examine the main effect of group selection and interaction on LBM, HSP70 concentrations, and 5RM with significance level at  $p \leq 0.05$ .

**Results**

All results are reported significant if  $p < 0.05$ . RT+S, RT+R and CON, respectively, changed LBM by  $0.99 \pm 2.0$  kg,  $0.30 \pm 1.0$  kg, and  $-0.48 \pm 0.8$  kg. See Table 2, Table 3, and Figure 1 below for body composition changes over the 6-week intervention. Time by group interaction for LBM was not significant ( $p=0.424$ ). The time interaction between subjects was also not significant ( $p=0.539$ ). There was a trend toward further increases in LBM from RT+S, but due to the small sample size and limited power (0.169), it was not statistically significant.

Table 2: Body composition measures for each group pre- and post-test via DEXA (mean  $\pm$  SD).

	Bodyweight (kg)		LBM (kg)		% Body Fat	
	Pre	Post	Pre	Post	Pre	Post
RT+S	82.98 $\pm$ 10.1	83.68 $\pm$ 8.5	61.46 $\pm$ 3.6	62.45 $\pm$ 3.2	21.82 $\pm$ 6.6	21.48 $\pm$ 7.2
RT+R	85.78 $\pm$ 14.2	85.84 $\pm$ 14.2	63.47 $\pm$ 7.3	63.77 $\pm$ 6.9	21.88 $\pm$ 11.2	19.62 $\pm$ 10.0
CON	74.37 $\pm$ 6.5	73.79 $\pm$ 6.0	64.00 $\pm$ 6.5	63.52 $\pm$ 7.3	10.13 $\pm$ 3.8	9.80 $\pm$ 4.3

Table 3: Change ( $\Delta$ : post – pre value) in body composition measures (mean  $\pm$  SD).

	$\Delta$ Bodyweight (kg)	$\Delta$ Lean Body Mass (kg)	$\Delta$ % Body Fat
<b>RT+S</b>	+ 0.70 $\pm$ 2.0	+ 0.99 $\pm$ 2.0	- 0.34 $\pm$ 1.6
<b>RT+R</b>	+ 0.05 $\pm$ 1.2	+ 0.30 $\pm$ 1.0	- 2.26 $\pm$ 3.4
<b>CON</b>	- 0.58 $\pm$ 0.5	- 0.48 $\pm$ 0.8	- 0.33 $\pm$ 1.4

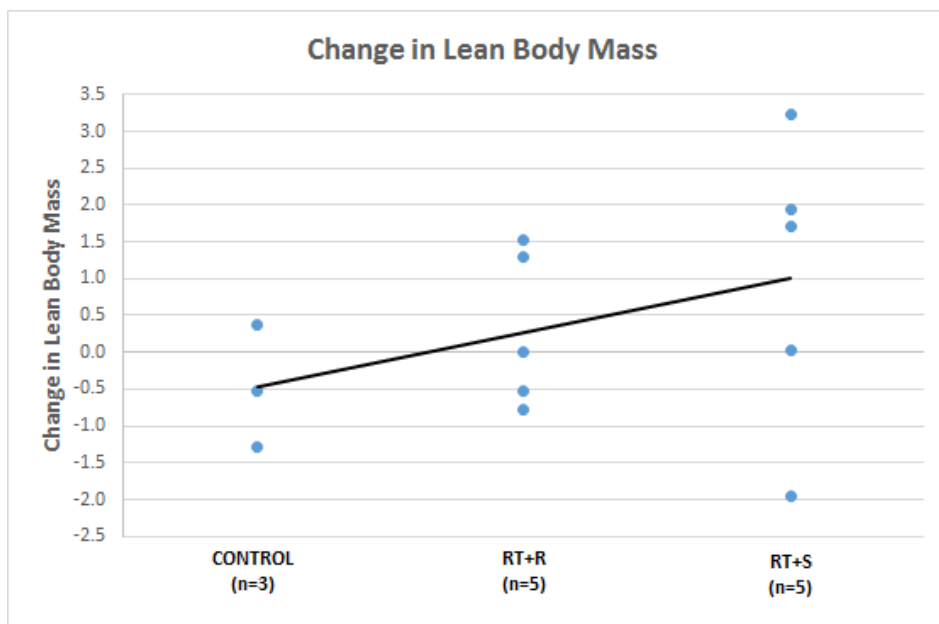


Figure 1: Change (post – pre tests) in lean body mass (LBM). RT+S, RT+R, and CON, respectively changed LBM 0.99  $\pm$  2.0 kg, 0.30  $\pm$  1.0 kg, and -0.48  $\pm$  0.8 kg. No significant main effect or interaction was detected ( $p=0.424$ )

RT+S, RT+R, and CON, respectively, changed HSP70 by 0.40  $\pm$  0.45 ng/ml, 0.09  $\pm$  0.13 ng/ml, and 0.04  $\pm$  0.35 ng/ml. See Table 4, Figure 2, and Table 5 for changes in HSP70. Time interaction between subjects for HSP70 was not significant ( $p=0.096$ ) with a 0.05  $\alpha$  level. The time by group interaction was also not significant ( $p=0.285$ ).

Table 4: HSP70 concentrations at pre-test, post-test, and post – pre ( $\Delta$ ) change (mean  $\pm$  SD).

Participant ID	Group	Pre-HSP70 (ng/ml) Mean $\pm$ SD	Post-HSP70 (ng/ml) Mean $\pm$ SD	$\Delta$ HSP70
1	RT+S	3.26 $\pm$ 0.06	4.45 $\pm$ 0.20	+ 1.19
2	RT+S	0.69 $\pm$ 0.09	0.89 $\pm$ 0.01	+ 0.20
3	RT+S	0.36 $\pm$ 0.01	0.49 $\pm$ 0.01	+ 0.13
4	RT+S	1.00 $\pm$ 0.02	1.37 $\pm$ 0.03	+ 0.37
5	RT+S	1.15 $\pm$ 0.55	1.25 $\pm$ 0.01	+ 0.10
6	RT+R	0.30 $\pm$ 0.01	0.50 $\pm$ 0.01	+ 0.20
7	RT+R	0.30 $\pm$ 0.01	0.41 $\pm$ 0.01	+ 0.11
8	RT+R	0.30 $\pm$ 0.02	0.40 $\pm$ 0.01	+ 0.10
9	RT+R	0.56 $\pm$ 0.60	0.40 $\pm$ 0.01	- 0.16
10	RT+R	0.58 $\pm$ 0.09	0.79 $\pm$ 0.01	+ 0.21
11	CON	0.88 $\pm$ 0.16	0.53 $\pm$ 0.01	- 0.35
12	CON	0.55 $\pm$ 0.03	0.79 $\pm$ 0.05	+ 0.24
13	CON	0.30 $\pm$ 0.10	0.54 $\pm$ 0.02	+ 0.24

\*HSP = heat shock protein.

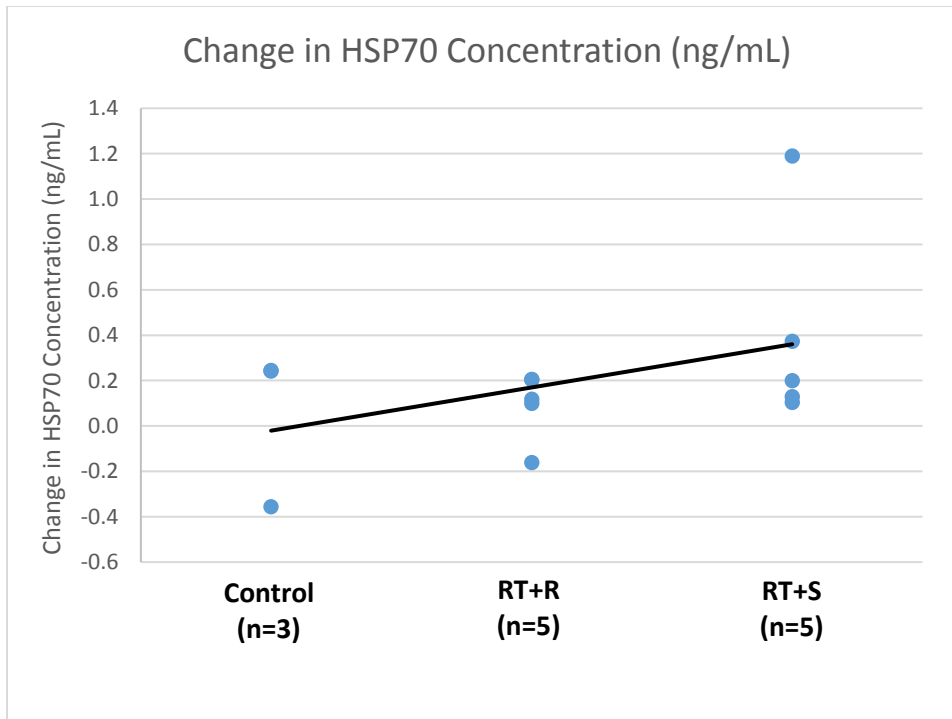


Figure 2: Change (post – pre tests) in heat shock protein (HSP) 70. RT+S, RT+R, and CON, respectively changed HSP70 0.40  $\pm$  0.45 ng/ml, 0.09  $\pm$  0.13 ng/ml, and 0.04  $\pm$  0.35 ng/ml. No main effect or interactions were detected.

Table 5: Change ( $\Delta$ : post – pre value) in each dependent variables (mean  $\pm$  SD).

	$\Delta$ HSP 70 (ng/ml)	$\Delta$ 5RM Squat (kg)	$\Delta$ LBM (kg)
RT+S	+ 0.40 $\pm$ 0.5	+ 23.15 $\pm$ 5.7	+ 0.99 $\pm$ 2.0
RT+R	+ 0.09 $\pm$ .01	+ 31.33 $\pm$ 8.2	+ 0.30 $\pm$ 1.0
CON	+ 0.04 $\pm$ 0.4	N/A	- 0.48 $\pm$ 0.8

\*HSP = heat shock protein; 5RM = 5 repetition maximum; LBM = lean body mass.

RT+S, RT+R respectively, changed 5RM by 23.15  $\pm$  5.7 kg and 31.22  $\pm$  8.2. See Table 5 and Figure 3. Group by time interactions for 5RM over the 6-week intervention was not significant ( $p=0.105$ ). Significance was found within subjects for time ( $p=0.001$ ). Although not statistically significant, it's prudent to observe RT+R had a higher weekly volume load (41,368  $\pm$  13,561) than RT+S (40,088  $\pm$  9,033), see Table 7. Also, average VAS was also greater in RT+R (3.1  $\pm$  1.1) than RT+S (2.9  $\pm$  0.6).

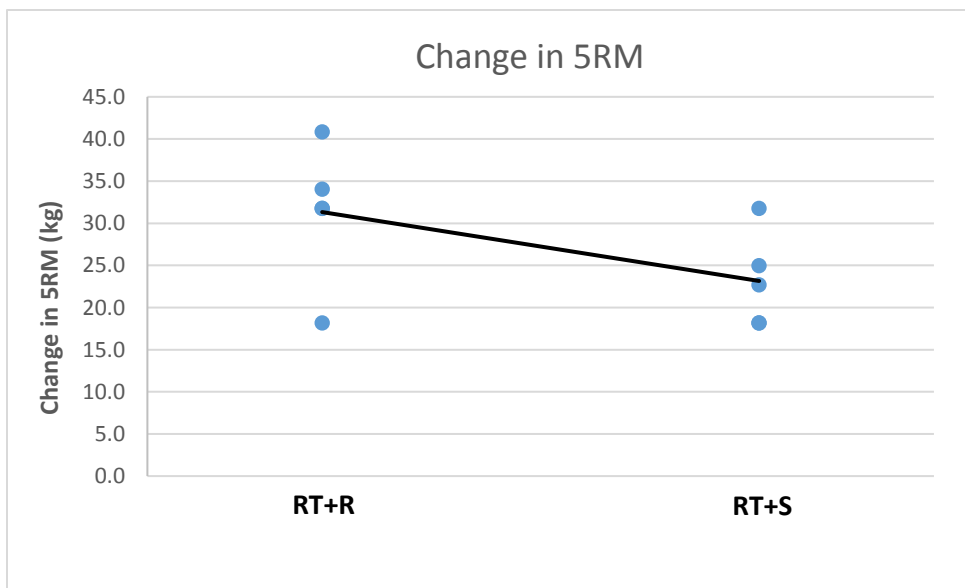


Figure 3: Change (post – pre tests) in 5RM. RT+S and RT+R, respectively changed 5RM 23.15  $\pm$  5.7 kg, 31.33  $\pm$  8.2 kg. A main effect of time was detected ( $p=0.001$ ) with time by group interaction not reaching significance ( $p=0.105$ )

Energy requirements and daily protein intake were recorded the first and last 7 days of the intervention (Table 6). RT+S, RT+R, and CON, respectively averaged  $2,466 \pm 391$  kcal,  $2,570 \pm 517$  kcal, and  $2,408 \pm 23$  kcal. Using the Cunningham equation [ $RMR = 500 + 2(\text{FFM})$ ], every participant was 1,300-2,300 kcal above their resting metabolic rate, which is sufficient for protein synthesis (6). Protein intake was also adequate for an anabolic state, averaging 1.66 grams/kg of bodyweight (5).

Table 6: Training volume load, kcal + protein intake, and pain scale measures for each group (mean  $\pm$  SD).

	Volume Load (lbs x week)	Daily kcal	Daily protein (g/kg)	VAS
<b>RT+S</b>	$40,088 \pm 9,033$	$2,466 \pm 391$	$1.43 \pm .3$	$2.94 \pm 0.6$
<b>RT+R</b>	$41,368 \pm 13,561$	$2,570 \pm 517$	$1.69 \pm .3$	$3.12 \pm 1.1$
<b>CON</b>	N/A	$2,408 \pm 23$	$2.00 \pm 1.3$	N/A

Weekly Volume Load= weight x reps x sets; VAS = visual analogy scale for pain assessment, 0 = no pain, 10 = worst possible pain.

\*CON was N/A for the 5 RM due to technique and injury risk of an untrained population

## Discussion

The primary hypothesis behind the benefit of a whole body heat stimulus during resistance training is an increased concentration of HSP70 concentrations. HSP70 plays a role as a molecular chaperone to protein in the process of skeletal muscle remodeling and has been hypothesized to act as a protective mechanism to muscle tissue (4,10). Analysis of the average body composition data demonstrated that the LBM for the RT+S group increased by 0.69 kg over the RT+R group. However this increase between groups was not significantly different ( $p=0.424$ ) due to the small sample and low power (0.169).

RT+S seemed to augment HSP70. HSP70 increased 0.40 ng/ml in RT+S group, but were only affected 0.09 ng/ml for RT+R and 0.04 ng/ml for CON. Despite time by group interaction not reaching significance ( $p=0.285$ ), LBM and HSP70 illustrated greater increases for RT+S during 6 weeks of training. The increased HSP70 concentration by sauna use may have aided the further enhancement of LBM. Increased intensity of hyperthermic stimulus and training duration may further support the interaction between HSP70 and muscle hypertrophy. However, if LBM is related to strength, RT+S should have increased 5RM strength more than RT+R (43). The outcome of the study did not see a further improvement in 5RM from RT+S despite enhanced HSP70 concentrations and LBM.

The RT+S group improved the 5RM by  $23.15 \pm 5.7$  kg, while RT+R increased  $31.33 \pm 8.2$  kg. CON was not measured on 5RM due to skill requirements and safety precautions. The strength improvements were not significant ( $p=0.105$ ). RT+R also had a higher VAS score of  $3.1 \pm 1.1$  compared to RT+S who averaged  $2.9 \pm 0.6$ . The larger degree of soreness, although not significant, likely reflects the higher volume load of RT+R (41,368 lbs/wk) per week when compared to RT+S (40,088 lbs/wk). The higher VAS assumes more delayed onset muscle soreness (DOMS) and skeletal muscle damage in RT+R, which could explain the greater increase in strength. A lower VAS score could also be explained by the saunas effect of muscle recovery. Anecdotally, most subjects reported a more relaxed sensation after sauna use that could stimulate a physiological or psychological benefit.

A greater LBM change in RT+S participants contradicts the 5RM results and speculates that strength gains were not directly related to increases in skeletal muscle mass. Future studies may require a longer training period ( $> 6$  weeks) and greater participation in order to produce sufficient measureable muscle hypertrophy outcomes. A possible explanation for the lower VAS

scores in RT+S may be due to hyperthermia causing vasodilation, which would increase oxygen availability and hydrogen ion removal (12,22), leading to a potential better recovery and subsequent workout. However, this remains speculative due to no statistical significance with the small sample size.

Hyperthermic stimuli (sauna) have many different physiological adaptations, such as increased heart rate, chronically reduced blood pressure, improved left ventricle heart function, reduced risk for cardiovascular disease (2,7), in addition to an increase in HSP70. Other members of the heat shock protein family are also involved in skeletal muscle remodeling and were not measured in this study. Most HSPs are released by the body in response to stress along with hormones (18). The endocrine adaptations are especially important to hyperthermia and body composition changes. Sauna use stimulates the sympathetic nervous system and stress response by activating the renin-angiotensin-aldosterone pathway, which incites the hypothalamus-pituitary-adrenal axis. Previous studies demonstrated an increase in epinephrine, norepinephrine, prolactin, ACTH, growth hormone, and beta endorphins with sauna use (13). Cortisol is also linked to the hypothalamus-pituitary-adrenal axis and has varying responses to hyperthermia with some studies showing an increased concentration, while others have shown a decreased concentration (12). Cortisol is an important hormone to skeletal muscle hypertrophy and its response needs to be examined further to elicit catabolic effects of hyperthermia. The degree of sympathetic stimulation is dependent on the temperature, humidity, and length of sauna sessions. Previous studies used higher temperatures (80-100° Celsius), therefore it is unclear if hormone concentration changed in this study. Future research is needed to examine how different amounts of hyperthermia effect hormones when used in combination with weight training.

The climate in Marquette, Michigan during the study length (January-March) may also need to be taken into account. The cold weather outside combined with the 15 minute sauna session could be considered contrast therapy. No instructions were given to the subjects after the sauna session, therefore it is unclear if the subjects were exposed to both extreme temperatures (hot and cold) during the training period in rapid succession (i.e., immediately after sauna some participants may have gone outside). Relaxation protocols and group meetings were held in the Physical Education Instruction Facility (70 °C, 25% humidity). The majority of the subjects using the sauna anecdotally commented on their feeling of relaxed muscles. Keep in mind placebo relaxation intervention was used for RT+R. It is unclear if mental relaxation had a significant effect on the study results.

Nutritional intake was adequate for all subjects to stay in an anabolic state for skeletal muscle hypertrophy. Using the Cunningham equation for resting metabolic rate (RMR), each individual reported a 1,300-2,300 kcal intake above their RMR, which is sufficient for protein synthesis (6). Protein intake was also adequate for all groups, averaging 1.66 grams/kg of bodyweight (5). However, the two, 7 day nutrition logs were self-reported by participants and lack of nutritional control was noted as a limitation. Further nutritional intervention is needed in future studies to fully understand if protein synthesis is effected by hyperthermia.

### *Practical Application*

This small, initial pilot study was not able to demonstrate a significant effect of sauna use on LBM, 5RM, or HSP70 concentrations in young males during six weeks of full body resistance training. LBM and HSP70 changed the most from use of a hyperthermic stimulus during resistance training that could potentially approach significance with a larger sample size. Therefore, additional research is required to investigate the interaction of HSP70 and LBM



during hyperthermic intervention. Future research should also utilize a longer training period for optimal skeletal muscle hypertrophy, determine the effect of different durations of sauna usage, and recruit a larger sample size with no resistance training experience.

The RT+R group (not using the sauna) tended to have increased strength enhancements in a 5RM back squat over the RT+S group (with sauna). This change was not statistically significant, but having a hyperthermic stimulus in a resistance training program could potentially over stress the body and therefore decrease strength adaptations. The high HSP70 concentrations for RT+S group could be interpreted as increased stress on the body. The further increases in LBM with the RT+S suggests the sauna was not creating a catabolic response. The increased strength improvements in the RT+R group could be attributed to the greater training volume load (although only slightly greater) and VAS scores despite not being statistically significant. Overall, supplementary research is needed to understand the use of a hyperthermic heat stimulus on skeletal muscle hypertrophy during resistance training. This remains an intriguing concept.

## CHAPTER II: LITERATURE REVIEW

### **Introduction**

Resistance training increases skeletal muscle size by creating small tears in the muscle cell, followed by the addition of muscle mass during the recovery period (44). The cellular damage caused by resistance training stimulates a stress response that assists the muscle to adapt to the stress and avoid extensive damage. Resistance training also increases satellite cell proliferation, synthesis of contractile proteins, and number of myofibrils resulting in muscle hypertrophy (1,17,20). Numerous physiological responses have been postulated to aid in this process; however, a relatively new concept involves the interaction of heat shock protein-70 (HSP70) with developing skeletal muscle (24). The exact physiological mechanism of this process is unknown but it has been suggested that HSP70 acts as a molecular chaperone which aid in the removal of denatured proteins and refold proteins into functional muscle mass (14,24). If this theory is correct, a whole body heat stimulus such as a sauna could aid skeletal muscle hypertrophy when used in combination with resistance training.

### **Structure and Function of Skeletal Muscle**

Skeletal muscle is composed of elongated muscle fibers that pull on the skeletal system to produce force. The muscle fiber is composed of numerous contractile units known as sarcomeres. Each sarcomere has thin actin myofibrils that are attached to the Z-line, which serves as a foundation for the molecular mechanism. Actin's active sites that attracts the myofibril myosin are not exposed due to the troponin/tropomyosin complex (32). When stimulated by calcium ions, troponin changes shape, moving tropomyosin and exposing the active site. Myosin's head attaches to the active site on actin and uses ATP to pull Z-lines closer

together and shorten sarcomeres. Huxley described this ratchet like motion between the myosin head and actin as the sliding filament theory (3).

When a muscle fiber is mechanically stressed, it increases the number and size of myofibrils. The increase in myofibrils can be seen as increases in the cross sectional area of a muscle fiber. Increases in cross sectional area have shown a strong relationship with muscular strength (32). The process of adding myofibrils to muscle fibers to increase diameter has been defined as skeletal muscle hypertrophy (3).

### **Skeletal Muscle Hypertrophy**

Muscular tension is the primary stimulus for hypertrophy of skeletal muscle mass (30). Damage of contractile proteins take place near the Z-line of the sarcomere, which alters gene expression and stimulates protein synthesis. Cellular components are then reconstructed with more myosin heavy chain and actin resulting in an increase in muscle diameter (41). Resistance training has demonstrated increased protein synthesis and satellite cell proliferation, which are necessary for hypertrophy. The myofibril protein synthesis can stay elevated for 4.5 hours after resistance training (34). If protein synthesis continues, the cross sectional area of the skeletal muscle will increase.

Assessing cross section area using a magnetic resonance imaging (MRI) is the gold standard when researching skeletal muscle hypertrophy in-vivo (5,33). Increases in cross sectional area have been observed after 4 weeks of resistance training (43,48) – partial justification for using 6 weeks in the current investigation. If MRI scans are not available a Dual-Energy X-ray Absorptiometry (DEXA) scan, used in the current study, of Lean Body Mass

(LBM) has been demonstrated to show a strong relationship to cross sectional area of skeletal muscle (5,33,39).

In order to increase muscle hypertrophy and LBM the body needs to be in a positive energy balance and consume a sufficient amount of protein (30). The Cunningham equation ( $REE = 500 + (22 \times FFM)$ ) has been validated as a research measurement for resting energy expenditure (6). Caloric intake can be compared to resting energy expenditure to estimate nutritional recovery. Sufficient protein intake is also necessary to stimulate protein synthesis and increases in muscle cross sectional area (30). Previous research has demonstrated a positive nitrogen balance during resistance training above 1.53 g/kg (23). A resistance training study with a primary goal of skeletal muscle hypertrophy needs to address caloric intake and protein consumption. Notably, the current researcher took this into account.

### **Resistance Training and Skeletal Muscle Hypertrophy**

A primary goal of resistance training is to increase the cross sectional area of a muscle fiber, defined as skeletal muscle hypertrophy (8). Mechanical load associated with resistance training, starts a cascade of intracellular processes that alter gene expression and promote protein synthesis (41). Magnitude of protein synthesis depends on degree of mechanical stress placed on the muscle fiber (1). For instance, when single-celled myoblasts from a chicken were placed under mechanical stretch of 7.5-13%, there was an increase in the accumulation of myofibril protein. At 20.8% stretch, protein levels were reduced due to a stress overload on the muscle fiber (47). In-vitro studies can easily quantify appropriate amounts of mechanical stress to induce hypertrophy but training volume is less clear in human training studies.

The training protocol is based on the National Strength and Conditioning Association's (NSCA) recommendations for hypertrophy. The NSCA suggests 6-12 repetitions for 3-6 sets and a rest period of 30-90 seconds (10). Multi-joint exercises and short rest periods are utilized to improve hormonal adaptations. An anabolic endocrine response is important to muscle hypertrophy and is further addressed in the endocrine response section (20). The protocol was completed 3 times per week for a total of 6 weeks. A direct increase in skeletal muscle cross sectional area has been observed from training periods 3-4 week's long, and hypertrophy is expected in participants completing the resistance training protocol (43,48). A visual analog scale (VAS) can be an important tool to measure muscular damage associated with Delayed-Onset Muscle Soreness (DOMS) (38). Differences in VAS scores between groups can elude to degree of effort during a training study and damage to the myofibril, hypothetically leading to muscle hypertrophy.

Muscle hypertrophy has shown a positive relationship to strength enhancements (43). Strength will also be a dependent variable in the current study to further understand hyperthermia's effect on physical performance. Ideally, strength is assessed by a one repetition maximum (1-RM) but due to the relatively untrained status of participants, a 5 repetition maximum (5RM) back squat was used (2,10). 1-RM requires adequate training status and significant stress is applied to the connective tissue and joint (29). Beyond 5RM technique can deteriorate and is a better assessment of muscular strength endurance (2).

### **HSP70 Stimulation**

An important part of the stress response that has been hypothesized to aid hypertrophy is an increase in the concentration of heat shock proteins (HSPs). There are many different members of the HSP family which respond to different stressors such as hyperthermia, hypoxia,

ischemia, and physical activity. However the most widely studied and abundant HSP that responds to stress in the human body is heat shock protein 70 (HSP70) (24). Its exact physiological mechanism of action is unknown, but it has been suggested that HSP70 acts as a molecular chaperone, aiding in the removal of denatured proteins and refolding other proteins into functional muscle mass (14,27). Importantly, by isolating HSPs in a rabbit's liver, researchers were able to show their involvement with the correct folding of actin within skeletal muscle leading to muscle hypertrophy. (50). Additionally, the role of HSPs in protein synthesis may play an important function in muscle hypertrophy and recovery.

HSP70 increases its concentration in responses to exercise in rats as well as humans (15,18,28,35). In humans, it appears that the intensity of the exercise and extent of muscular damage is an important factor in the amount of HSP70 stimulation (24,26,37). Due to this interaction between the intensity of exercise, resulting cellular damage and HSP70 stimulation, it has been hypothesized that HSPs may play an important role in muscular recovery and muscular hypertrophy after intense exercise. It remains unclear what stimulates the increase in HSP70 during exercise but it has been suggested that the heat produced by the muscle during intense exercise may be the primary stimulus (26).

An alternative method of increasing the concentrations of HSP70 is by creating a whole body hyperthermic environment. Rats exposed to intermittent hyperthermia alone responded with a dramatic increase of HSP concentration (19,36,42,45). This increase in HSPs in response to heat is important because it may suggest heat could be used in combination with cellular damaging exercise to aid or augment muscular development. A case in point relates to a group of rats exposed to heat stress demonstrating increased HSPs to be strongly correlated with proliferation of satellite cells and increased protein concentrations in the cell (19). This particular

study directly relates to hyperthermia leading to muscular development and possibly muscle hypertrophy, further supporting the main purpose and methodology of the current investigation.

On another note, HSP stimulation through hyperthermia has been shown to help the recovery of rats that have undergone skeletal muscle atrophy through forced inactivity. The muscle weight of the soleus decreased significantly less when rats were exposed to hyperthermic conditions (35,36,42,49). This suggests that using hyperthermic conditions to stimulate HSPs may protect the muscle against cellular damage, even when inactive. If hyperthermia can assist with the recovery from muscle atrophy, it may also protect the skeletal muscle from excessive damage during resistance training and lead to heightened muscle hypertrophy. In fact, an in-vitro study of rat skeletal muscle with applied heat stress and mechanical stress of stretching the muscle cell, lead to a larger increases in cell protein concentrations than with either method alone (9). Still, heat stress alone was shown to increase protein concentrations, but not as significantly as heat stress in combination with mechanical stress, underscoring the effect of external heat application on muscle development. Currently, this is the most direct study to suggest that the combination of heat and mechanical stress (e.g., resistance training) compound muscle hypertrophy. Other researchers, using a rat model in-vivo, supported the prior mentioned concept by observing increased weight of the soleus muscle after seven days of heat stress (46). This suggests that heat stress alone could promote muscle cell generation and induce muscular hypertrophy. If mechanical stress is combined with the heat stress there is a greater possibility for HSP stimulation and therefore further muscle hypertrophy.

Previous studies on the use of the sauna in humans have typically focused on the cardiovascular aspects of hyperthermic conditions. These researchers demonstrated that the use of the sauna produced cardiovascular effects which were similar to moderate exercise (e.g.,

increased heart rate, chronically reduced blood pressure, improved left ventricle heart function, reduced risk for cardiovascular disease) (12,22). To the best of my knowledge, no study in humans has investigated the effect of sauna use in combination with resistance training and its effect on muscle hypertrophy. As suggested by this literature review, the protective function of HSPs and their stimulation during the hyperthermic conditions of a sauna may assist recovery and lead to further gains in muscle mass. Hence, the purpose of this study was to elucidate the effects of hyperthermic whole body heat stimulus (sauna) on HSPs (i.e., HSP70) and skeletal muscle hypertrophy following resistance training in humans. If the findings of this study were found to be similar to those observed in animal models, it could significantly change the methods used to increase muscle mass during resistance training. However, as mentioned in Chapter I, our findings were not significant and further research is warranted.

### **Endocrine Response to Hyperthermia**

Heat stress that occurs during a sauna session has demonstrated an acute endocrine response (16). The exact response is individualized but has shown a strong link to the duration of hyperthermia. Cortisol will decrease for the initial 15 minutes of a sauna session and then increase for longer durations (16). Cortisol has shown to cause a catabolic effect and inhibit protein synthesis (7). If cortisol is decreased from brief sauna session, it would aid skeletal muscle hypertrophy and decrease catabolism. An increase in testosterone would also enhance the anabolic process of muscle hypertrophy (11). Testosterone drives protein synthesis and increases in response to the demand of exercise (4). Using hyperthermia testosterone can also positively affect testosterone levels and anabolic stimulus (21). The combination of decreased cortisol and increased testosterone from hyperthermia may improve muscle hypertrophy.



Growth hormone elucidates a strong relationship with muscle hypertrophy and most studies report an increase with resistance training. Growth hormone increases protein synthesis and amino acid transport across cell membranes (31). Sauna use stimulates the stress response and has shown up to a 16-fold increase in growth hormone (21). Changes in growth hormone produced by hyperthermia could aid anabolism and assist skeletal muscle hypertrophy. Plasma prolactin and adrenocorticotrophic hormone (ACTH) have demonstrated a steady increase in concentrations after a sauna session (21). Future research needs to examine how hormonal changes from sauna use interact with resistance training. Alone hyperthermia shows a hormonal advantage that would improve hypertrophy.

### CHAPTER III: CONCLUSIONS AND RECOMMENDATIONS

This study was not able to demonstrate a significant improvement between and within participants using a traditional Finish sauna with concomitant changes in LBM during 6-weeks of resistance training. There were further increases in LBM for RT+S but it was not statistically significant. RT+S also increased HSP70 concentrations, which may be related to further LBM improvements, but not in this investigation. Further research is needed to uncover if HSP70 truly has a relationship with changes in LBM in humans, using a larger sample size and training duration. HSP70 interaction with skeletal muscle hypertrophy in humans is still unclear and could be interpreted as a catabolic response to hyperthermic physiological stress; although, again, this was not significant. Currently, the greater change in LBM and HSP70 for RT+S suggests HSP stimulation is an anabolic response. Nevertheless, the changes in LBM and HSP70 were not indicative of changes in 5RM observed. Future research should monitor bodily hormone fluctuation to properly assess HSP70 interactions, including the observation of other HSPs.

The RT+R group (i.e., not using the sauna) had the greatest strength enhancements in a 5RM back squat. The change was not statistically significant, but having a hyperthermic stimulus in a resistance training program could over stress the body and decrease strength adaptations, such as in the RT+S lifters. Changes in LBM demonstrated that the strength differences between groups were not likely related to skeletal muscle hypertrophy. The higher strength increases in the RT+R group could possibly be attributed to greater training volume load and VAS scores; although this may have been an arbitrary finding. Skill involvement, neurological changes, and individual variation could also play a role in a 5RM test that may have skewed the results in this study.

The outcomes of this study cannot directly support the hypothesis that sauna use can aid skeletal muscle hypertrophy. Recall, there was a further increase in LBM and HSP70 concentrations in participants using the sauna, but not significant. Ultimately, a relationship between HSP70 and hypertrophy may exist, but future research with a larger sample size and greater training duration is needed for validation.

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## Appendix I: Informed Consent

### Study Title: The Effect of Hyperthermic Whole Body Heat Stimulus (Sauna) on Heat Shock Protein 70 and Skeletal Muscle Hypertrophy in Young Males during Weight Training

#### Informed Consent

- (1) In signing this informed consent, I agree to all of the following conditions for this study.
  - a. I agree to voluntarily participate in a seven-week long study that intends to research whether or not sauna use can increase skeletal muscle hypertrophy in young resistance training males.
  - b. I acknowledge that I will be randomly selected into a group that uses the sauna and resistance trains three times per week.
  - c. The other two groups will selectively just sauna and stretch or only resistance train.
  - d. If resistance training, it will involve: barbell and dumbbell exercises that will focus on multi-joint, full body exercises, and working out at NMU in the PEIF.
  - e. If using the sauna, it will be at the PEIF on the NMU campus.
  - f. I understand my time commitment is 100% voluntary and I am able to withdrawal from the study for any reason at any time without penalty.
  - g. I understand I will have to travel at the start and end of the study to Dr. Bryan Dixon's office, Advanced Orthopedic Center, at the UP Medical Center (1414 W Fair Ave #344, Marquette, MI 49855) for a DEXA scan looking at percent body fat, lean mass, bone density, among other limb characteristics.
  - h. I understand I will have blood drawn at the beginning and end of the study to measure a particular protein in my blood, heat shock protein 70 (HSP-70), which helps with muscle growth.
  - i. I understand I will utilize a visual analog scale provided by the researchers and used weekly to judge muscle soreness and pain.
- (2) I acknowledge that there is some minimal risk involved with the training program and sauna usage. Proper instruction will be provided for both to ensure safety. Any exercise has some physical risks but weight training has a statistically low injury rate. If any ill effects are observed, please let the researchers know immediately. The researchers will take personal responsibility to get proper medical treatment if necessary.
- (3) Using a sauna effects heat regulation in the body and stresses the body in a similar manner to exercise. Negative health effects of saunas are rare and normally include alcohol or pre-dehydration. If at any time I feel dizzy, weak, light headed, or not right please contact the researcher immediately. All subjects will be given the lead authors email at the beginning of the study.
- (4) To confirm, during the first week and last week of the study blood will be taken to measure HSP-70 and a DEXA scan will be performed to determine lean body mass. These are safe procedures that will be completed by trained professionals in a clinical setting. This can be beneficial health information that can be used in the future.



(5) I give permission to the author of this study to use this information in a published scholarly article and thesis. My identity will be coded by subject numbers to everyone except the authors that are directly involved with the study.

Participants Name: \_\_\_\_\_

Participants Signature: \_\_\_\_\_

Date: \_\_\_\_\_

Researcher Signature: \_\_\_\_\_

## Appendix II: Canadian Society for Exercise Physiology PAR-Q

Physical Activity Readiness  
Questionnaire - PAR-Q  
(revised 2002)

# PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of <u>any other reason</u> why you should not do physical activity?

If  
you  
answered

### YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

### NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

### DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

**PLEASE NOTE:** If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

**Informed Use of the PAR-Q:** The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

**No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.**

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME \_\_\_\_\_

SIGNATURE \_\_\_\_\_

DATE \_\_\_\_\_

SIGNATURE OF PARENT  
or GUARDIAN (for participants under the age of majority) \_\_\_\_\_

WITNESS \_\_\_\_\_

**Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.**



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### Appendix III: Health Questionnaire

Name:

Age:

Height:

Weight:

#### Over the Past Year, Exercise Experience:

- (1) On average how many times per week of aerobic (e.g., walking, running, cycling, swimming, other) activity? \_\_\_\_\_
- (2) On average what was your duration, in minutes, of aerobic activity per session?: \_\_\_\_\_
- (3) On average how many times per week of resistance training or weight training? \_\_\_\_\_
- (4) On average what was your duration, in minutes, of resistance training per session? \_\_\_\_\_
- (5) During resistance training, on average per session, did you perform a full body or split body part routine? \_\_\_\_\_

#### Supplements Currently Being Used or Recent Use:

## Appendix IV: Weekly Protocol Validation

Session 1

~8-10

~3

~60 sec

Exercise	Weight	Reps	Sets	Rest
1. BB Back Squat				
2. BB Bench Press				
3. DB Row				
4. DB Split Squat				
5. DB Shoulder Press				
6. BB Romanian Deadlift				

Sauna for 20 min

Session 2

~8-10

~3

~60 sec

Exercise	Weight	Reps	Sets	Rest
1. BB Back Squat				
2. BB Bench Press				
3. DB Row				
4. DB Split Squat				
5. DB Shoulder Press				
6. BB Romanian Deadlift				

Sauna for 20 min

## Appendix IV: Weekly Protocol Validation Cont...

Session 3

~8-10

~3

~60 sec

Exercise	Weight	Reps	Sets	Rest
1. BB Back Squat				
2. BB Bench Press				
3. DB Row				
4. DB Split Squat				
5. DB Shoulder Press				
6. BB Romanian Deadlift				

Sauna for 20 min

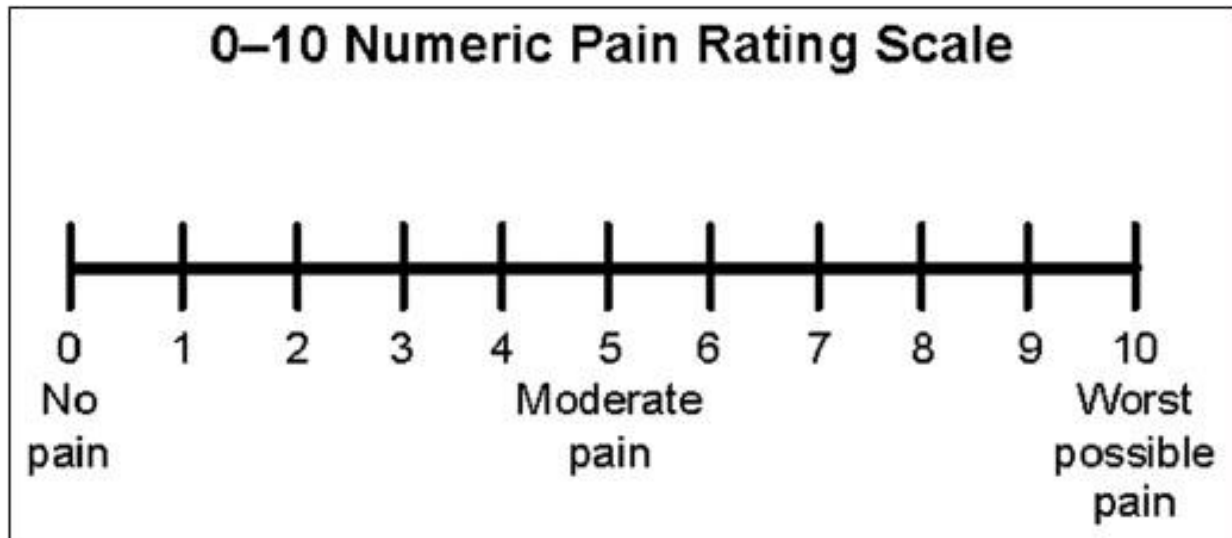
**Appendix V: 24 Hour Nutrition Log (x7 days)**

Nutrition Log (Total kcal (Protein, Carbohydrate, Fat Intake)

<b>Time</b>	<b>Food</b>	<b>Amount</b>

**Appendix VI: Visual Analog Scale (Muscular Pain/Soreness)**

Please rate your level of muscular soreness/pain on the scale below



## **Appendix VII: Relaxation Protocol**

### Relaxation protocol instructions for RT+R participants

- Please sit quietly in a room of your choice for 15 minutes
- You are free to complete any mindfulness/relaxation activities that you like, but **MUST** be inactive
- The room must be at comfortable temperature (NOT Hot or Cold)
- Free application “Headspace” can be used for guided relaxation



## Appendix VIII: Resistance Training Protocol

\*BB= Barbell

\*DB=Dumbbell

\*Weight should be maximal weight the subject can use for repetition range with good form

- All subjects will be instructed to move concentrically as fast as possible and control the eccentric portion of all lifts
- The National strength and conditioning association (NSCA) suggests that a program involving multi-joint compound movements is most efficient for strength and muscle hypertrophy. The repetition range was chosen to maximize hypertrophy along with the moderate amount of sets for subjects new to resistance training. The rest is kept short to increase hormonal adaptations.
- A brief (~2 min) biking warm up will begin each resistance training day
- Subjects are instructed to do no other planned exercise outside of the program.
- Done three times per week for six weeks

Exercise	Weight	Reps	Sets	Rest
1. BB Back Squat	Max for reps	8 - 10	3	~60 sec
2. BB Bench Press	Max for reps	8 - 10	3	~60 sec
3. DB Row	Max for reps	8 – 10 per arm	3	~60 sec
4. DB Split Squat	Max for reps	8 – 10 per leg	3	~60 sec
5. DB Shoulder Press	Max for reps	8 - 10	3	~60 sec
6. BB Romanian Deadlift	Max for reps	8-10	3	~60 sec