

The effect of infrared radiation emitting garments on the acute skeletal muscle molecular signalling responses to high-intensity interval exercise

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ABSTRACT

Infrared radiation (IR) has previously been demonstrated to activate signalling pathways involved in mitochondrial biogenesis and angiogenesis. We tested the hypothesis that IR emitting fabric (IREF) would enhance translocation and phosphorylation of proteins, and mRNA expression of genes, associated with mitochondrial biogenesis and angiogenesis in response to high-intensity interval exercise (HIIE) compared to non-IREF. Eleven males (age, 22 ± 4 yrs, $\dot{V}O_{2\text{peak}}$, 48.0 ± 4.6 ml·kg⁻¹·min⁻¹) completed two HIIE sessions consisting of 10 x 3 min intervals at a power output equivalent to 60% of the difference between gas exchange threshold and peak power output. Each interval was interspersed by 3 min at 25 W. In a repeated-measures, counter balanced, single-blind design, participants wore IREF and non-IREF clothing (SHAM) before, during and following HIIE. Muscle biopsies were obtained pre (Baseline), immediately post (PE) and 3 hours post exercise (3hPE). Cytosolic and Nuclear *p*-CaMKII content were both greater PE in IREF compared to SHAM ($P = 0.001$ and $P = 0.003$ respectively). Cytosolic and Nuclear *p*-p38 MAPK content increased from baseline to PE in IREF ($P = 0.008$ and $P = 0.005$, respectively) but not SHAM. *NRF1* and *TP53* mRNA expression were greater in IREF compared to SHAM PE ($P \leq 0.013$) and 3hPE in *NRF1* only ($P = 0.002$). There was no change in the phosphorylated protein content or gene expression of any angiogenesis related target in IREF compared to SHAM. Wearing IREF before, during and following HIIE augments some of the acute cellular signalling cascades associated with mitochondrial biogenesis.

Keywords: Skeletal muscle adaptation; Mitochondrial biogenesis; Angiogenesis; Gene expression; Protein phosphorylation

46 **NEW & NOTEWORTHY**

47 Infrared radiation (IR) has been demonstrated to activate skeletal muscle cellular signalling
48 pathways. We show that wearing IR emitting fabric (IREF) before, during and following high-
49 intensity interval exercise (HIIE) augmented some of the acute cellular signalling cascades
50 associated with mitochondrial biogenesis. Specifically, IREF enhanced the phosphorylation of
51 CaMKII and the mRNA expression of *NRF1* and *TP53* compared to a non-IREF sham condition.
52 Whether these acute changes translate to enhanced chronic adaptation remains to be determined.

INTRODUCTION

Exercise is a potent stimulus for skeletal muscle adaptation that involves the activation of cellular signalling pathways associated with mitochondrial biogenesis, the formation of new components of the mitochondrial reticulum, and angiogenesis, the formation of new capillaries from the existing capillary bed. Numerous interventions have been utilised to augment these cellular signalling responses to enhance physiological adaptation and ultimately improve endurance exercise performance (1, 2). These interventions include but are not limited to; i) exercise training in a glycogen depleted state (3), ii) exogenous dietary supplementation (e.g., caffeine, bicarbonate and β -alanine) (4) and iii) blood flow restriction (5). Another novel intervention that has the potential to augment the exercise induced cellular signalling response involves the use of infrared (IR) radiation.

When used within safety limits (6), IR is a non-ionising electromagnetic wave that can be separated into IR-A (0.78 – 1.4 μm), IR-B (1.4 – 3.0 μm) and IR-C (3 – 1000 μm) wavelengths (7). At an intensity $> 5 \text{ mW} \cdot \text{cm}^{-2}$ IR radiation can heat tissue to exert an effect on cellular signalling cascades (8–10). Alternatively, lower powered irradiation ($< 5 \text{ mW} \cdot \text{cm}^{-2}$) passes energy from IR-C waves to increase the dielectric friction of water bound to subcellular components, such as ion channels or proteins, which initiates vibration and alters their structure and/or function (9, 11–13). Consequently, the effects of IR-C radiation may be passed indirectly through tissue to activate cellular signalling cascades. Such mechanisms are important for IR-C wavelengths to influence muscle tissue as this wavelength range does not directly penetrate epidermal layers (14–16).

Previous work investigating the potential for IR to alter cell signalling pathways have used IR lamps and radiators that can emit up to $30 \text{ mW} \cdot \text{cm}^{-2}$. For example, 15-60 minutes IR exposure in cultured endothelial cells and murine models, has been demonstrated to increase NO production, in addition to the phosphorylation of Ca^{2+} /Calmodulin (CaM)-dependant kinase II (CaMKII) and p38 mitogen-activated protein kinase (MAPK) (8, 17–20) both of which are required for nuclear translocation and subsequent production of new components of the mitochondrial reticulum (1, 21, 22). Increased nuclear factor erythroid 2-related factor 2 (Nrf2) and p53 protein content has also been observed with IR exposure, which enables transduction of homeostatic disturbances to enhance nuclear respiratory factor 1 (*NRF1*) and mitochondrial transcription factor A (*TFAM*) expression (23–26). These are two key factors required to increase mitochondrial DNA and protein synthesis (27, 28). Increased Ca^{2+} and CaM nuclear translocation have also been demonstrated following up to 30-minutes of IR radiator exposure (17, 29). These factors are essential for the downstream signalling cascade involving CaMKII and peroxisome proliferator-activated receptor-gamma coactivator-1 α (PGC-1 α) which work concurrently with TFAM and NRF1 to increase mitochondrial DNA production (27, 28).

In addition to the mitochondrial biogenesis pathways, IR may also activate key angiogenic pathways. These involve the release of vascular endothelial growth factor (VEGF) from subsarcolemmal vesicles into the muscle interstitium and subsequent signal transduction through VEGF receptor 2 (*KDR*) (30, 31). Furthermore, endothelial nitric oxide synthase (eNOS) phosphorylation (30–32) and hypoxia-inducible factor 1 α (*HIF-1* α) expression are important for increasing *VEGFA* mRNA abundance for angiogenesis (33). Indeed, this was demonstrated following 3 days of IR incubation in human umbilical vein

endothelial cells (HUVEC) in which both VEGF secretion and *HIF-1 α* mRNA expression were increased (34). Additionally, increased eNOS phosphorylation and NO production were demonstrated following up to 60-minutes IR exposure in endothelial cells (8, 17–20).

Despite these observations, such IR emitters have limited practical application during exercise. A novel, more accessible and convenient method of delivering IR radiation is IR emitting fabric (IREF). IREF contains metal oxides (i.e., silicon oxide, aluminium oxide and titanium oxide) that are embedded into the yarn fibres. These compounds absorb radiation (e.g., body heat and sunlight) that is re-emitted to its surroundings, including into the body, predominantly in the IR-C wavelength range (35). Predominantly used for clinical applications (36), commercially available IREF can deliver 0.25 mW·cm⁻² of IR in the 2.5–16.7 μ m wavelength range at 35°C (37). Such technology has primarily been used in the context of improving acute exercise performance and recovery (38, 39), while enhanced *PPARGC1 α* mRNA expression has been demonstrated following 72 hours and 1–4 weeks IREF exposure in L6 murine muscle cells (40) and in Sprague-Dawley rats, respectively (41). However, there is limited information on the effect of IREF on adaptive molecular signalling cascades in skeletal muscle after exercise. Furthermore, the effects of IREF on mitochondrial and angiogenic cellular signalling pathways in humans are currently unknown.

The aim of this study was to determine whether IREF would enhance protein translocation and phosphorylation, and gene expression, associated with mitochondrial biogenesis and angiogenesis in response to high-intensity interval exercise (HIIE) compared to a non-IREF sham condition in humans. Specifically, the phosphorylation of AMPK, CaMKII, p38 MAPK, Nrf2, and eNOS,

122 cytosolic-nuclear translocation of AMPK, CaMKII, p38 MAPK, PGC-1 α and Nrf2 and gene
123 expression of *PPARGC1 α* , *NRF1*, *TFAM*, *TP53*, *VEGFA*, *KDR*, *NOS3* and *HIF-1 α* were
124 investigated. We tested the hypothesis that wearing IREF would augment these acute adaptive
125 signals to HIIE compared to a non-IREF sham condition.

126

METHODS

Participants

An *a priori* sample size calculation was conducted in G*power (v3.1.9.2; (42)). An effect size was generated by our laboratory when the effect of IREF on cultured skeletal muscle cells (C2C12) was assessed. A significant interaction for CaMKII phosphorylation (partial eta squared (η^2) = 0.108) was observed which was converted to Cohen's f (f = 0.348) and used alongside an α of 0.05 and a power ($1 - \beta$) of 80%. From these data, the required sample size for the primary muscle cellular signalling outcome was deemed to be 10. Eleven recreationally active males (age, 22 ± 4 yrs; height, 1.72 ± 0.04 m; body mass, 68.5 ± 5.2 kg; mean $\pm$ SD) volunteered to participate in this study following recruitment via posters and online posts. All completed health and muscle biopsy screening questionnaires prior to participation to mitigate for contraindications to maximal exercise and muscle biopsy procedures. Inclusion criteria consisted of, i) undertaking at least one hour of training per week, ii) no history of smoking/vaping, iii) no history of cardiovascular, metabolic, neuromuscular, haematological or musculoskeletal abnormalities or injuries that may have affected study outcomes, iv) no contraindications to the muscle biopsy procedure, including allergy to Lidocaine hydrochloride administration, and v) were not using pharmacological treatments or dietary supplements during the study period. One female volunteered but was excluded from the study due to ongoing pharmacological treatment. Participants were fully informed of the risks and discomforts associated with all experimental trials before providing written, informed consent. All experimental procedures were approved by the Loughborough University Ethics Approvals Human Participants Sub-Committee (2023-16032-16038), and conformed to the Declaration of Helsinki, except for pre-registration in a trial database.

Experimental design

In a repeated-measures, counter-balanced, single-blind design participants performed HIIE wearing either IR-emitting (IREF) or non-IR emitting (SHAM) garments. No resting control condition was undertaken as previous work from our laboratory demonstrated that there was no effect of IREF on acute cellular signalling cascades (phosphorylation of AMPK, CaMKII, p38 MAPK and eNOS and mRNA expression of *PPARGC1 α* and *VEGFA*) following 3 hours wear at rest ($N = 8$; (94)). Participants visited the laboratory on four occasions over a minimum 3-week period. The first visit involved preliminary testing of pulmonary gas exchange threshold (GET), $\dot{V}O_{2peak}$, and peak power output (W_{peak}) followed (at least 2 days later) by a familiarization to the HIIE protocol. Approximately 7 days later participants returned to the laboratory to perform the first experimental trial, followed at least 7 days and less than 2 weeks later by the second experimental trial.

All exercise tests were conducted on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode, Gronigen, The Netherlands). Ergometer saddle and handlebar configurations were recorded during preliminary testing and standardized for the remainder of the testing period. Prior to all visits, participants refrained from ingesting caffeine for 12 hours and alcohol for 24 hours and avoided strenuous exercise (no more than an easy jog/cycle/walk or a light gym session with no high load resistance exercise) for 48 hours. Additionally, 24 hours prior to experimental visits participants refrained from ingesting nitrate-rich foods (e.g., beetroot, green leafy vegetables and cured meats). All tests were conducted in constant laboratory ambient conditions (18-21°C, 40-50% humidity).

Preliminary testing and familiarization

Upon arrival to the laboratory for the first time, height (274 stadiometer, Seca GmbH, Hamburg, Germany), body mass and fat percentage (mBCA 515 bioimpedence scales, Seca GmbH, Hamburg, Germany) were measured. Participants then completed a ramp incremental cycling test to exhaustion to assess GET, $\dot{V}O_{2\text{peak}}$, and W_{peak} . Participants began cycling, at a freely chosen constant pedal cadence, at 25 W with power output increasing linearly at a ramp rate of $30 \text{ W} \cdot \text{min}^{-1}$ (i.e., 1 W every 2 s) until volitional exhaustion or when cadence fell 10% below the chosen cadence for more than 5 s, despite strong verbal encouragement. Pulmonary gas exchange was measured continuously throughout exercise (Vyntus CPX; Carefusion, San Diego, California, USA). $\dot{V}O_{2\text{peak}}$ was defined as the highest $\dot{V}O_2$ for a 30 s period. W_{peak} was defined as the highest work-rate achieved during the test. GET was determined using three criteria (43): 1) the first disproportionate increase in rate of carbon dioxide production ($\dot{V}CO_2$) in proportion to $\dot{V}O_2$; 2) an increase in expired ventilatory equivalent of oxygen ($\dot{V}E/\dot{V}O_2$) in the absence of a concomitant increase in the expired ventilatory equivalent of carbon dioxide ($\dot{V}E/\dot{V}CO_2$); 3) the first increase in end-tidal O_2 tension with no fall in end-tidal CO_2 . To determine the power output that evoked GET (WR_{GET}) the linear interpolation method was used with account taken for the mean response time for $\dot{V}O_2$ during the ramp protocol (i.e., two-thirds of the ramp rate (20 W) deducted from the WR_{GET} to account for the muscle-to-lung gas transit time). From these data the power output equivalent to $60\%\Delta$ (60% of the difference between W_{GET} and $W_{\text{peak}} + W_{\text{GET}}$) was calculated to be used in the HIIE protocol. At least 2 days later, participants were familiarised with the HIIE protocol by performing 3 x 3 minutes of cycling at the $60\%\Delta$ power output.

195

196 **Experimental protocol**

197 A meal was provided on the evening before the visit (i.e., pizza and a chocolate bar providing, 109
198 g carbohydrate, 39 g protein, 40 g fat) with no other food consumed after 7 pm and water consumed
199 *ad libitum*. Participants were asked to record their physical activity for 48 hours and dietary intake
200 for 24 hours preceding the first experimental visit and to repeat this prior to the second
201 experimental visit.

202

203 On the morning of the trial, participants arrived at 8 am ($\pm$ 1 hour) and were provided with a
204 standardised breakfast ($1.5 \text{ g}\cdot\text{kg}^{-1}$ of body mass (BM) carbohydrate, $0.4 \text{ g}\cdot\text{kg}^{-1}$ BM protein, 0.4
205 $\text{g}\cdot\text{kg}^{-1}$ BM fat, with a total energy intake of $\sim 50 \text{ kJ}\cdot\text{kg}^{-1}$ BM) consisting of cereal, milk, toast and
206 a cereal bar, and water *ad libitum*. Thirty minutes after finishing breakfast, participants rested in a
207 supine position for approximately 10 minutes before a baseline blood sample and muscle biopsy
208 were obtained. Skin temperature (T_{skin}) sensors were then attached to the contralateral side to
209 which the muscle biopsy was taken. Following this, either the IREF or SHAM clothing was
210 donned which comprised of a t-shirt and cycling leggings (bib-tights). Participants remained in a
211 resting position (seated or semi-supine) for 75 minutes. At this point, participants returned to a
212 supine position for 10 minutes before another blood sample was obtained. These procedures
213 resulted in a 90-minute period of pre-exercise clothing wear prior to commencing the HIIE to
214 reflect the wear time suggested to increase tissue oxygen saturation in previous studies utilising
215 IREF (44, 45).

216

Participants then commenced the exercise protocol. This consisted of an initial warmup of 5 min at 25 W followed by 10 x 3 min intervals at 60%Δ with 3 min recovery at 25 W between each interval. Participants were instructed to maintain a constant cadence, and water was available *ad libitum* throughout the exercise. Heart rate (HR) was measured within the final 15 s of each interval. Capillary blood samples for the measurement of lactate concentration ($[La^-]$) were obtained pre-exercise, within the final 15 s of the 1st, 5th and 10th interval of the HIIE and 10 minutes post-exercise (post₁₀).

Immediately after completing the exercise participants dismounted the cycle ergometer and quickly moved to an adjacent laboratory where post-exercise (PE) venous and capillary blood samples and a muscle biopsy were obtained. This process took approximately 10 minutes. Participants were then provided with a snack bar containing 15 g of carbohydrate and 15 g of protein (Maximuscle Protein Bar, Maximuscle, Nantwich, UK) and allowed to shower, after which they were given a fresh set of experimental (IREF or SHAM) clothing (t-shirt and leggings). Participants then rested (in a seated or semi-supine position) for 3 hours. During this period, they were able to work or use an electronic device for entertainment. Finally, a 3 hour PE (3hPE) venous blood sample and muscle biopsy were obtained. One participant was exhausted after 8 intervals of HIIE during their first experimental visit and therefore repeated this number of intervals on their second experimental visit. Their muscle tissue samples were included at all post-exercise timepoints as the session was deemed sufficient to elicit the desired stimulus. A schematic depicting the timeline for an experimental visit is shown in Figure 1.

Experimental clothing

In the IREF experimental trial participants wore garments (Kymira Ltd, Reading, UK) containing IR emitting polyester (Celliant[®], Hologenix LLC, CA, USA) with metal oxides (i.e., silicon oxide, aluminium oxide and titanium oxide) contained within the yarn fibres. IR emission occurred at an intensity of 0.25 mW·cm⁻² in the 2.5-16.7 µm wavelength range at 35°C (37) (Table 1). IR emission was verified to be within this wavelength range from fabric used in this study. In the SHAM trial participants wore garments (Kymira Ltd, Reading, UK; Danish Endurance, Copenhagen, Denmark; Nike, Oregon, USA) that were matched as closely as possible for material composition, fit and colour (Table 1).

Physiological measures

Skin temperature

T_{skin} sensors (DS1922L ibuttons; Measurement Systems Ltd, Newbury, UK) were placed on the chest, upper arm, thigh and calf on the same side of the body with measurements pooled for determination of weighted mean skin temperature (46). Data were sampled every minute with an average of 5 min of data used at each respective timepoint: baseline, 90 min following the donning of clothing pre-exercise (Pre-ex) and 3hPE values. The data point corresponding to the last minute of each HIIE interval repetition was used to analyse T_{skin} during exercise.

Capillary blood sampling and analysis

Capillary blood samples (20 µL) were collected from the finger into capillary tubes and immediately placed into haemolysing solution (EKF Diagnostics, Cardiff, UK). Blood [La⁻] was determined using a Biosen C-Line analyzer (EKF Diagnostics, Cardiff, UK) within 4 hours of collection.

262 *Heart rate*

263 HR was recorded using a Polar Unite watch with a Polar H10 HR sensor (Polar, Kempele, Finland)
264 placed around the chest, below the sternum.

265

266 **Venous blood sampling and analysis**

267 Venous blood was obtained from an antecubital vein using a 21G gauge needle (BD Valu-Set;
268 Becton-Dickinson, Helsingborg, Sweden). Samples were collected (6 ml, Lithium Heparin
269 Vacutainer, Becton-Dickinson, Helsingborg, Sweden) and, within 2 minutes, centrifuged ($3500 \times$
270 g at 4°C for 10 min). Plasma was aliquoted and stored at -80°C for subsequent analysis.

271 *Plasma $[\text{NO}_2^-]$*

272 Plasma nitrite concentration ($[\text{NO}_2^-]$) was measured using 500 μL injections as previously
273 described (47). The CV for this analysis was 7.3%.

274 *Plasma cGMP*

275 Plasma cGMP concentration was measured using a commercially available ELISA (cat. No.
276 581021; Cayman Chemical Company, MI, USA) according to the manufacturer's instructions.
277 Briefly, prior to plate loading, samples were mixed 1:1 with ethanol and vacuum dried (miVac
278 Quattro Concentrator, GenevacTM, Ipswich, UK). Samples were then re-suspended in supplied
279 buffer prior to plate loading. Once loaded in a 96-well plate, samples were incubated at room
280 temperature for 18 hours before absorbance was measured on a Varioskan LUX multimode
281 microplate reader (Thermo Fisher Scientific, Loughborough, UK) at 405 and 420 nm. The intra-
282 assay CV was 6.4%.

283

284 **Muscle sampling and analysis**

285

Skeletal muscle biopsies were obtained from the lateral portion of the vastus lateralis under local anesthesia (1% lidocaine, 4 mL) using the percutaneous needle biopsy technique (48) with suction. Experimental clothing was pulled below the knee to allow access to the biopsy site. In each experimental trial, samples were obtained from the same leg, ~2 cm apart and in the second experimental trial, from the contralateral leg. Muscle samples (~150 mg) were blotted on filter paper to remove excess blood and cleaned of fat and connective tissue and portioned for subsequent analysis before storage at -80°C until analysis.

Cytosolic and nuclear protein fractions

Approximately 20-50 mg (wet weight) of tissue was fractionated into cytosolic and nuclear components using a NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Fisher Scientific, Loughborough, UK). Samples were homogenized in cytoplasmic extraction reagent (CER)-I buffer using a glass pestle and mortar (DWK Life Sciences, Wertheim, Germany) prior to centrifugation at 16,000 g in CER-II buffer. Supernatant (crude cytosolic fraction) was then removed and stored at -80°C. Remaining pellets were washed 5 times in phosphate-buffered saline (PBS) before addition of nuclear extraction reagent and centrifugation at 21,100 g. Supernatant was then removed (crude nuclear fraction) and stored at -80°C. All buffers and PBS were supplemented with protease and phosphatase inhibitors (Halt™, Thermo Fisher, USA). Purification of all cytosolic and nuclear fractions was confirmed by LDHA (cytosolic) and Histone H3 (nuclear) protein content. Purity was calculated as the ratio between LDHA and Histone H3 in an individual target compartment divided by the sum of cytosolic and nuclear compartments.

Immunoblotting

Cytosolic and nuclear sample protein concentration was quantified using a commercial colorimetric assay according to the manufacturer's instructions (Pierce™ BCA Protein Assay,

Thermo Fisher Scientific, Loughborough, UK). Samples were prepared at 0.75 or 1.5 $\mu\text{g}/\mu\text{L}$ of protein for nuclear and cytosolic fractions, respectively, and combined with 4x LDS buffer (Invitrogen, Loughborough, UK) and β -mercaptoethanol (Sigma-Aldrich, Dorset, UK) as required. Samples were boiled for 5 min at 95 °C then stored at -20 °C until analysis. For SDS-PAGE, protein loading for each target was pre-determined (using serial dilution optimisations) with either 5 μL (AMPK, p38 MAPK, PGC-1 α , LDHA, Histone H3, and Nrf2) or 10 μL (eNOS & CaMKII) of cytosolic sample and 10 μL (AMPK, p38 MAPK, PGC-1 α , LDHA, Histone H3 and Nrf2) or 13.3 μL (CaMKII) of nuclear sample loaded per lane. Membranes were first probed for phosphorylated (*p*-) eNOS, AMPK, CaMKII, p38 MAPK and Nrf2 before being stripped for 2 hours at 37°C (Restore™ stripping buffer, Thermo Fisher Scientific, Loughborough, UK) and probed for the requisite total (*t*-) protein target. PGC-1 α , LDHA and Histone H3 were probed for the *t*- target only.

Samples were loaded into a 15 lane 4–15% Mini-PROTEAN™ TG Stain-free™ protein gel (Bio-Rad, Hertfordshire, UK) and run for 1 hour at 150 V. The gel was activated by UV exposure for 1 min before protein was transferred to a PVDF membrane (Bio-Rad, Hertfordshire, UK), that was pre-activated for 1 min in methanol, using a Trans-blot Turbo Transfer System (Bio-Rad, Hertfordshire, UK) at 25 V for 30 min in transfer buffer (80% dH₂O, 10% 10x Tris/Glycine buffer [Bio-Rad, Hertfordshire, UK], 10% methanol). Membranes were then imaged using a Stain Free Blot setting to determine total protein for use as a loading control before being washed in Tris Buffered Saline with Tween (TBST) for 5 min. Subsequently, membranes were blocked (Supplemental Table S1) and cut at the molecular weight of the protein of interest. Following overnight incubation at 4°C in the requisite primary antibody, membranes were incubated with

secondary antibody for 1 hour at room temperature (Supplemental Table S1). Clarity Western ECL Substrate (Bio-Rad, Hertfordshire, UK) was applied to membranes for 5 min in the dark prior to imaging in a ChemidocTM imaging system (Bio-Rad, Hertfordshire, UK). Band densities were quantified using image analysis software (ImageLabTM v6.0, Bio-Rad, Hertfordshire, UK). Membranes were washed for 3 x 5 min in TBST before and after each antibody incubation. Antibodies were purchased from Cell Signalling Technologies (MA, USA) and Abcam (Cambridge, UK) (Supplemental Table S1). Phosphorylated proteins were expressed relative to total protein and total protein relative to total membrane protein.

RNA isolation

Approximately 10-30 mg (wet weight) of tissue was homogenized (Tissue lyser Qiagen, Hilden, Germany) in TRIzolTM reagent (Invitrogen, Loughborough, UK) for 2 x 2 min at 20 Hz. The solution was separated into an organic and aqueous phase following the addition of chloroform and centrifugation. RNA was isolated from the aqueous phase following precipitation with 2-propanolol. The RNA pellet was washed with 75% ethanol, air dried, and suspended in RNA Storage solution (Thermo Fisher, USA). The quantity and quality of RNA was determined spectrophotometrically using a Nanodrop 2000 (Thermo Fisher Scientific, Loughborough, UK) at optical densities of 260 and 280 nm. RNA was extracted with a yield of $241.7 \pm 82.1 \text{ ng} \cdot \mu\text{l}^{-1}$ and 260/280 nm absorbance of 2.05 ± 0.03 .

RT-qPCR

Two-step RT-qPCR was used to analyse the gene expression of *GAPDH*, *PPARGC1 α* , *NRF1*, *TFAM*, *TP53*, *VEGFA*, *KDR*, *NOS3* and *HIF-1 α* . Genomic DNA was removed from 750 ng of RNA by addition of gDNA removal mix prior to a 2 min incubation at 45°C. RNA was then reverse transcribed to cDNA using a commercially available kit (QuantiNova[®] Reverse Transcription Kit,

Qiagen, Hilden, Germany) according to manufacturer's instructions. cDNA reactions were diluted tenfold and stored at -20°C before addition to the PCR cocktail. RT-qPCR was performed using a QuantiTect Studio 6 Pro cycler (Thermo Fisher Scientific, Loughborough, UK) in 10 µL reactions consisting of 5 µl QuantiNova® SYBR® Green master mix (Qiagen, Hilden, Germany), 100 nM forward and reverse primer, 0.05 µl carboxyrhodamine and 1 µL cDNA template. Reactions were performed in triplicate consisting of 2 min at 95°C followed by 40 cycles of 5 sec at 95°C and 10 sec at 60°C. Target gene cycle threshold (C_T) values were normalized to GAPDH and presented as a fold change from the individual within condition baseline using the $2^{(-\Delta\Delta C_T)}$ method (49). The reference gene GAPDH showed low within-subject variability, with a mean CV of 2.7% (range 1.8 - 3.6%) across all time points. Primers optimised for use with two-step PCR are displayed in Supplemental Table S2. Outliers were identified as samples with a value greater than 3 SD from the mean. Where two or more samples were identified as outliers for one participant, the individual was removed from the analysis of the gene target.

Statistical analysis

Data were analysed using Jamovi v2.4.11 (50). A two-way condition (IREF or SHAM) x time linear mixed model (LMM) was used to analyse data with participants as a random factor. The number of time points for different analyses are indicated in brackets: immunoblot and PCR (3), cGMP and plasma $[NO_2^-]$ (4), T_{skin} (13), blood $[La^-]$ (5), and HR (11). Holm-Bonferroni corrected *post-hoc t*-tests were carried out where significant main effects or interactions were detected. Normality was assessed using the Shapiro-Wilk statistic, however non-normal data were still included due to the robustness of the LMM (51). Statistical significance was accepted at $P < 0.05$. η^2 effect sizes were calculated for omnibus tests (small: 0.01, medium: 0.06, large: ≥ 0.14) and

Hedges g (g) bias correction effect sizes were calculated for *post hoc t*-tests (small: 0.2, medium: 0.5, large: ≥ 0.8) (52). All data are $N = 11$ and presented as mean $\pm$ SD unless otherwise stated.

RESULTS

Physiological responses to HHE

Physiological and performance parameters from preliminary testing were as follows: $\dot{V}O_{2\text{peak}}$, $48.0 \pm 4.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; W_{peak} , $316 \pm 34 \text{ W}$; W_{GET} , $132 \pm 16 \text{ W}$; 60% Δ , $226 \pm 27 \text{ W}$.

Skin temperature

There was a main effect of time and a condition $\times$ time interaction, but no main effect of condition for T_{skin} (Fig. 2). There was no difference between IREF and SHAM at any timepoint.

Heart rate and blood lactate

There were main effects of time but no main effects of condition or condition $\times$ time interactions for HR or blood $[\text{La}^-]$ (Supplemental Figure S1 & S2).

Plasma $[\text{NO}_2^-]$ and cGMP

There was a main effect of time but no main effect of condition or condition $\times$ time interaction for plasma $[\text{NO}_2^-]$ and cGMP responses (Supplemental Figure S3 & S4).

Muscle protein phosphorylation and content

Fraction quality analysis confirmed the attainment of highly specific cytosolic and nuclear fractions of skeletal muscle tissue (Supplemental Table S3 & Supplemental Figure S5). Representative Western blot membrane images and a stain free gel image are shown in Fig. 3. Due to limited tissue availability, one SHAM 3hPE data point was missing from cytosolic fractions and

one data point from IREF and SHAM baseline and one from SHAM 3hPE were missing from nuclear fractions.

AMPK

There was a main effect of time but no main effect of condition or condition x time interaction for *p*-AMPK content in the cytosolic fraction (Fig. 4A). There was a main effect of time and a main effect of condition but no condition x time interaction for *p*-AMPK content in the nuclear fraction (Fig. 4B).

CaMKII

There was no main effect of time but there was a main effect of condition and condition x time interaction for cytosolic *p*-CaMKII content (Fig. 4C) which was greater PE in IREF compared to SHAM (4.04 ± 1.15 a.u. vs 2.42 ± 0.61 a.u.; $P = 0.001$, $g = 1.691$). There was no main effect of time but there was a main effect of condition and condition x time interaction for nuclear *p*-CaMKII content (Fig. 4D) which was greater PE in IREF compared to SHAM (5.58 ± 1.78 a.u. vs 2.41 ± 0.67 a.u.; $P = 0.003$, $g = 2.267$).

p38 MAPK

There was no main effect of time or condition but there was a significant condition x time interaction for cytosolic *p*-p38 MAPK content (Fig. 4E) which increased from baseline to PE in IREF (3.25 ± 1.60 a.u. vs 6.59 ± 2.55 a.u.; $P = 0.008$, $g = 1.509$) but not SHAM (4.47 ± 1.91 a.u. vs 4.15 ± 1.53 a.u.; $P = 1.000$, $g = 0.178$). There was no main effect of condition but there was a main effect of time and a significant condition x time interaction for nuclear *p*-p38 MAPK content (Fig. 4F) which increased from baseline to PE in IREF (1.50 ± 1.08 a.u. vs 3.87 ± 1.80 a.u.; $P = 0.005$, $g = 1.514$) but not SHAM (2.24 ± 0.90 a.u. vs 2.56 ± 1.01 a.u.; $P = 1.000$, $g = 0.322$).

Nrf2

There was no main effect of time, condition or condition x time interaction for cytosolic *p*-Nrf2 content (Fig. 4G). There was no main effect for time, condition or condition x time interaction for nuclear *p*-Nrf2 content (Fig. 4H).

PGC-1 α

There was no main effect for time, condition or condition x time interaction for cytosolic *t*-PGC-1 α content (Fig. 4I). There was a main effect of time but no main effect of condition or condition x time interaction for nuclear *t*-PGC-1 α content (Fig. 4J).

eNOS

As expected, eNOS was detected in the cytosol only. There was a main effect of time but no main effect for condition or condition x time interaction for *p*-eNOS content (Fig. 4K).

Muscle gene expression

One participant was removed from this analysis due to being an outlier in multiple targets, therefore gene analysis data is reported from a total of 10 participants.

PPARGC1 α

There was a main effect of time but no main effect of condition or condition x time interaction for *PPARGC1 α* mRNA expression (Fig. 5A).

NRF1

There was no main effect of time, but there was a main effect of condition and condition x time interaction for *NRF1* mRNA expression (Fig. 5B) which was greater PE (2.16 ± 1.24 vs 0.69 ± 0.53 fold change from baseline; $P = 0.013$, $g = 1.478$) and 3hPE (2.34 ± 1.91 vs 0.60 ± 0.51 fold change from baseline; $P = 0.002$, $g = 1.191$) in IREF compared to SHAM. For IREF but not SHAM there was an increase from baseline to 3hPE ($P = 0.030$, $g = 0.950$) but not PE ($P = 0.076$, $g = 1.267$).

448 *TP53*

449 There was a main effect of time, condition and a condition x time interaction for *TP53* mRNA
450 expression (Fig. 5C) which increased from baseline to PE in IREF only (6.41 ± 6.98 fold change
451 from baseline; $P < 0.001$, $g = 1.283$). Furthermore, *TP53* mRNA expression was greater PE in
452 IREF compared to SHAM (6.41 ± 6.98 vs 2.03 ± 3.14 fold change from baseline; $P < 0.001$, $g =$
453 1.278).

454 *TFAM*

455 There was no main effect of time, condition or condition x time interaction for *TFAM* mRNA
456 expression (Fig. 5D).

457 *NOS3*

458 There was a main effect of time but no main effect of condition or condition x time interaction for
459 *NOS3* mRNA expression (Fig. 6A).

460 *HIF-1 α*

461 There was a main effect of time but no main effect of condition or condition x time interaction for
462 *HIF-1 α* mRNA expression (Fig. 6B).

463 *VEGFA*

464 There was a main effect of time and condition (3.55 ± 2.76 vs 1.49 ± 1.21 fold change from
465 baseline) but no condition x time interaction for *VEGFA* mRNA expression (Fig. 6C).

466 *KDR*

467 There was a main effect of time but no main effect of condition or condition x time interaction for
468 *KDR* mRNA expression (Fig. 6D).

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DISCUSSION

The main findings of this study were: 1) in cytosolic and nuclear fractions, content of *p*-CaMKII were greater following HIIE in IREF compared to SHAM; 2) the content of *p*-AMPK, *p*-p38 MAPK, *p*-Nrf2, *p*-eNOS, and *t*-PGC-1 α were not different between IREF and SHAM; 3) mRNA expression of *NRF1*, *TP53* were greater following HIIE in IREF compared to SHAM; 4) mRNA expression of *PPARGC1 α* , *TFAM*, *VEGFA*, *KDR*, *NOS3* or *HIF-1* were not different in IREF compared to SHAM.

Mitochondrial biogenesis involves the phosphorylation and nuclear translocation of sensor proteins including AMPK, CaMKII, and p38 MAPK (27, 28). Wearing IREF before, during and following HIIE resulted in a greater cytosolic and nuclear *p*-CaMKII immediately following HIIE compared to the SHAM condition. The potential mechanism by which IREF may enhance *p*-CaMKII content is uncertain but will likely involve increased $[Ca^{2+}]_i$ (53–55). Exposure to an IR emitting radiator for 30 minutes increased $[Ca^{2+}]_i$ and *p*-CaMKII content simultaneously in endothelial cells (17). An increased $[Ca^{2+}]_i$ may result from greater action potential derived Ca^{2+} release from the sarcoplasmic reticulum (SR). Indeed, previous observations in cultured pancreatic β cells demonstrated that 30 minutes exposure to an IR emitting generator concomitantly increased $[Ca^{2+}]_i$ and the mRNA expression and protein abundance of Cav1.2, a key protein in SR calcium release (56). Alternatively, it may be that the IREF increased the activity of transient receptor potential vanilloid (TRPV) channels, which allow an influx of extracellular Ca^{2+} to the cytoplasm. Such an increase has been demonstrated in tonsil-derived mesenchymal stem cells following 30 minutes of exposure to an IR emitting radiator (9). However, it is unclear if one or more of the mentioned mechanisms or another, yet to be discerned mechanism, were responsible for the

alterations to cellular signalling observed here in human skeletal muscle tissue. Future work should assess SR Ca^{2+} release and reuptake rates (57) which would help determine the effect of IREF on Ca^{2+} flux following HIIE.

Further investigations into $[\text{Ca}^{2+}]_i$ adaptations may also uncover the mechanism associated with the increased cytosolic and nuclear *p*-p38 MAPK content observed in IREF. Previous work has highlighted the association between raised $[\text{Ca}^{2+}]_i$ and *p*-p38 MAPK whereby rat epitrochlearis muscle incubated with caffeine to induce SR Ca^{2+} release, was shown to have greater *p*-p38 MAPK content. Moreover, when incubated with KN93, a CaMKII-specific inhibitor, this effect was abolished, suggesting p38 MAPK phosphorylation is mediated by CaMKII in response to raised $[\text{Ca}^{2+}]_i$ (58). Oxidative stress is also a regulator of p38 MAPK, in addition to inflammatory cytokines and mechanical stress (59–61) and we cannot rule out an effect of these stimuli on p38 MAPK phosphorylation in the current study.

Phosphorylation of AMPK is often considered to be one of the critical steps in the initiation of mitochondrial biogenesis in response to exercise, particularly given the nature of energy transduction that occurs during HIIE (62). In the present study, there was no difference in *p*-AMPK content in IREF compared to SHAM. The only other study to have measured the effect of IR on *p*-AMPK content *in vivo* was Li et al. (63) where 2 weeks of exposure to a 30 W graphene-based IR emitting film resulted in an increased *p*-AMPK content in both exercised and rested mouse gastrocnemius muscle. These observations suggest repeated exposure or a greater ‘dose’ of IR may be required to elicit significant increases in cytosolic and/or nuclear *p*-AMPK content.

The observations of increased *p*-CaMKII and *p*-p38 MAPK content with IREF exposure need to be considered against the context that no increase in the phosphorylation of these sensor proteins were observed PE in the SHAM condition, where an increase in phosphorylation might have been expected (64–70). A possible explanation is the ~10 min delay in obtaining the PE biopsy whereby it was previously demonstrated that 1 minute of recovery following an exhaustive incremental cycling test was sufficient to restore *p*-CaMKII content to resting levels (71, 72). Hence, the current study likely missed the immediate post-exercise signalling response. However, as this appears to have been the case for SHAM and not for IREF, this could suggest that IREF may sustain post-exercise *p*-CaMKII and *p*-p38 MAPK signalling. Subsequently, prolonging the phosphorylation of these kinases could extend the activation of downstream transcription factors involved in mitochondrial biogenesis, although it remains unclear whether this would increase mitochondrial protein abundance (73).

When in the nucleus, CaMKII, p38 MAPK and AMPK phosphorylate intermediary proteins that bind to the PGC-1 α promoter and initiate *PPARGC1 α* transcription (28, 74). However, transcription is not instantaneous, and expression of *PPARGC1 α* mRNA typically peaks 3 h post-exercise (75–80), a finding corroborated in the present study, across both SHAM and IREF conditions. Despite the increased phosphorylation and nuclear accumulation of upstream kinases PE, *PPARGC1 α* mRNA expression 3hPE was not greater in IREF compared to SHAM. The lack of increase in *PPARGC1 α* mRNA expression with IREF contrasts with previous studies employing IREF that observed enhanced *PPARGC1 α* mRNA expression in L6 murine muscle cells following 72 h incubation (40) and following 4 weeks exposure in rats (41). However, as the time course of the upstream signal for *PPARGC1 α* mRNA expression, namely *p*-CaMKII content, appears to be

altered with IREF, it may be that this is also the case for *PPARGC1 α* mRNA expression at a time point other than those measured here. Moreover, *NRF1* mRNA expression was increased in IREF compared to SHAM at both PE and 3hPE. Increases in *NRF1* expression were earlier than the expected 10–24 h time course typical for mitochondrial protein encoding genes post-exercise (81, 82). The signal for the increased *NRF1* mRNA expression is unclear given there was no change in *PPARGC1 α* expression or nuclear PGC1 α protein abundance with IREF wear compared to SHAM. Given the snapshot of muscular signalling provided by muscle biopsies, further study is required to determine the effect of IREF on the time course of acute adaptive signals to HIIE.

Furthermore, there was a greater *TP53* mRNA expression PE with IREF which returned to baseline at 3hPE. Mitochondrial biogenesis is not solely regulated by PGC-1 α with p53 being a putative regulator activated by p38 MAPK and AMPK post-exercise (83). The expression of *TP53* would be expected to peak as much as 48 h post-exercise (84), thus the increased expression seen PE in IREF further suggests an alteration to the time course of mRNA expression compared to SHAM. *TFAM* mRNA expression, a regulator of mitochondrial DNA synthesis that lies downstream of *NRF1* and *TP53*, did not change following HIIE with IREF suggesting the purported acceleration to mRNA expression did not lead to the subsequent increased expression of *TFAM*. The mechanism and time course of mitochondrial gene expression with IREF during HIIE remains to be fully elucidated.

The acute angiogenic response to HIIE with IREF was also investigated. IREF did not influence *p*-eNOS content, or *NOS3* and *HIF-1 α* mRNA expression. These targets are important in shear stress and hypoxia-induced angiogenesis (30, 31, 85, 86). Such a finding contrasts previous work

suggesting 30 minutes of IR exposure in endothelial cells may augment *p*-eNOS and *HIF-1α* mRNA expression (87, 88). In the present study there was no increase in circulating plasma [NO₂⁻], a surrogate of eNOS-derived NO, or [cGMP], a marker of eNOS signalling (89). NO has a VEGF-independent role in angiogenesis, and these findings suggest IREF does not augment NO-dependant angiogenesis in humans following HIIE.

Despite no changes in these angiogenic signals, *VEGFA* mRNA expression indicated a main effect of condition with a large effect size ($\eta p^2 = 0.130$). As baselines were normalised to 1, this indicates *VEGFA* mRNA expression may be increased across both post exercise timepoints in IREF. An increase in *VEGFA* mRNA expression is in contrast to the only other study to assess the effect of IR radiation on *VEGFA* mRNA expression where there was no increase following up to 30 minutes exposure to an electrified ceramic IR emitting plate in endothelial cells (18). PGC-1α may be responsible for the increase in *VEGF* mRNA expression as crosstalk between these signals have been identified post-exercise (90). However, as there was no change to PGC-1α protein expression nor mRNA expression with IREF at the timepoints measured here, further work is required to identify the upstream signal for the increased *VEGF* mRNA expression.

The biophysical mechanisms by which IREF can alter cellular signalling cascades during HIIE in humans is unclear but may be mediated through heat-dependent and/or heat-independent processes. The ‘dose’ of IR supplied by the IREF in the present study is similar to the optimal intensity discerned from a dose response study in cultured endothelial cells which were subjected to up to 90 minutes of irradiation at either 0.13, 0.8, 1.8 and 7.2 mW·cm⁻². Irradiation at 0.13 mW·cm⁻² for 30-minutes was deemed to be most effective for influencing cellular signalling.

Moreover, this irradiation intensity and duration did not result in any change to cell culture media temperature, an increase in which was said to be inhibitory to the effect of IR-C radiation (20). In the present study, IR radiation did not induce any greater increase in T_{skin} compared to SHAM, and whilst measuring intramuscular temperature would confirm if heating occurred at the site of protein signalling, the low intensity emission from an IREF ($0.25 \text{ mW} \cdot \text{cm}^{-2}$) makes bulk tissue heating unlikely (16, 91). Therefore, the observed effects may be heat independent and possibly a result of IR radiation-mediated water vibration (8, 29, 92). IR waves are postulated to increase the dielectric friction of water bound to subcellular components, such as ion channels, initiating vibration and consequently altering the structure and/or function of the associated assembly (11, 93). As IR-C waves do not penetrate directly beyond epidermal layers (14, 15), this mechanism allows a transfer of IR energy deeper into tissue (16). Furthermore, heat production from humans during exercise would warm IREF to a greater extent than at rest. According to the Stefan-Boltzmann Law, higher black body temperature increases IR emission. Thus, there may have been a higher effective ‘dose’ of IR delivered to participants compared to rest. Indeed, in a parallel study, it was demonstrated that there were no changes in the phosphorylation (AMPK, CaMKII, p38 MAPK and eNOS) or mRNA expression (*PPARGC1a* and *VEGFA*) of key proteins measured in this study when IREF was worn over a 3-hour time course at rest (94). Therefore, the observations in the present study appear to be mediated by exercise *per se* through increased IR delivery and/or an effect of IR on the activated signalling cascades.

There are several limitations to the present study. Although protein phosphorylation and content were measured in cytosolic and nuclear fractions from whole muscle, we did not consider any muscle fibre type-specific differences. Type II muscle fibres experience greater rates of glycogen

611 utilisation during HIIE, leading to increased *p*-AMPK content (95). Additionally, type II fibres
612 exhibit larger $[Ca^{2+}]_i$ transients (96) and differential expression of Ca^{2+} handling proteins (97, 98).
613 Thus, it might be possible that the effect of IREF on the adaptive signal processes may be fibre
614 type specific. Unfortunately, no females that volunteered for the study met all inclusion criteria,
615 therefore the observations are unable to be generalised to both biological sexes (99, 100). It is also
616 unclear **if** the acute signalling responses detected in the current study may translate to longer term
617 training adaptations.

618
619 In conclusion, IREF enhanced the phosphorylation of cytosolic and nuclear CaMKII and the
620 mRNA expression of *NRF1* and *TP53* compared to SHAM following HIIE. Wearing IREF before,
621 during and following HIIE appears to augment some of the acute cellular signalling cascades
622 associated with mitochondrial biogenesis. Whether these acute changes translate to enhanced
623 chronic adaptation remains to be determined.

DATA AVAILABILITY

All data are available as Supplemental Material: <https://doi.org/10.17028/rd.lboro.33296322.v1>

SUPPLEMENTAL MATERIAL

Supplemental Figures S1-S5: <https://doi.org/10.17028/rd.lboro.33287259.v3>

Supplemental Tables S1-S3: <https://doi.org/10.17028/rd.lboro.33287472>

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DISCLOSURES

T.B is the founder of Kymira Ltd and had no input into the data collection, analysis or
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AUTHOR CONTRIBUTIONS

J.W.B., T.B. and R.A.F. conceived and designed research; J.W.B. and R.A.F. performed experiments; J.W.B., P.T.H. and R.A.F. analyzed data; J.W.B., H.F.D., N.R.W.M., S.J.B. and R.A.F. interpreted results of experiments; J.W.B. prepared figures; J.W.B. and R.A.F. drafted manuscript; J.W.B., P.T.H., H.F.D., N.R.W.M., S.J.B., T.B. and R.A.F. edited and revised the manuscript; J.W.B., P.T.H., H.F.D., N.R.W.M., S.J.B., T.B. and R.A.F. approved final version of manuscript.

REFERENCES

1. **Hood DA.** Mechanisms of exercise-induced mitochondrial biogenesis in skeletal muscle. *Applied Physiology, Nutrition and Metabolism* 34: 465–472, 2009. doi: 10.1139/H09-045.
2. **Ross M, Kargl CK, Ferguson R, Gavin TP, Hellsten Y.** Exercise-induced skeletal muscle angiogenesis: impact of age, sex, angiocrines and cellular mediators. *Eur J Appl Physiol* 123: 1415–1432, 2023. doi: 10.1007/s00421-022-05128-6.
3. **Bartlett JD, Hawley JA, Morton JP.** Carbohydrate availability and exercise training adaptation: Too much of a good thing? *Eur J Sport Sci* 15: 3–12, 2015. doi: 10.1080/17461391.2014.920926.
4. **Rothschild JA, Bishop DJ.** Effects of Dietary Supplements on Adaptations to Endurance Training. *Sports Medicine* 50: 25–53, 2020. doi: 10.1007/s40279-019-01185-8.
5. **Ferguson RA, Mitchell EA, Taylor CW, Bishop DJ, Christiansen D.** Blood-flow-restricted exercise: Strategies for enhancing muscle adaptation and performance in the endurance-trained athlete. *Exp. Physiol.* 106 Blackwell Publishing Ltd: 837–860, 2021.
6. **ICNIRP.** ICNIRP Guidelines on Limits of Exposure to Incoherent Visible and Infrared Radiation. *Health Phys* 105: 74–96, 2013. doi: 10.1097/HP.0b013e318289a611.
7. **CIE V. Commission Internationale del’ Eclairage (International Commission on Illumination).** International lighting vocabulary. 2nd edition. International Commission on Illumination (CIE).
8. **Hwang S, Lee DH, Lee IK, Park YM, Jo I.** Far-infrared radiation inhibits proliferation, migration, and angiogenesis of human umbilical vein endothelial cells by suppressing secretory clusterin levels. *Cancer Lett* 346: 74–83, 2014. doi: 10.1016/j.canlet.2013.12.011.

9. **Kim HY, Oh SY, Choi YM, Park JH, Kim HS, Jo I.** Transient receptor potential vanilloid 2 mediates the inhibitory effect of far-infrared irradiation on adipogenic differentiation of tonsil-derived mesenchymal stem cells. *Stem Cell Res* 53: 102291, 2021. doi: 10.1016/j.scr.2021.102291.
10. **Blick DW, Adair ER, Hurt WD, Sherry CJ, Walters TJ, Merritt JH.** Thresholds of microwave-evoked warmth sensations in human skin. *Bioelectromagnetics* 18: 403–409, 1997. doi: 10.1002/(SICI)1521-186X(1997)18:6<403::AID-BEM1>3.0.CO;2-6.
11. **Cooke R, Kuntz ID.** The properties of water in biological systems. *Annu Rev Biophys Bioeng* 1: 95–126, 1974. doi: 10.1007/s11483-009-9103-1.
12. **Tait MJ, Franks F.** Water in Biological Systems. *Nature* 230: 91–94, 1971. doi: 10.1038/230091a0.
13. **Samanta T, Matyushov D V.** Dielectric friction, violation of the Stokes-Einstein-Debye relation, and non-Gaussian transport dynamics of dipolar solutes in water. *Phys Rev Res* 3: 023025, 2021. doi: 10.1103/physrevresearch.3.023025.
14. **Bachem A, Reed CI.** The Penetration of Light Through Human Skin. *American Journal of Physiology-Legacy Content* 97: 86–91, 1931. doi: 10.1152/ajplegacy.1931.97.1.86.
15. **Schieke SM, Schroeder P, Krutmann J.** Cutaneous effects of infrared radiation: From clinical observations to molecular response mechanisms. *Photodermatol Photoimmunol Photomed* 19: 228–234, 2003. doi: 10.1034/j.1600-0781.2003.00054.x.
16. **Vatansever F, Hamblin MR.** Far infrared radiation (FIR): Its biological effects and medical applications. *Photonics Lasers Med* 1: 255–266, 2012. doi: 10.1515/plm-2012-0034.

- 718 17. **Park JH, Lee S, Cho DH, Park YM, Kang DH, Jo I.** Far-infrared radiation acutely
719 increases nitric oxide production by increasing Ca²⁺ mobilization and Ca²⁺/calmodulin-
720 dependent protein kinase II-mediated phosphorylation of endothelial nitric oxide synthase
721 at serine 1179. *Biochem Biophys Res Commun* 436: 601–606, 2013. doi:
722 10.1016/j.bbrc.2013.06.003.
- 723 18. **Rau CS, Yang JCS, Jeng SF, Chen YC, Lin CJ, Wu CJ, Lu TH, Hsieh CH.** Far-
724 infrared radiation promotes angiogenesis in human microvascular endothelial cells via
725 extracellular signal-regulated kinase activation. *Photochem Photobiol* 87: 441–446, 2011.
726 doi: 10.1111/j.1751-1097.2010.00853.x.
- 727 19. **Lin CC, Liu XM, Peyton K, Wang H, Yang WC, Lin SJ, Durante W.** Far infrared
728 therapy inhibits vascular endothelial inflammation via the induction of heme oxygenase-1.
729 *Arterioscler Thromb Vasc Biol* 28: 739–745, 2008. doi: 10.1161/ATVBAHA.107.160085.
- 730 20. **Hsu Y-H, Chen Y-C, Chen T-H, Sue Y-M, Cheng T-H, Chen J-R, Chen C-H.** Far-
731 Infrared Therapy Induces the Nuclear Translocation of PLZF Which Inhibits VEGF-
732 Induced Proliferation in Human Umbilical Vein Endothelial Cells. *PLoS One* 7: e30674,
733 2012. doi: 10.1371/journal.pone.0030674.
- 734 21. **Miller BF, Hamilton KL.** A perspective on the determination of mitochondrial
735 biogenesis. *Am J Physiol Endocrinol Metab* 302: 496–499, 2012. doi:
736 10.1152/ajpendo.00578.2011.-The.
- 737 22. **Ryan MT, Hoogenraad NJ.** Mitochondrial-nuclear communications. *Annu Rev Biochem*
738 76: 701–722, 2007. doi: 10.1146/annurev.biochem.76.052305.091720.
- 739 23. **Piantadosi CA, Carraway MS, Babiker A, Suliman HB.** Heme oxygenase-1 regulates
740 cardiac mitochondrial biogenesis via nrf2-mediated transcriptional control of nuclear

respiratory factor-1. *Circ Res* 103: 1232–1240, 2008. doi:

10.1161/01.RES.0000338597.71702.ad.

24. **Park JY, Wang PY, Matsumoto T, Sung HJ, Ma W, Choi JW, Anderson SA, Leary SC, Balaban RS, Kang JG, Hwang PM.** P53 improves aerobic exercise capacity and augments skeletal muscle mitochondrial DNA content. *Circ Res* 105: 705–712, 2009. doi: 10.1161/CIRCRESAHA.109.205310.
25. **Bartlett JD, Close GL, Drust B, Morton JP.** The emerging role of p53 in exercise metabolism. *Sports Medicine* 44: 303–309, 2014.
26. **Kensler TW, Wakabayashi N, Biswal S.** Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 47: 89–116, 2007. doi: 10.1146/annurev.pharmtox.46.120604.141046.
27. **Miller KN, Clark JP, Anderson RM.** Mitochondrial regulator PGC-1 α —Modulating the modulator. *Curr Opin Endocr Metab Res* 5: 37–44, 2019. doi: 10.1016/j.coemr.2019.02.002.
28. **Egan B, Sharples AP.** Molecular responses to acute exercise and their relevance for adaptations in skeletal muscle to exercise training. *Physiol Rev* 103: 2057–2170, 2023. doi: 10.1152/physrev.00054.2021.
29. **Cho DH, Lee HJ, Lee JY, Park JH, Jo I.** Far-infrared irradiation inhibits breast cancer cell proliferation independently of DNA damage through increased nuclear Ca²⁺/calmodulin binding modulated-activation of checkpoint kinase 2. *J Photochem Photobiol B* 219: 112188, 2021. doi: 10.1016/j.jphotobiol.2021.112188.
30. **Egginton S.** Invited review: Activity-induced angiogenesis. *European Journal of Physiology* 457: 963–977, 2009. doi: 10.1007/s00424-008-0563-9.

31. **Hoier B, Hellsten Y.** Exercise-induced capillary growth in human skeletal muscle and the dynamics of VEGF. *Microcirculation* 21: 301–314, 2014. doi: 10.1111/micc.12117.
32. **Prior BM, Yang HT, Terjung RL.** What makes vessels grow with exercise training? *J Appl Physiol* 97: 1119–1128, 2004. doi: 10.1152/japplphysiol.00035.2004.-Exercise.
33. **Gustafsson T, Puntschart A, Kaijser L, Jansson E, Sundberg CJ.** Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. *Am J Physiol Heart Circ Physiol* 276: 679–685, 1999. doi: 10.1152/ajpheart.1999.276.2.h679.
34. **Chen X, Zhang H, Zeng W, Wang N, Lo HH, Ip CK, Yang LJ, Hsiao WLW, Sin WM, Xia C, Law BYK, Wong VKW.** Far infrared irradiation suppresses experimental arthritis in rats by down-regulation of genes involved inflammatory response and autoimmunity. *J Adv Res* 38: 107–118, 2022. doi: 10.1016/j.jare.2021.08.015.
35. **Kyselovic J, Masarik J, Kechemir H, Koscova E, Turudic II, Hamblin MR.** Physical properties and biological effects of ceramic materials emitting infrared radiation for pain, muscular activity, and musculoskeletal conditions. *Photodermatol Photoimmunol Photomed* 39: 3–15, 2023. doi: 10.1111/phpp.12799.
36. **Liau BY, Leung TK, Ou MC, Ho CK, Yang A, Lin YS.** Inhibitory effects of far-infrared ray-emitting belts on primary dysmenorrhea. *International Journal of Photoenergy* 2012: 1–6, 2012. doi: 10.1155/2012/238468.
37. **Anderson DM, Fessler JR, Pooley MA, Seidel S, Hamblin MR, Beckham HW, Brennan JF.** Infrared radiative properties and thermal modeling of ceramic-embedded textile fabrics. *Biomed Opt Express* 8: 1698, 2017. doi: 10.1364/boe.8.001698.

- 786 38. **Nunes RFH, Cidral-Filho FJ, Flores LJF, Nakamura FY, Rodriguez HFM, Bobinski**
787 **F, De Sousa A, Petronilho F, Danielski LG, Martins MM, Martins DF, Guglielmo**
788 **LGA.** Effects of Far-Infrared Emitting Ceramic Materials on Recovery During 2-Week
789 Preseason of Elite Futsal Players. *J Strength Cond Res* 34: 235–248, 2020. doi:
790 10.1519/JSC.0000000000002733.
- 791 39. **Mantegazza V, Contini M, Botti M, Ferri A, Dotti F, Berardi P, Agostoni P.**
792 Improvement in exercise capacity and delayed anaerobic metabolism induced by far-
793 infrared-emitting garments in active healthy subjects: A pilot study. *Eur J Prev Cardiol*
794 25: 1744–1751, 2018. doi: 10.1177/2047487318768598.
- 795 40. **Seo Y, Kim YW, Lee D, Kim D, Kim K, Kim T, Baek C, Lee Y, Lee J, Lee H, Jang G,**
796 **Jeong W, Choi J, Hwang D, Suh JS, Kim SW, Kim HK, Han J, Bang H, Kim JH,**
797 **Zhou T, Ko JH.** Far-infrared rays enhance mitochondrial biogenesis and GLUT3
798 expression under low glucose conditions in rat skeletal muscle cells. *Korean Journal of*
799 *Physiology and Pharmacology* 25: 167–175, 2021. doi: 10.4196/KJPP.2021.25.2.167.
- 800 41. **Lee D, Kim YW, Kim JH, Yang M, Bae H, Lim I, Bang H, Go KC, Yang GW, Rho**
801 **YH, Park HS, Park EH, Ko JH.** Improvement characteristics of bio-active materials
802 coated fabric on rat muscular mitochondria. *Korean Journal of Physiology and*
803 *Pharmacology* 19: 283–289, 2015. doi: 10.4196/kjpp.2015.19.3.283.
- 804 42. **Faul F, Erdfelder E, Lang A-G, Buchner A.** G*Power 3: A flexible statistical power
805 analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*
806 39: 175–191, 2007. doi: 10.3758/BF03193146.

- 807 43. **Beaver WL, Wasserman K, Whipp BJ, Whipp BJA.** A new method for detecting
808 anaerobic threshold by gas exchange. *J Appl Physiol* 60: 2020–2027, 1986. doi:
809 10.1152/jappl.1986.60.6.2020.
- 810 44. **Washington K, Wason J, Thein M, Lavery L, Hamblin M, Gordon I.** Randomized
811 Controlled Trial Comparing the Effects of Far-Infrared Emitting Ceramic Fabric Shirts
812 and Control Polyester Shirts on Transcutaneous PO₂. *J Text Sci Eng* 08, 2018. doi:
813 10.4172/2165-8064.1000349.
- 814 45. **Gordon IL, Casden S, Vangel M, Hamblin MR.** Effect of Shirts with 42% Celliant™
815 Fiber on tcPO₂ Levels and Grip Strength in Healthy Subjects: A Placebo-controlled
816 Clinical Trial. *J Text Sci Eng* 9, 2019. doi: <https://doi.org/10.1108/rjta-03-2021-0032>.
- 817 46. **Ramanathan NL.** A new weighting system for mean surface temperature of the human
818 body. *J Appl Physiol* 19: 531–533, 1964. doi: 10.1152/jappl.1964.19.3.531.
- 819 47. **Rowland SN, James LJ, O'Donnell E, Bailey SJ.** Influence of acute dietary nitrate
820 supplementation timing on nitrate metabolism, central and peripheral blood pressure and
821 exercise tolerance in young men. *Eur J Appl Physiol* 124: 1381–1396, 2024. doi:
822 10.1007/s00421-023-05369-z.
- 823 48. **Bergström J.** Percutaneous Needle Biopsy of Skeletal Muscle in Physiological and
824 Clinical Research. *Scand J Clin Lab Invest* 35: 609–616, 1975. doi:
825 10.1080/00365517509095787.
- 826 49. **Livak KJ, Schmittgen TD.** Analysis of Relative Gene Expression Data Using Real-Time
827 Quantitative PCR and the 2^{-C T} Method. *METHODS* 2: 402–408, 2001. doi: 10.1006/m.
- 828 50. **Jamovi (Version 2.6) [Computer Software].** The Jamovi Project.
829 <https://www.jamovi.org>: 2025.

- 830 51. **Schielzeth H, Dingemanse NJ, Nakagawa S, Westneat DF, Allegate H, Teplitsky C,**
831 **Réale D, Dochtermann NA, Garamszegi LZ, Araya-Ajoy YG.** Robustness of linear
832 mixed-effects models to violations of distributional assumptions. *Methods Ecol Evol* 11:
833 1141–1152, 2020. doi: 10.1111/2041-210X.13434.
- 834 52. **Lakens D.** Calculating and reporting effect sizes to facilitate cumulative science: A
835 practical primer for t-tests and ANOVAs. *Front Psychol* 4: 1–12, 2013. doi:
836 doi.org/10.3389/fpsyg.2013.00863.
- 837 53. **Chin ER.** Intracellular Ca²⁺ Signaling in Skeletal Muscle. *Exerc Sport Sci Rev* 38: 76–
838 85, 2010. doi: 10.1097/JES.0b013e3181d495d2.
- 839 54. **Chin ER.** The role of calcium and calcium/calmodulin-dependent kinases in skeletal
840 muscle plasticity and mitochondrial biogenesis. *Proceedings of the Nutrition Society* 63:
841 279–286, 2004. doi: 10.1079/pns2004335.
- 842 55. **Coffey VG, Hawley JA.** The Molecular Bases of Training Adaptation. *Sports Medicine*
843 37: 737–763, 2007. doi: https://doi.org/10.2165/00007256-200737090-00001.
- 844 56. **Hsu Y-H, Chen Y-C, Chen Y-W, Chiu T-H, Kuo Y-T, Chen C-H.** Far-infrared
845 radiation prevents decline in β -cell mass and function in diabetic mice via the
846 mitochondria-mediated Sirtuin1 pathway. *Metabolism* 104: 154143, 2020. doi:
847 10.1016/j.metabol.2020.154143.
- 848 57. **Ørtenblad N, Nielsen J, Saltin B, Holmberg HC.** Role of glycogen availability in
849 sarcoplasmic reticulum Ca²⁺ kinetics in human skeletal muscle. *Journal of Physiology*
850 589: 711–725, 2011. doi: 10.1113/jphysiol.2010.195982.
- 851 58. **Wright DC, Geiger PC, Han DH, Jones TE, Holloszy JO.** Calcium induces increases in
852 peroxisome proliferator-activated receptor γ coactivator-1 α and mitochondrial biogenesis

by a pathway leading to p38 mitogen-activated protein kinase activation. *Journal of Biological Chemistry* 282: 18793–18799, 2007. doi: 10.1074/jbc.M611252200.

59. **Kyriakis JM, Avruch J.** Mammalian Mitogen-Activated Protein Kinase Signal Transduction Pathways Activated by Stress and Inflammation. *Physiol Rev* 81: 807–869, 2001. doi: 10.1152/physrev.2001.81.2.807.
60. **Nordgaard C, Vind AC, Stonadge A, Kjøbsted R, Snieckute G, Antas P, Blasius M, Reinert MS, Del Val AM, Bekker-Jensen DB, Haahr P, Miroshnikova YA, Mazouzi A, Falk S, Perrier-Groult E, Tiedje C, Li X, Jakobsen JR, Jørgensen NO, Wojtaszewski JF, Mallein-Gerin F, Andersen JL, Pennisi CP, Clemmensen C, Kassem M, Jafari A, Brummelkamp T, Li VS, Wickström SA, Olsen JV, Blanco G, Bekker-Jensen S.** ZAK β is activated by cellular compression and mediates contraction-induced MAP kinase signaling in skeletal muscle. *EMBO J* 41, 2022. doi: 10.15252/embj.2022111650.
61. **Kim JS, Saengsirisuwan V, Sloniger JA, Teachey MK, Henriksen EJ.** Oxidant stress and skeletal muscle glucose transport: Roles of insulin signaling and p38 MAPK. *Free Radic Biol Med* 41: 818–824, 2006. doi: 10.1016/j.freeradbiomed.2006.05.031.
62. **Chen ZP, Stephens TJ, Murthy S, Canny BJ, Hargreaves M, Witters LA, Kemp BE, McConell GK.** Effect of exercise intensity on skeletal muscle AMPK signaling in humans. *Diabetes* 52: 2205–2212, 2003. doi: 10.2337/diabetes.52.9.2205.
63. **Li S, Miao XY, Zhang JS, Wei DD, Dong HJ, Xue R, Li JC, Zhang Y, Feng XX, Li J, Zhang YZ.** Far-infrared therapy promotes exercise capacity and glucose metabolism in mice by modulating microbiota homeostasis and activating AMPK. *Sci Rep* 14: 16314, 2024. doi: 10.1038/s41598-024-67220-5.

- 876 64. **Egan B, Carson BP, Garcia-Roves PM, Chibalin A V., Sarsfield FM, Barron N,**
877 **McCaffrey N, Moyna NM, Zierath JR, O’Gorman DJ.** Exercise intensity-dependent
878 regulation of peroxisome proliferator-activated receptor γ coactivator-1 α mRNA
879 abundance is associated with differential activation of upstream signalling kinases in
880 human skeletal muscle. *Journal of Physiology* 588: 1779–1790, 2010. doi:
881 10.1113/jphysiol.2010.188011.
- 882 65. **Rose AJ, Kiens B, Richter EA.** Ca²⁺-calmodulin-dependent protein kinase expression
883 and signalling in skeletal muscle during exercise. *Journal of Physiology* 574: 889–903,
884 2006. doi: 10.1113/jphysiol.2006.111757.
- 885 66. **Fiorenza M, Gunnarsson TP, Hostrup M, Iaia FM, Schena F, Pilegaard H, Bangsbo**
886 **J.** Metabolic stress-dependent regulation of the mitochondrial biogenic molecular
887 response to high-intensity exercise in human skeletal muscle. *Journal of Physiology* 596:
888 2823–2840, 2018. doi: 10.1113/JP275972.
- 889 67. **Alessio HM, Hagerman AE, Fulkerson BK, Ambrose J, Rice RE, Wiley RL.**
890 Generation of reactive oxygen species after exhaustive aerobic and isometric exercise.
891 *Med Sci Sports Exerc* 32: 1576–1581, 2000. doi: 10.1097/00005768-200009000-00008.
- 892 68. **Quindry JC, Stone WL, King J, Broeder CE.** The effects of acute exercise on
893 neutrophils and plasma oxidative stress. *Med Sci Sports Exerc* 35: 1139–1145, 2003. doi:
894 10.1249/01.MSS.0000074568.82597.0B.
- 895 69. **Powers SK, Criswell D, Lawler J, Ji LL, Martin D, Herb RA, Dudley G.** Influence of
896 exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. *American*
897 *Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 266: R375–
898 R380, 1994. doi: 10.1152/ajpregu.1994.266.2.R375.

- 899 70. **Fisher G, Schwartz DD, Quindry J, Barberio MD, Foster EB, Jones KW, Pascoe DD.**
900 Lymphocyte enzymatic antioxidant responses to oxidative stress following high-intensity
901 interval exercise. *J Appl Physiol* 110: 730–737, 2011. doi:
902 10.1152/jappphysiol.00575.2010.
- 903 71. **Gallego-Selles A, Martin-Rincon M, Martinez-Canton M, Perez-Valera M, Martín-**
904 **Rodríguez S, Gelabert-Rebato M, Santana A, Morales-Alamo D, Dorado C, Calbet**
905 **JAL.** Regulation of Nrf2/Keap1 signalling in human skeletal muscle during exercise to
906 exhaustion in normoxia, severe acute hypoxia and post-exercise ischaemia: Influence of
907 metabolite accumulation and oxygenation. *Redox Biol* 36: 101627, 2020. doi:
908 10.1016/j.redox.2020.101627.
- 909 72. **Gallego-Selles A, Galvan-Alvarez V, Martinez-Canton M, Garcia-Gonzalez E,**
910 **Morales-Alamo D, Santana A, Gonzalez-Henriquez JJ, Dorado C, Calbet JAL,**
911 **Martin-Rincon M.** Fast regulation of the NF- κ B signalling pathway in human skeletal
912 muscle revealed by high-intensity exercise and ischaemia at exhaustion: Role of
913 oxygenation and metabolite accumulation. *Redox Biol* 55: 102398, 2022. doi:
914 10.1016/j.redox.2022.102398.
- 915 73. **Bishop DJ, Hawley JA.** Reassessing the relationship between mRNA levels and protein
916 abundance in exercised skeletal muscles. *Nat Rev Mol Cell Biol* 23: 773–774, 2022. doi:
917 10.1038/s41580-022-00541-3.
- 918 74. **Taylor DF, Bishop DJ.** Transcription Factor Movement and Exercise-Induced
919 Mitochondrial Biogenesis in Human Skeletal Muscle: Current Knowledge and Future
920 Perspectives. *Int J Mol Sci* 23: 1517, 2022. doi: 10.3390/ijms23031517.

- 921 75. **Trewin AJ, Parker XL, Shaw CS, Hiam DS, Garnham A, Levinger I, Mcconell GK,**
922 **Stepito NK.** Acute HIIE elicits similar changes in human skeletal muscle mitochondrial H
923 2 O 2 release, respiration, and cell signaling as endurance exercise even with less work.
924 *Am J Physiol Regul Integr Comp Physiol* 315: 1003–1016, 2018. doi:
925 10.1152/ajpregu.00096.
- 926 76. **Edgett BA, Foster WS, Hankinson PB, Simpson CA, Little JP, Graham RB, Gurd**
927 **BJ.** Dissociation of Increases in PGC-1 α and Its Regulators from Exercise Intensity and
928 Muscle Activation Following Acute Exercise. *PLoS One* 8, 2013. doi:
929 10.1371/journal.pone.0071623.
- 930 77. **Percival ME, Martin BJ, Gillen JB, Skelly LE, MacInnis MJ, Green AE,**
931 **Tarnopolsky MA, Gibala MJ.** Sodium bicarbonate ingestion augments the increase in
932 PGC-1 α mRNA expression during recovery from intense interval exercise in human
933 skeletal muscle. *J Appl Physiol* 119: 1303–1312, 2015. doi:
934 10.1152/jappphysiol.00048.2015.
- 935 78. **Nordsborg NB, Lundby C, Leick L, Pilegaard H.** Relative workload determines
936 exercise-induced increases in PGC-1 α mRNA. *Med Sci Sports Exerc* 42: 1477–1484,
937 2010. doi: 10.1249/MSS.0b013e3181d2d21c.
- 938 79. **Scribbans TD, Edgett BA, Bonafiglia JT, Baechler BL, Quadrilatero J, Gurd BJ.** A
939 systematic upregulation of nuclear and mitochondrial genes is not present in the initial
940 postexercise recovery period in human skeletal muscle. *Applied Physiology, Nutrition and*
941 *Metabolism* 42: 571–578, 2017. doi: 10.1139/apnm-2016-0455.
- 942 80. **Granata C, Oliveira RSF, Little JP, Renner K, Bishop DJ.** Training intensity
943 modulates changes in PGC-1 α and p53 protein content and mitochondrial respiration, but

not markers of mitochondrial content in human skeletal muscle. *FASEB Journal* 30: 959–970, 2016. doi: 10.1096/fj.15-276907.

81. **Leick L, Lyngby SS, Wojtasewski JFP, Pilegaard H.** PGC-1 α is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle. *Exp Gerontol* 45: 336–342, 2010. doi: 10.1016/j.exger.2010.01.011.
82. **Perry CGR, Lally J, Holloway GP, Heigenhauser GJF, Bonen A, Spriet LL.** Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *Journal of Physiology* 588: 4795–4810, 2010. doi: 10.1113/jphysiol.2010.199448.
83. **Saleem A, Adhiketty PJ, Hood DA.** Role of p53 in mitochondrial biogenesis and apoptosis in skeletal muscle. *Physiol Genomics* 37: 58–66, 2009. doi: 10.1152/physiolgenomics.90346.2008.-p53.
84. **Kuang J, McGinley C, Lee MJ-C, Saner NJ, Garnham A, Bishop DJ.** Interpretation of exercise-induced changes in human skeletal muscle mRNA expression depends on the timing of the post-exercise biopsies. *PeerJ* 10: e12856, 2022. doi: 10.7717/peerj.12856.
85. **Lindholm ME, Rundqvist H.** Skeletal muscle hypoxia-inducible factor-1 and exercise. *Exp Physiol* 101: 28–32, 2016. doi: 10.1113/EP085318.
86. **Tang K, Breen EC, Wagner H, Brutsaert TD, Gassmann M, Wagner PD.** HIF and VEGF relationships in response to hypoxia and sciatic nerve stimulation in rat gastrocnemius. *Respir Physiol Neurobiol* 144: 71–80, 2004. doi: 10.1016/j.resp.2004.04.009.
87. **Hsu YH, Chen YC, Chen TH, Sue YM, Cheng TH, Chen JR, Chen CH.** Far-infrared therapy induces the nuclear translocation of PLZF which inhibits VEGF-induced

proliferation in human umbilical vein endothelial cells. *PLoS One* 7, 2012. doi:
10.1371/journal.pone.0030674.

88. **Chen C, Chen M, Hsu Y, Chou T.** Far-infrared radiation alleviates cisplatin-induced
vascular damage and impaired circulation via activation of HIF-1 α . *Cancer Sci* 113: 1–13,
2022. doi: 10.1111/cas.15371.

89. **Hobbs AJ.** Soluble guanylate cyclase: the forgotten sibling. *Trends Pharmacol Sci* 18:
484–491, 1997. doi: 10.1016/S0165-6147(97)01137-1.

90. **Chinsomboon J, Ruas J, Gupta RK, Thom R, Shoag J, Rowe GC, Sawada N,
Raghuram S, Arany Z.** The transcriptional coactivator PGC-1 mediates exercise-induced
angiogenesis in skeletal muscle. *Proceedings of the National Academy of Sciences* 106:
21401–21406, 2009. doi: 10.1073/pnas.090913110.

91. **Tsai S-R, Hamblin MR.** Biological effects and medical applications of infrared radiation.
J Photochem Photobiol B 170: 197–207, 2017. doi: 10.1016/j.jphotobiol.2017.04.014.

92. **Wang JL, Lin YC, Young TH, Chen MH.** Far-infrared ray radiation promotes neurite
outgrowth of neuron-like PC12 cells through AKT1 signaling. *Journal of the Formosan
Medical Association* 118: 600–610, 2019. doi: 10.1016/j.jfma.2018.08.015.

93. **Sheppard AR, Swicord ML, Balzano Q.** Quantitative Evaluations of Mechanisms of
Radiofrequency Interactions with Biological Molecules and Processes. *Health Phys* 93:
365–396, 2008. doi: 10.1097/01.HP.0000319903.20660.37.

94. **Bond JW.** The effect of infrared radiation emitting garments on acute skeletal muscle
adaptive responses; Chapter 6. Loughborough University: 2025.
doi.org/10.26174/thesis.lboro.29270324

95. **Kristensen DE, Albers PH, Prats C, Baba O, Birk JB, Wojtaszewski JFP.** Human muscle fibre type-specific regulation of AMPK and downstream targets by exercise. *Journal of Physiology* 593: 2053–2069, 2015. doi: 10.1113/jphysiol.2014.283267.
96. **Baylor SM, Hollingworth S.** Sarcoplasmic reticulum calcium release compared in slow-twitch and fast-twitch fibres of mouse muscle. *Journal of Physiology* 551: 125–138, 2003.
97. **Tripp TR, Frankish BP, Lun V, Wiley JP, Shearer J, Murphy RM, MacInnis MJ.** Time course and fibre type-dependent nature of calcium-handling protein responses to sprint interval exercise in human skeletal muscle. *J Physiol* 600: 2897–2917, 2022. doi: 10.1113/JP282739.
98. **Berchtold MW, Brinkmeier H, Müntener M.** Calcium ion in skeletal muscle: Its crucial role for muscle function, plasticity, and disease. *Physiol Rev* 80: 1215–1265, 2000. doi: 10.1152/physrev.2000.80.3.1215.
99. **Hunter SK.** Age and sex differences in the limits of human performance: fatigability and real-world data. *J Appl Physiol* 136: 659–676, 2024. doi: 10.1152/jappphysiol.00866.2023.
100. **Ansdell P, Thomas K, Hicks KM, Hunter SK, Howatson G, Goodall S.** Physiological sex differences affect the integrative response to exercise: acute and chronic implications. *Exp Physiol* 105: 2007–2021, 2020. doi: 10.1113/EP088548.

FIGURE LEGENDS

Figure 1. Schematic depicting the timeline for an experimental visit. Created in BioRender.

Bond, J. (2026) <https://BioRender.com/yqyv6jw>.

Figure 2. Skin temperature (T_{skin}) at baseline, pre-exercise, repetitions 1-10 and 3hPE in infrared emitting fabric (IREF) and non-infrared emitting fabric (SHAM) conditions. Data are mean $\pm$ SD. $N = 10$ for repetitions 9 and 10 due to one participant completing 8 repetitions, otherwise $N = 11$. # $P < 0.05$ compared to baseline; † $P < 0.05$ compared to Pre-ex; ? $P < 0.05$ compared to repetition 3; ‡ $P < 0.05$ compared to repetition 7; § $P < 0.05$ compared to repetition 8; ^ $P < 0.05$ compared to repetition 9; □ $P < 0.05$ compared to repetition 10.

Figure 3. Representative Western blot membrane and stain free gel images at baseline, PE and 3hPE in infrared emitting fabric (IREF) and non-infrared emitting fabric (SHAM) conditions. A: Cytosolic and nuclear *p*- and *t*- protein targets for mitochondrial biogenesis and representative stain free gel image. B: Cytosolic *p*- and *t*- protein targets for angiogenesis

Figure 4. Cytosolic and nuclear content of phosphorylated (*p*-) and total (*t*-) proteins at baseline, PE and 3hPE in infrared emitting fabric (IREF) and non-infrared emitting fabric (SHAM) conditions. A & B: *p*-AMPK. C & D: *p*-CaMKII. E & F: *p*-p38 MAPK. G & H: *p*-Nrf2. I & J: *t*-PGC-1 α . K: cytosolic *p*-eNOS. Box and whisker plots are presented with black symbols representing individual responses. Whiskers extend to minimum and maximum values and the bar within each box represents the median value. Boxes represent the interquartile range. One sample

was missing from all targets 3hPE in SHAM and at baseline in IREF and SHAM in the nuclear fraction due to insufficient muscle tissue. Another sample was missing from cytosolic *p*-Nrf2 analysis 3hPE in IREF due to insufficient sample. $N = 7$ for nuclear p-AMPK and $N = 11$ for all other targets at all other timepoints. * $P < 0.05$ between conditions; # $P < 0.05$ compared to baseline; † $P < 0.05$ compared to post-exercise.

Figure 5. Changes in mitochondrial gene expression from baseline to PE and 3hPE in infrared emitting fabric (IREF) and non-infrared emitting fabric (SHAM) conditions. A: *PPARGC1 α* . B: *NRF1*. C: *TP53*. D: *TFAM*. Box and whisker plots are presented with black symbols representing individual responses. Whiskers extend to minimum and maximum values and the bar within each box represents the median value. Boxes represent the interquartile range. The dotted line represents the within condition baseline value of 1, to which each value was normalised using the $2^{(-\Delta\Delta C_T)}$ method. $N = 9$ for *TP53* due to a participant having multiple outliers in this data set. Another sample was removed as an outlier from IREF 3hPE. $N = 10$ for all other targets. * $P < 0.05$ between conditions; # $P < 0.05$ compared to baseline; † $P < 0.05$ compared to post-exercise.

Figure 6. Changes in angiogenic gene expression from baseline to PE and 3hPE in infrared emitting fabric (IREF) and non-infrared emitting fabric (SHAM) conditions. A: *NOS3*. B: *HIF-1 α* . C: *VEGFA*. D: *KDR*. Box and whisker plots are presented with black symbols representing individual responses. Whiskers extend to minimum and maximum values and the bar within each box represents the median value. Boxes represent the interquartile range. The dotted line represents the within condition baseline value of 1, to which each value was normalised using

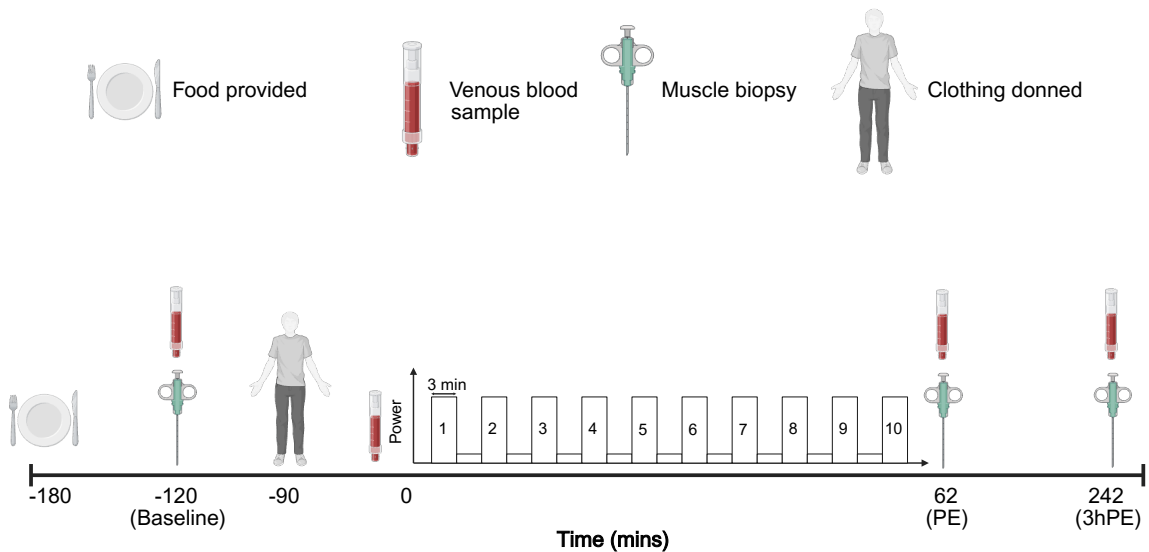
1058 the $2^{(-\Delta\Delta C_T)}$ method. A single sample was removed from *NOS3* in SHAM PE and *KDR* in IREF
1059 3hPE due to being identified as an outlier. $N = 10$ for all other targets. # $P < 0.05$ compared to
1060 baseline; † $P < 0.05$ compared to post-exercise.

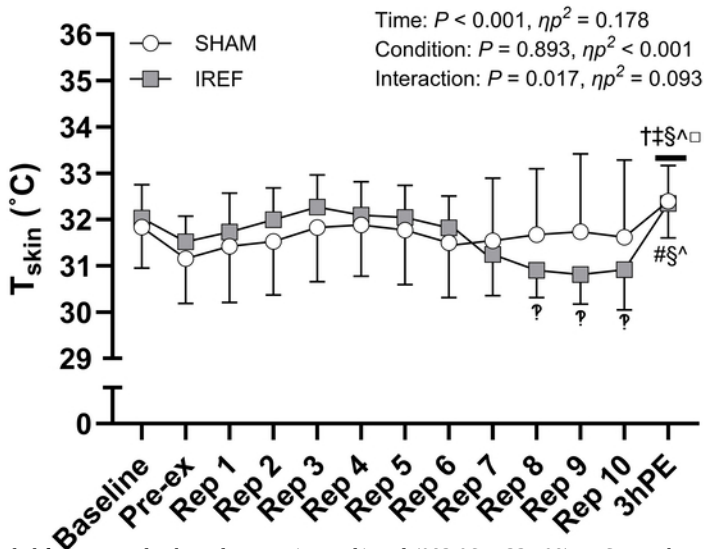
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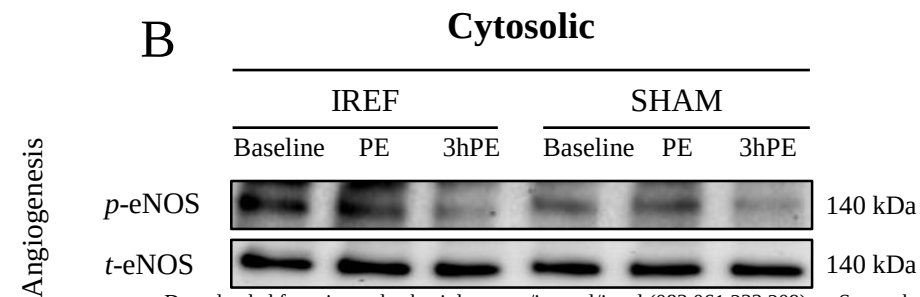
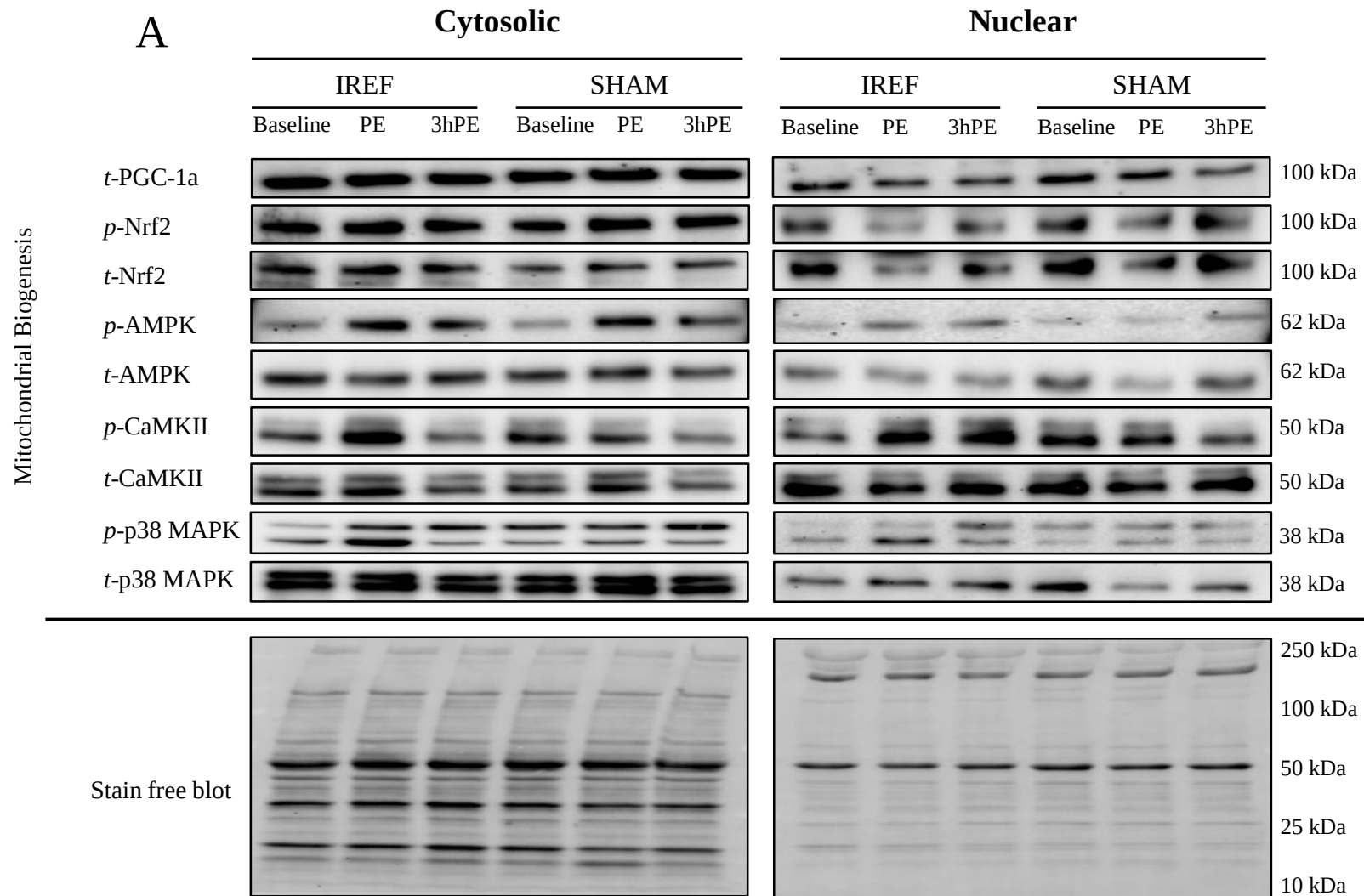
1062 **TABLE LEGEND**

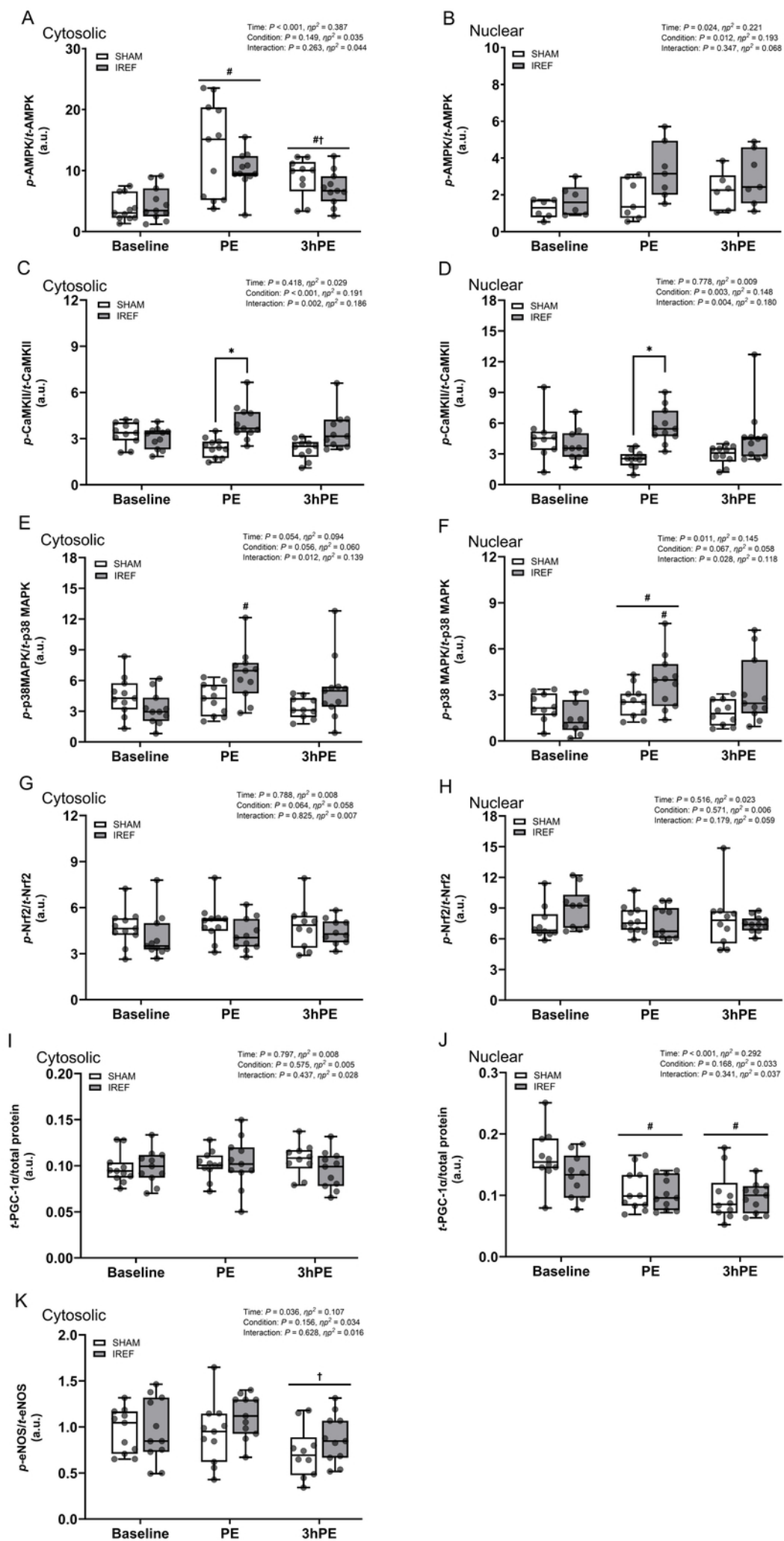
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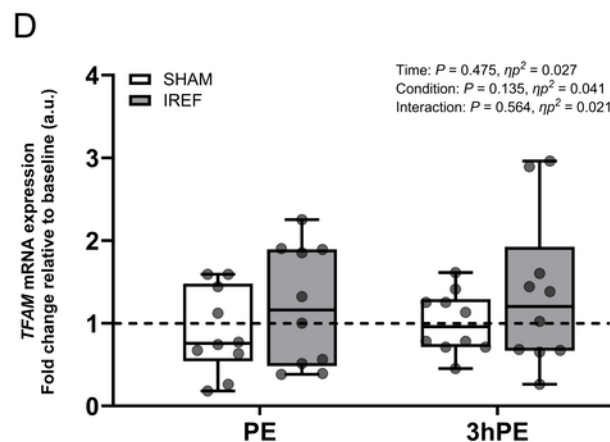
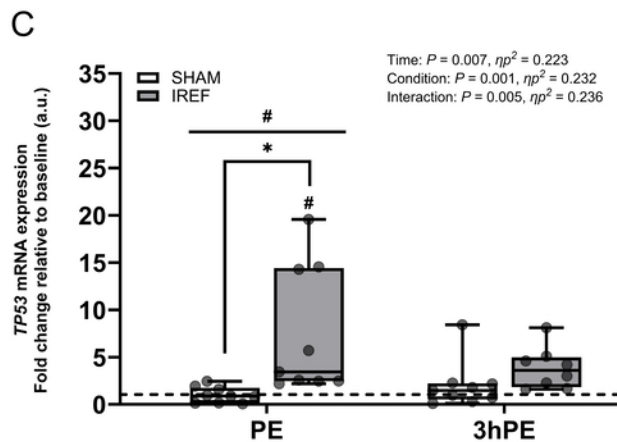
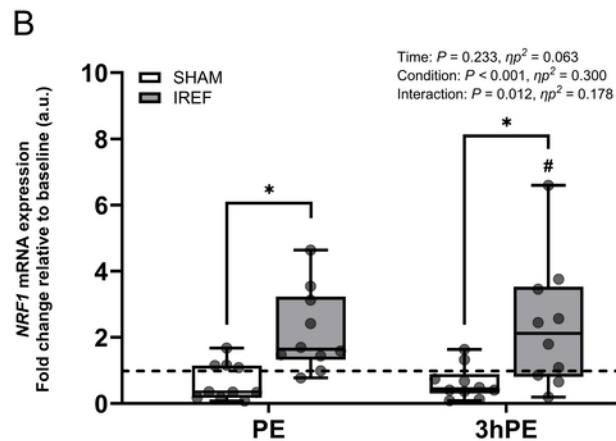
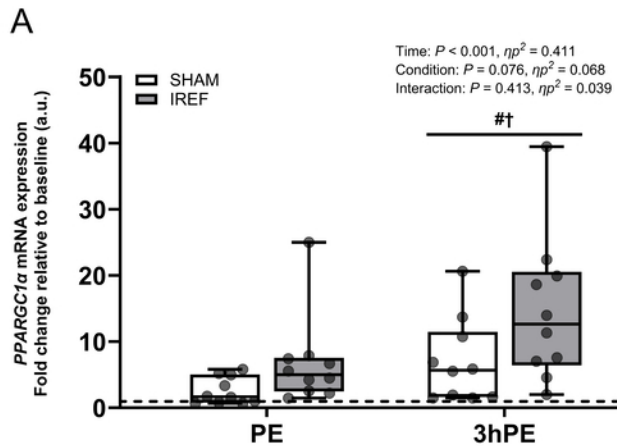
1064 **Table 1.** Composition of IREF and SHAM garments.

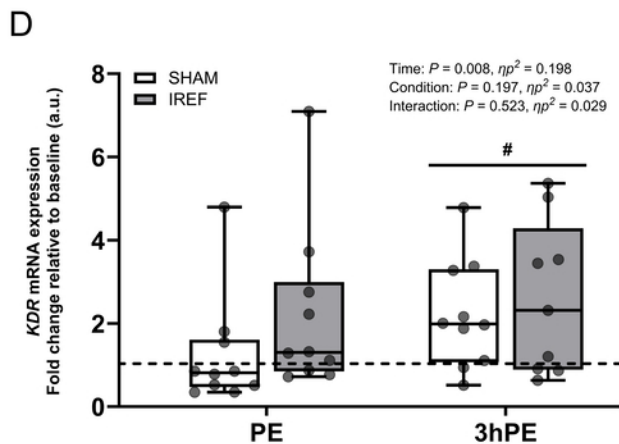
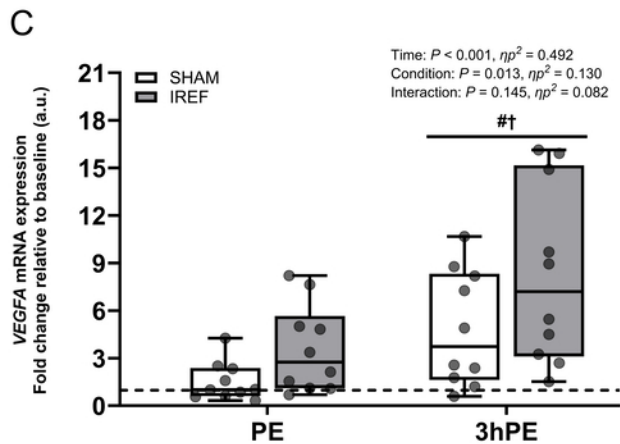
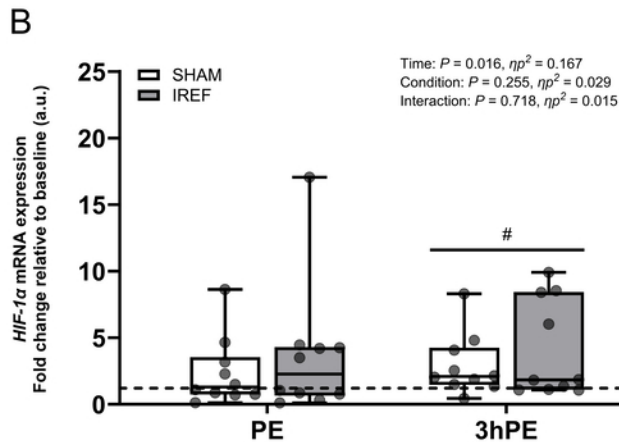
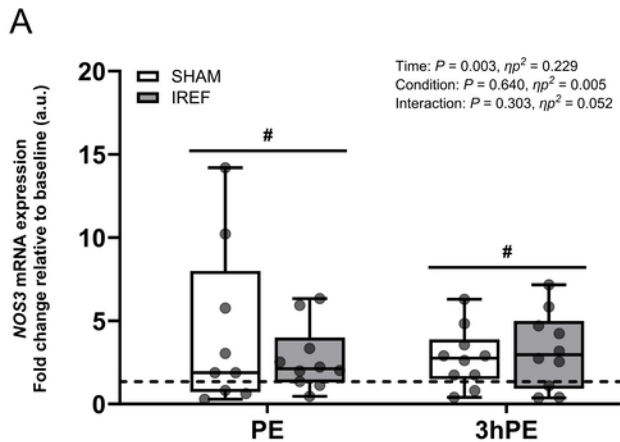












Garment	IREF	SHAM
	(KYnergy polyester®/polyester/elastane, %)	(polyester/elastane, %)
T-shirt	50/50/0 (front) 69/22/9 (back)	94/6
Cycling leggings	50/30/20	85/15
Leggings	50/30/20	83/17

KYnergy polyester® is IR emitting and was supplied by Kymira Ltd. IREF, infrared emitting fabric; SHAM, clothing without infrared emitting properties.

Infrared emitting fabric wear augments acute cellular signalling cascades in response to high-intensity cycling interval exercise

METHODS:

Infrared emitting fabric:



PARTICIPANTS:

11 Males

Age 22 ± 4 yrs

VO_{2peak} , $48.0 \pm 4.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$

SHAM fabric wear



Infrared fabric wear

SHAM fabric wear

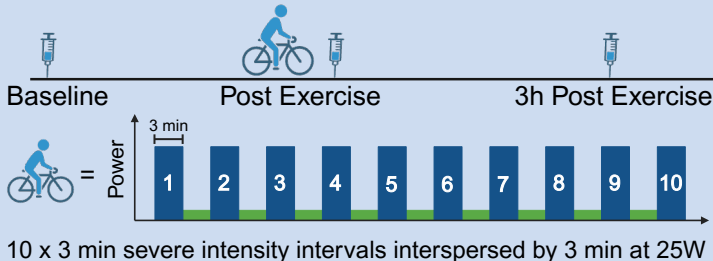


Infrared fabric wear



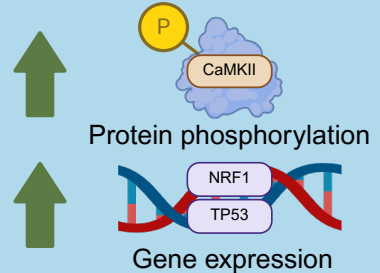
7-day
washout

MUSCLE BIOPSY TIMEPOINTS:



OUTCOME:

POST EXERCISE INFRARED EMITTING FABRIC WEAR



3H POST EXERCISE INFRARED EMITTING FABRIC WEAR



CONCLUSION:

Wearing infrared emitting fabric during high-intensity interval exercise augments post exercise protein phosphorylation and gene expression associated with mitochondrial biogenesis.